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Deletions in the SMN and NAIP Genes in Patients with Spinal Muscular Atrophy in Croatia

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ABSTRACT

Two genes, i.e. survival motor neuron (SMN) and neuronal apoptosis inhibitory protein (NAIP) have been mapped to the SMA region of chromosome 5q13. Both genes are frequently deleted or truncated in SMA patients. We have studied 26 patients with SMA types I–III, 29 first relatives, and 14 subjects with mild adult-onset type IV. DNA deletion genotypes were determined by PCR techniques amplifying exons 7 and 8 of SMN, and exon 5 of NAIP gene which distinguish SMN and NAIP telomeric copy from a non-pathogenic gene homologue as a centromeric copy.

Results revealed the homozygous deletions of exon 7 and 8 of the SMN gene and exon 5 of the NAIP gene in 3/3 infants with SMA I and in 1/20 with SMA type II. Exons 7 and 8 of the SMN gene were homozygously deleted in 10/20 and only exon 7 in 6/20 children with SMA type II. The overall percentage of deletion cases observed was 77% in children with SMA types I–III. Adult patients with type IV SMA showed no homozygous deletion of exons 7, 8 and 5 of the SMN and NAIP genes. Also, all relatives had both a telomeric and centromeric SMN and NAIP copy. Deletion analysis of SMN and NAIP genes are a significant diagnostic tool, because there are clinical entities resembling SMA which most likely have another pathogenetic background.

Introduction

Spinal muscular atrophy (SMA) is a clinically heterogeneous group of neuromuscular disorders characterized by degeneration of the anterior horn cell in the spinal cord and brainstem motor nuclei. The International Consortium on SMA has distinguished three forms of childhood-onset SMA (types I, II, and III) on the basis of age of onset and severity of the clinical course as assessed by clinical examination, muscle biopsy, and electromyography; type I (Werdnig-Hoffmann disease) is the most acute and severe form, with onset before the age of 6 months and death usually before the age of 2 years, type II (intermediate chronic

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form) has onset before the age of 2 years, type III (Kugelberg-Welander disease) is a mild, chronic form, with onset after the age of 18 months. Adult-onset form, termed SMA type IV, is also known. Cases with adult-onset SMA have normal muscle strength in childhood, simptoms are manifested after the age of 18 years and progression of muscle weakness is very slow[1–4]. Spinal muscular atrophy (SMA) is a recessively inherited neuromuscular disorder with a worldwide distribution, with estimated incidence of approximately 1/10000 liveborn, whereas adult SMA is less frequent with prevalence of 0.32 per 100000 in the general population[5]. SMA region is mapped to chromosome 5q 11.2–13.3[6–8]. International investigations demonstrated that SMA region is highly variable and unstable. By a combination of genetic and physical mapping, a yeast artificial chromosome contig of the 5q 13 region spanning the disease locus was constructed to show the presence of low copy repeats in this region. Inherited and de novo deletions were observed in SMA patients, too[9,10]. Two candidate genes NAIP and SMN have recently been reported in SMA patients. SMN gene, telomeric copy (telSMN) is highly homologous with centromeric copy (cenSMN or ᶜBCD541). Both copies show identical sequences, except for five exchanges of a base pair at the 3'-end of the gene, intron 6 to exon 8[7]. However, only deletion/mutation in telSMN seem to cause SMA. According to different type of SMA 80%–98% of SMA patients show homozygous deletions/interruptions of exons 7 and 8 of telSMN, whereas homozygous deletions of cenSMN was found in about 2%–3% of carriers and control[11]. However, since the first six exons of the two copies cannot be detected, question is whether the whole or only a part of the gene is deleted in SMA patients. The neuronal apoptosis inhibitory protein (NAIP) gene, which lies in the region adjacent to the SMN gene, is deleted in 45% of patients with type I SMA and 18% of those with type II and III, while centromeric NAIP copy is a pseudogene. The frequency of homozygous deletion of NAIP exon 5 has been found to be clearly correlated with the severity of disease[12]. SMN deletion analysis has proven useful for establishing the diagnosis in individuals with adult-onset mild muscle weakness[13,14]. Question is whether adult-onset SMA, which is the mildest form of the disease is the separate genetic entity from infantile SMA. Within the last year, there have been several reports of SMN and NAIP gene deletions in SMA patients in many countries[11,16]. We present analysis of deletions in NAIP and SMN genes in children with SMA type I–III and adult spinal muscular atrophy patients from Croatia.

Material and methods

We studied 26 Croatian children at the age from 1 month to 8 years with proximal SMA, and their first relatives for deletions of exons 7 and 8 of SMN gene and exon 5 of the NAIP gene. Diagnosis of SMA type I was established in 3 children according to the International SMA Consortium[17] at the age of 5–6 months. The diagnosis in 20 children with SMA type II was usually established in the second year of life, and in three children with type III between the 3–8 years of age. We also examined 29 of their healthy first degree relatives and 14 adult-onset SMA patients. DNA isolation from blood was performed by the phenol/chloroform or salting-out procedure[18]. PCR/RFLP DNA analysis of SMN uses amplification of exons 7 and 8, digestion with restriction enzymes (exon 7/DdeI; exon 8/DraI), separation of the restriction fragment on agarose gel electrophoresis, and staining with ethidium bromide[19]. PCR products of exon 8 of the SMN gene and the copy gene can be distinguished since the copy

gene contains a recognition site for the DdeI, which is absent in exon 8 of SMN, while for exon 7 a restriction site is created for DraI in the PCR product to distinguish SMN from a non-pathogenic gene homologue. M-PCR analysis of NAIP gene amplified genomic DNA with specific oligonucleotide for exons 5 and 13 in a multiplex PCR reaction. Exon 13 is present in both functional and pseudogene copies of NAIP, and can be used as a positive control for exon 5 which is present only in the functional NAIP gene. PCR was carried out using the standard protocol. The absence of PCR reaction product for exon 7 and/or 8 of SMN and 5 of NAIP indicates positive results and an SMA phenotype[8].

Results

Deletion analysis of SMN and NAIP genes in infantile and adult SMA patients using PCR/RFLP and M-PCR methodology are shown in Figure 1. SMN/exon 7/DraI digestion results in two fragments corresponding to the SMN gene and ^{c}BCD541 copy. SMN/exon 8/DdeI digestion gives three fragments: the one with higher molecular weight corresponds to SMN, and the other two correspond to ^{c}BCD541 copy. In NAIP analysis only control band presentation indicates deletions of exon 5, which is specific for the functional gene. The results thus obtained for analyzed patients and relatives are summarized in Table 1. Homozygous deletions of exon 7 and/or 8 of SMN, and exon 5 of NAIP genes were detected in 20/26 children with SMA types I–III. Exon 7 and 8 of the SMN gene and exon 5 of the NAIP gene were homozygously deleted in all children with SMA type I, and in one child with SMA type II. 10 patients showed a homozygous deletion of exon 7 and 8, while 6 lacked the SMN exon 7, but retained the exon 8. None of the relatives and adult-onset showed homozygous deletion of the telomeric SMN gene. In addition, centromeric region of the SMN exon 7 and 8 were present in all individuals.

Discussion

We have studied the incidence of deletions in both SMN and NAIP genes in a clinically heterogeneous group including children with proximal SMA (type I–III) and subjects with mild adult-onset type IV SMA. Genotyping of the patients established the 3/3 SMA type I while 17/20 SMA type II patients, who fulfilled the diagnostic criteria of the International SMA Consortium, shared homozygous deletion of exons 7 and/or 8 of the telomeric copy of the SMN gene. It means that SMN and NAIP genes have been found to be homozygously deleted in 77% in children with proximal SMA in Croatia. Exons 7 and 8 of the SMN gene and exon 5 of the NAIP gene were homozyously deleted in 3/3 infants with SMA type I and in 1/20 with type II. Exons 7 and 8 of the SMN gene were homozygously deleted in 10/20 children with type II, and only exon 7 was deleted in 6/20 SMA patients. We report an increased deletion frequency for exon 7 (23%) and lower for exons 7 and 8 (54%); Rodrigues et al. found SMN deletions in 8.7% and 73.9%, respectively[11]. Our results are consistent with those reported in Europe and America[3,20]. Within the last three years several reports of gene deletions in SMA patients have been published for many ethnic groups. Samilchuk et al. found that SMA gene deletions are more common in Arab patients than in patients of other ethnic origin[16]. The situation, however, is different in those with onset beyond 30 years of age (SMA type IV). No homozygous deletion of the exons 7 and 8 of SMN and exon 5 of NAIP genes was found in 14 patients belonging to this group. Three SMA patients type III did not show deletions of the SMN and NAIP genes. The difference in clinical ex-

TABLE 1
SMN AND NAIP DELETIONS IN CLINICAL SUBTYPES OF SMA

SMA genotype			SMA phenotype				
SMN exon 7	SMN exon 8	NAIP exon 5	Type I	Type II	Type III	Type IV	Relatives
del	del	del	3	1			
del	del	n		10			
del	n	n		6			
n	n	n		3	3	14	29

del = deleted
n = not deleted

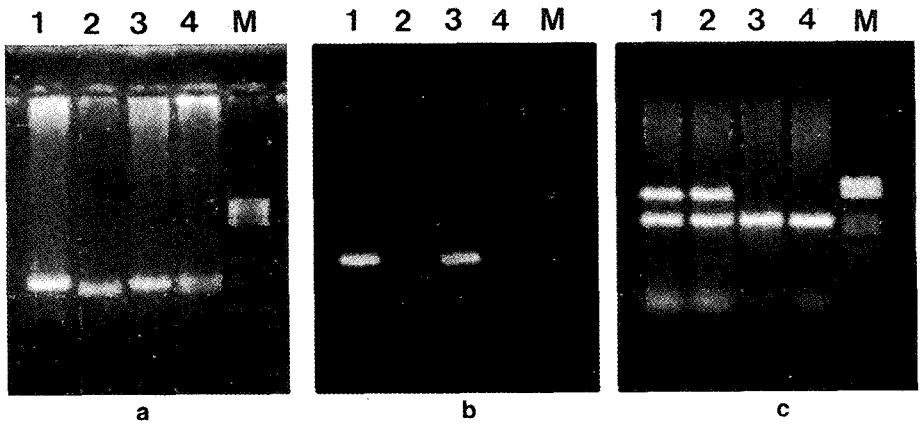

Fig. 1. PCR analysis of SMN and NAIP genes.
***a)** SMN exon 7,* **b)** *SMN exon 8*
(upper band SMN – telomeric gene; lower band ^{c}BCD541 – centromeric gene)
Lanes 1, 3 *undigested PCR product*
Lanes 2–4 *Dra I (exon 7); DdeI (exon 8) digests*
Lane 2 *SMA patient with deletion of SMN gene*
Lane 4 *control with both the SMN and ^{c}BCD541*

***c)** NAIP exon 5*
(upper band exon 5 and lower band exon 13)
Lanes 1, 2 *control with both NAIP exons 5 and 13*
Lanes 3, 4 *SMA patients showing absence of exon 5*

M *DNA molecular weight markers 8–587 bp*

pression may be due to homozygosity or compound heterozygosity for point mutations, microdeletions or frame-shift deletions, de novo deletions, or repeat elements[6,8,20–23]. In this study, we did not find gene deletions in asymptomatic relatives of SMA patients. Our study showed that no deletions of SMN and NAIP genes could be detected in 29 asymptomatic relatives. Some doubt was related to the

SMN gene in determining SMA phenotype by homozygous deletions in an unaffected sibling. Hannen et al. reported on an unaffected mother and five siblings of SMA type II who showed homozygous deletions of SMN exons 7 and 8[21]. The presence of deletions in healthy siblings of affected individuals is difficult to explain. Brahe et al. found neurogenic EMG pattern in the asymptomatic patients compatible with chronic SMA, which suggests that those patients could develop SMA and become symptomatic afterwards[12]. Results obtained on our adult-onset SMA patients with mild muscle weakness are similar to those reported by Zerres et al., who did not find any deletion in adult-onset SMA patients, suggesting the possibility of locus heterogeneity between adult and childhood disease[22]. Clement et al. found deletion in a 73-year-old woman who developed SMA type IV at the age of 47 years. Three of her five children had SMA type II. This finding provides evidence for allelic homogeneity betteen childhood and adult forms of SMA[14]. SMA region is highly unstable, suggesting that de novo mutation of alleles is responsible for some cases of SMA, too. Although there are questions regarding the phenotype-genotype correlation, there is no doubt about the importance of SMA DNA analysis for homozygous deletion of the SMN gene. SMN and NAIP DNA test is a significant diagnostic finding which in the proper clinical setting may supplement other confirmatory tests now in use. The advantage of PCR/RFLP and M-PCR methodology is that it is fast, noninvasive, and results are clear and unambiguous. The genetic investigation of SMA region is an important step in understanding of SMA at the molecular level as a clinical and genetic entity, but elucidating the function of the gene product is important for the understanding of the pathogenesis of SMA.

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DELECIJE SMN I NAIP GENA U BOLESNIKA SA SPINALNOM MIŠIĆNOM ATROFIJOM U HRVATSKOJ

S A Ž E T A K

Dva SMA genska kandidata nalaze se na kromosomu 5q13; SMN i NAIP. Intragenske promjene (delecije) su nađene kod bolesnika sa SMA. U ovom radu prikazani su rezultati genskih ispitivanja 26 SMA bolesnika tipa I–III, 14 odraslih bolesnika tipa IV i 29 zdravih članova obitelji u hrvatskoj populaciji. Delecijski genotip je utvrđen PCR analizom egzona 7 i 8 SMN i egzona 5 NAIP gena čime se SMN i NAIP telomerna kopija gena razlikuje od nepatogene centromerne kopije. Rezultati genotipizacije otkrivaju učestalost delecija SMN i NAIP gena u 77% SMA bolesnika tipa I–III. Homozigotna delecija egzona 7 i 8 SMN gena i egzona 5 NAIP gena nađena je u 3/3 SMA bolesnika tipa I i u jednog SMA bolesnika tipa II. Delecija egzona 7 i 8 SMN gena nađena je kod 10/20, a delecija samo egzona 7 kod 6/20 SMA bolesnika tipa II, dok su kod ostalih ispitanika rezultati bili negativni. Kod odraslih bolesnika s tipom IV SMA nije nađena homozigotna delecija egzona 7, 8 i 5 SMN i NAIP gena. Također kod članova obitelji nađene su i telomerna i centromerna kopija SMN i NAIP gena. Delecijska analiza SMN i NAIP gena je važna u dijagnostici SMA zbog sličnih kliničkih entiteta koji imaju drugu patogensku osnovu.