Molecular Analysis of Survival Motor Neuron (SMN) and Neuronal Apoptosis Inhibitory Protein (NAIP) Genes in a Chinese Type III Spinal Muscular Atrophy (SMA) Family

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ABSTRACT

In this report we present the genetic studies in a Chinese type III spinal muscular atrophy (SMA) family. The survival motor neuron (SMN) gene exons 7 and 8 and neuronal apoptosis inhibitory protein (NAIP) gene exon 5 were amplified by sequence-specific oligonucleotide primer and polymerase chain reaction (PCR). The SMN gene deletion was detected by restriction endonuclease digestion and single strand conformation polymorphism (SSCP) analysis of PCR-amplified products. The deletion in NAIP gene was detected directly by agarose gel electrophoresis of multiplex PCR-amplified products. All three affected siblings are homozygous for telomeric SMN gene deletion, whereas no NAIP gene deletion was found. Both parents, maternal uncle, and paternal grandmother of affected sibs showed heterozygosity for telomeric SMN deletions. Two unaffected sibs are non-carriers for SMN deletions.

Key words: spinal muscular atrophy, SMN, NAIP

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disorder with an overall incidence of 1 in 10,000 live births and a carrier frequency of 1/50 (Pearn, 1980). SMA is characterized by loss of α-motor neurons in the spinal cord, leading to progressive muscular weakness and atrophy. The affected individuals are classified into three groups on the basis of their clinical severity and the age of onset (Dubowitz, 1978). Type I is the most severe with clinical onset before age of 6 months. These children are never able to sit unsupported and the majority of them die from respiratory failure within the first three years of life. In the intermediate type II, onset is usually before age of 15 months. These children are able to sit without support but never manage to stand or walk unaided. They survive beyond 4 years of age until adolescence or later, depending on the degree of respiratory muscle involvement. The mild type III has onset of muscle weakness that occurs between 18 months to 18 years. These patients manage to stand and walk unsupported, although their walking distance is limited. The long term survival is good, again dependent mainly on respiratory function, and the affected person may have a near-normal life span.

On the basis of linkage studies, all three types of SMA map to chromosome 5q13 (Brzustowicz et al., 1990; Gilliam et al., 1990; Melki et al., 1990a, 1990b). Studies of microsatellite markers which show linkage disequilibrium with the disease indicate a high frequency of null alleles and deletions in SMA patients (DiDonato et al., 1994; Melki et al., 1994; Daniels et al., 1995). Recently, the genomic structure of the SMA locus was reported (Figure 1A) (for review, see Lewin, 1995). The presence of multiple copy repetitive sequences, pseudogenes, and retrotransposon-like sequences underlies the instability of this chromosomal region (Theodosiou et al., 1994; Francis et al., 1995).

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Two candidate genes, the survival motor neurone (SMN) gene and the neuronal apoptosis inhibitory protein (NAIP) gene, were isolated from the region.

The SMN gene contains 8 exons extending over approximately 20 kb (Lefebvre et al., 1995). Two copies of the SMN gene, centromeric and telomeric, have been identified. They are virtually identical at their genomic sequence (only five nucleotide changes along 20 kb) and both are transcribed (Lefebvre et al., 1995). The vast majority (98.6%) of patients have a deletion in the telomeric copy of SMN gene, whereas point mutations have been identified in the remaining 3 of 229 patients (Lefebvre et al., 1995). Mutation in the telomeric SMN causing SMA is further supported by a number of other studies reported (Brahle et al., 1995a; Bussaglia et al., 1995; Chang et al., 1995; Cobben et al., 1995; Matthijs et al., 1996; Rodrigues et al., 1995b, 1999; Velasco et al., 1996; Zappata et al., 1996).

The NAIP gene lies adjacent to the SMN gene and contains 16 exons spanning 60 kb (Roy et al., 1995). The two first coding exons (exons 5 and 6) of NAIP were deleted in 45% of type I patients and 18% of types II and III (Roy et al., 1995). Studies of Spanish SMA patients showed that NAIP exon 5 was deleted in 67.9% type I patients and 16.2% types II and III (Velasco et al., 1996). Thus, loss of NAIP is not sufficient to cause the disease. It was hypothesised that mutations in the telomeric SMN gene are the major determinant of the SMA phenotype, whereas the extent of the deletions, which may include the NAIP gene, may correlate with the severity of the disease (Lefebvre et al., 1995; Roy et al., 1995).

We used a PCR-based DNA test to investigate a Chinese SMA III family for the presence of deletions at both loci. The results revealed that exons 7 and 8 of the telomeric SMN gene are deleted in all three affected sibs. No deletions were detected in the NAIP gene within the family.

Materials and Methods

Subjects and family

A family with three affected sibs were referred to our laboratory for molecular analysis. Clinically, the affected sibs in the family are classified as SMA type III as defined by the International SMA Consortium (Munsat and Davies, 1992). The proposita (III-1 of Figure 2) is 27 years old. Her 19 years old brother (III-5 of Figure 2) had onset of symptoms at the age of five years. Both her 23 and 21 years old sisters (III-3 and III-4 of Figure 2) had onset of symptoms at the age of eight years. All three affected sibs are wheelchair-bound now.

DNA preparation

Peripheral blood (5-10 ml) from subjects were collected into tubes containing EDTA and lysed in
buffer containing 0.32 M sucrose - 10 mM Tris pH 7.5 - 5 mM MgCl₂ - 1 % Triton X-100. Nuclei were collected by centrifugation and digested in buffer containing 50 µg/ml proteinase K - 10 mM Tris pH 7.8 - 5 mM EDTA - 0.5 % SDS. Genomic DNA was then isolated and quantified using standard procedures (Sambrook et al., 1989).

PCR and restriction endonuclease analysis of SMN exons 7 and 8

Amplification of the SMN gene was carried out by using exon 7 primers (R111 and X7-Dra) and exon 8 primers (541C960 and 541C1120) (Figure 1B) as previously described (van der Stege et al., 1995; Lefebvre et al., 1995). Samples of DraI digested exon 7 product were loaded on to a 2.0 % agarose minigel containing ethidium bromide and electrophoresed at 100 V for 60 min to resolve 184 bp (telomeric copy) and 160 bp (centromeric copy) fragments. Samples of DdeI digested exon 8 product were separated in a 1.8 % agarose mini gel to resolve 187 bp (telomeric copy) and 121 bp (centromeric copy) fragments.

Nonisotopic SSCP analysis of SMN exons 7 and 8

Ten microliters of the PCR products were mixed with an equal volume of formamide, denatured for 10 minutes at 95 °C, loaded onto a nondenaturing polyacrylamide gel (0.5 × Hydrolink MDE (J.T.Baker) in 0.6 × TBE). The acrylamide minigel was run at 4 °C for 2.5 hours at 250 V on the Novex Xcell II (Novex). The gels were stained with ethidium bromide (0.5 µg/ml) for 20 min, destained for 15 min, and then visualized and photographed under UV light.

PCR analysis of NAIP exon 5

Deletion of NAIP gene was analyzed by using exon 5 primers (1863 and 1864) and exon 13 primers (1258 and 1343) (Figure 1C) in a multiplex PCR reaction as described (Roy et al., 1995). Samples were loaded on to a 14 % agarose minigel containing ethidium bromide and electrophoresed at 100 V for 60 min to resolve 435 bp (exon 5) and 241 bp (exon 13) fragments.

Results

Restriction endonuclease analysis of deletions in the SMN gene

The two copies of SMN gene differ in their exons by only two base pairs, one in exon 7 and the other in exon 8, thus making distinction of the telomeric copy
obtained (data not shown).

**Analysis of deletions in the NAIP gene**

A multiplex PCR reaction to amplify exon 5 and exon 13 was conducted. Exon 13 is present in both functional and pseudogene copies of NAIP and therefore can be used as an amplification control for exon 5 which is present only in the functional NAIP gene. Exon 5 (435 bp) and exon 13 (241 bp) were detected on a 1.4 % agarose gel in all family members tested (Figure 4). Thus no exon 5 deletion was found in the affected sibs.

**Discussion**

Two different genes (NAIP and SMN) have been recently reported to be associated with the SMA disease (Lefebvre et al., 1995; Roy et al., 1995). The SMN gene encodes a protein of 294 amino acids. Both the telomeric copy and the centromeric copy are widely expressed and their predicted amino acid sequence is identical. Moreover, exon 7 of the centromeric copy is shown alternatively spliced (Lefebvre et al., 1995). NAIP is expressed in motor neurons and is homologous with baculovirus proteins that inhibit virally induced insect cell apoptosis (Roy et al., 1995). NAIP has been shown to have an anti-apoptotic effect in mammalian cells (Liston et al., 1996). The motor neuron depletion observed in SMA suggests that a genetic defect in NAIP may result in a pathologic persistence or reactivation of normally occurring apoptosis.

The incidence of deletions of both telomeric SMN and NAIP genes was higher in type I SMA in comparison with types II and III (Cobben et al., 1995; Rodrigues et al., 1996; Velasco et al., 1996). Thus patients suffering from the milder forms of the disease showed smaller deletions predominantly in the telomeric SMN but not in NAIP. High frequency of deletions in the telomeric SMN makes an important tool for SMA diagnosis. In our study, the three mild type III affected sibs had deletions of telomeric SMN exons 7 and 8 (Figures 2 and 3), but no deletion of NAIP exon 5 was detected (Figure 4).

Two deletion assays were used in this study to examine the SMN gene in the affected family. The restriction enzyme digestion analysis is based on the presence or absence of Drel or Ddel restriction site in exon 7 or 8 that allows distinction of the telomeric SMN gene from its centromeric copy. The SSCP analysis is based on the alternatively folded conformation assumed by the mutated single strand DNA, as indicated by a single base difference in both exons 7 and 8 of the two genes. By restriction enzyme digestion and SSCP
analysis, we have identified carriers in the family. Although assays of exons deletions are not sensitive enough to detect the precise extent of the deletions or to differentiate between inframe or out of frame deletions, the methods has allowed us to confirm directly the clinical diagnosis of SMA in patients. It has also been used for presymptomatic diagnosis (Brahe et al., 1995b) and prenatal diagnosis (Rodrigues et al., 1995a) in families at risk of SMA.

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References


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台灣一個第三型脊椎性肌肉萎縮症 (Type III SMA) 家族致病基因的分子研究

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摘要

本研究中，我們檢視了台灣一個第三型脊椎性肌肉萎縮症 (Type III spinal muscular atrophy) 家族致病基因的分子構造。我們以 DNA 序列專一的寡核苷酸引子 (sequence-specific oligonucleotide primer) 及聚合酶鍵反應 (polymerase chain reaction) 放大 survival motor neuron (SMN) 基因的表現子 7 和 8 及 neuronal apoptosis inhibitory protein (NAIP) 基因的第九表現子。SMN 基因的缺失可藉限制酵素切割 (restriction endonuclease digestion) 及單股核酸摺形多型性 (single strand conformation polymorphism) 分析聚合酶鍵反應放大的產物而檢測之。NAIP 基因的缺失則可直接以瓊脂糖電泳 (agarose gel electrophoresis) 檢測之。三位患者之 telomeric SMN 基因皆為同型合子 (homozygous) 的缺失，而 NAIP 基因則無缺失。患者的雙親、舅舅及祖母皆為異型合子 (heterozygous) 的 telomeric SMN 基因缺失，其二位正常的姪姪則不帶有 SMN 基因缺失。

關鍵詞：脊椎性肌肉萎縮症、SMN、NAIP