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Analysis of point mutations in the SMN1 gene in SMA patients bearing a single SMN1 copy

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Abstract

Spinal muscular atrophy (SMA) is caused by homozygous deletion of the SMN1 gene in approximately 96% of cases. Four percent of SMA patients have a combination of the deletion or conversion on one allele and an intragenic mutation on the second one. We performed analysis of point mutations in a set of our patients with suspicion of SMA and without homozygous deletion of the SMN1 gene. A quantitative test determining SMN1 copy number (using real-time PCR and/or MLPA analysis) was performed in 301 patients and only 1 SMN1 copy was detected in 14 of them. When these 14 patients were screened for the presence of point mutations we identified 6 mutations, p.Y272C (in three patients) and p.T274I, p.I33IfsX6, and p.A188S (each in one case). The mutations p.I33IfsX6 and p.A188S were found in two SMAI patients and were not detected previously. Further, evaluation of the relationship between mutation type, copy number of the SMN2 gene and clinical findings was performed. Among our SMA patients with a SMN1 homozygous deletion, we found a family with two patients: the son with SMAII possesses 3 SMN2 copies and the nearly asymptomatic father has a homozygous deletion of SMN1 exon 7 and carries 4 SMN2 copies. Generally, our results illustrate that an increased SMN2 gene copy number is associated with a milder SMA phenotype.

Keywords: SMA; SMN1; SMN2; Real-time PCR; Copy number; Point mutation

1. Introduction

Spinal muscular atrophy (SMA), with an incidence of 1/6000–1/10,000 and a carrier frequency of 1/40, is the second most frequent lethal autosomal recessive disease in Europeans next to cystic fibrosis [1,2]. The SMA-determining gene, termed survival motor neuron (SMN), is present on 5q13 in two copies, a telomeric SMN1 gene and a centromeric SMN2 gene which are highly homologous and contain only five base-pair differences [3]. The SMN protein plays a crucial role in the generation of the pre-mRNA splicing machinery and thus in mRNA biogenesis [4]. Lefebvre concluded that the SMN gene has 8 exons [3]; however Burglen characterized the gene in more detail and showed that it consists of 9 exons [5] and in order not to confuse

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previously published mutation data, exon 2 was referred to as exon 2a and 2b. The stop codon occurs in exon 7.

Exon 7 of the SMN1 gene is not detectable in approximately 96% of SMA patients, owing to either deletion of SMN1 or conversion of the SMN1 sequence to SMN2. Approximately 4% of patients have a combination of the deletion or conversion on one allele and an intragenic mutation on the second one. The centromeric SMN2 gene cannot compensate for the SMN1 defect because single nucleotide difference in exon 7 causes exon skipping in about 90% of SMN2 transcripts [6]. However, increased SMN2 gene copy number, which can occur as the result of gene conversion events, is associated with a milder SMA phenotype [7].

The C terminus of SMN, including the sequence encoded by exon 7 containing a highly conserved tyrosine/glycine-rich sequence (Y/G box), is required for SMN's nucleic acid and protein binding activity as well as for its oligomerization [8]. Several missense mutation clusters have been described in and around the Y/G box, including p.S262I, p.Y272C, p.T274I, p.G275S, and p.G279V. One of these mutations, p.Y272C, is associated with the most severe form of the disease (type SMAI), and among different subtle SMN1 mutations p.Y272C is the most frequent (20% of SMN1 mutations) [9].

We present results of molecular genetic analyses performed in the set of our patients who do not have homozygous deletion of the SMN1 exon 7, and in whom a quantitative test showed a single copy of this exon. By using long-range PCR, PCR and sequencing we identified six mutations (four different types). Two of these, p.I33IfsX6 and p.A188S, are novel ones not described so far. Further, we performed analysis of SMN2 copy number in these patients and evaluated relationships among the detected mutation, SMN2 copy number, and clinical manifestation of the disease. Moreover, we performed also analysis of SMN2 copy number in 70 patients with SMAI, II, and III. We found a family with two SMA patients where the son has been diagnosed as SMAII and possesses 3 SMN2 copies, and the nearly asymptomatic father has also homozygous deletion of SMN1 exon 7 and carries 4 SMN2 copies.

2. Patients and methods

2.1. Patients

Patients with supposed SMA diagnosis were tested for the presence of homozygous deletion of SMN1. This test is based on the single nucleotide differences in exons 7 and 8 that distinguish SMN1 and SMN2 [5]. Genomic DNA was isolated from peripheral blood by the salting-out method [10]. DNA concentration was initially determined from UV absorbance at 260 nm using a NanoDrop instrument (NanoDrop Technologies), and after dilution to ~10 ng/μl the exact concentration was established from UV absorbance.

2.2. Real-time PCR for determination of SMN1 gene copy number

Real-time detection of PCR products was performed with TaqMan hybridization probes labelled in the case of the SMN1 gene with FAM reporter dye, and in the case of a reference gene (the ALB gene, albumin) with JOE reporter dye. Sequences of primers and probes used are given in Table 1. Multiplex PCR was performed in a total volume of 25 μl, containing 50 ng of genomic DNA, 400 nM primers specific for the SMN1 gene, 400 nM primers specific for the ALB gene, 200 nM each of probes, and 1× TaqMan Universal PCR Master Mix (Roche). Real-time PCRs were carried out using a RotorGene 3000 instrument (Corbett Research). The following PCR conditions were used: 95 °C/10 min, followed by 40 cycles of 95 °C/15 s, and 60 °C/30 s. DNA samples were amplified in two parallel PCRs and each run contained two control genomic DNAs (the calibrators), the first with one SMN1 copy and the second with two SMN1 copies per genome. The number of SMN1 gene copies was determined using the comparative C_t method [11]. The result of this analysis determines the normalized SMN1 gene copy

Table 1
Primers and probes used for real-time PCR

Fragment	Name	Sequence (5′ → 3′ direction)
SMN1	SMN1-F	ACTTCCTTTTATTTTCCTTACAGGGTTTC
	SMN1-R	AATGCTGGCAGACTTACTCCTTAATTTAA
	SMN1-probe	FAM-ACAAAATCAAAAAGAAGGAAGGTGCTCACATTC-BHQ1
SMN2	SMN2-F	AATGCTTTTTAACATCCATATAAAGCT
	SMN2-R	CCTTAATTTAAGGAATGTGAGCACC
	SMN2-probe	FAM-TGATTTGTCTAAAACCC-MGB
Albumin	ALB-F	GCTGTCATCTCTTGTGGGCTGT
	ALB-R	ACTCATGGGAGCTGCTGGTTC
	ALB-probe	JOE-CCTGTCATGCCCACACAAATCTCTCC-BHQ1