Neurogenetics (1998) 1:273–276 © Springer-Verlag 1998

Original article

Identification of a novel missense mutation of the *SMN*[T] gene in two siblings with spinal muscular atrophy

Ching H. Wang · Brett D. Papendick · Paul Bruinsma · J. Kevin Day

Accepted: 19 March 1998

ABSTRACT

Spinal muscular atrophy (SMA) is a motor neuron disease caused by mutations in the telomeric copy of the survival motor neuron (*SMN*^{T}) gene. Over 90% of SMA patients harbor a deletion of *SMN*^{T}, but relatively few base-pair mutations have been reported. We report here a novel G279C mutation with a G to T transversion on exon *7* (nucleotide position 868) of *SMN*^{T}. Another missense mutation has been reported recently on position 869. The fact that two mutations on the same codon both result in SMA suggest a functional significance of this amino acid within the SMN protein.

Key words Spinal muscular atrophy · *SMN* gene · Missense mutation

INTRODUCTION

Spinal muscular atrophy (SMA) is a common childhood neuromuscular disorder characterized by degeneration of the motor neurons and muscular atrophy. Recent advances in the molecular genetics of SMA have led to the identification of the survival motor neuron (*SMN*) gene [1]. The *SMN* gene is present in multiple copies: one telomeric (*SMN*^{T}) and several centromeric (*SMN*^{C}) copies [2–5]. Only the *SMN*^{T} copy is associated with SMA. Over 90% of SMA patients harbor a homozygous deletion of the *SMN*^{T} copy [1, 6–9]. Several small-scale DNA rearrangements, such as microdeletions and duplications, have been identified [1, 10, 11]. However, few base-pair mutations have been reported [3, 12–14], possibly due to the extreme homology of the *SMN* gene copies posing a technical difficulty in the detection of *SMN*^{T}–specific mutations. We report here a novel base-pair mutation of the *SMN*^{T} gene in two SMA siblings. This missense mutation on the paternal chromosome in combination with a possible deletion or gene conversion on the maternal chromosome resulted in SMA.

C. H. Wang (✉) · B. D. Papendick
Departments of Psychiatry and Neurology, University of Missouri, Columbia, MO 65212, USA

C. H. Wang
Department of Biochemistry, University of Missouri, Columbia, MO 65212, USA

C. H. Wang · P. Bruinsma · J. Kevin Day
Genetic Area Program, University of Missouri, Columbia, MO 65212, USA

MATERIAL AND METHODS

The two patients were diagnosed with SMA type II (7237) and type III (7236) according to the criteria of the International SMA Consortium [15]. The single-strand conformation polymorphism (SSCP) assays were performed using primers flanking exons *7* and *8* as previously reported [1, 16]. Polymerase chain reaction (PCR) bands were separated by electrophoresis through an MDE gel (AT Biochem, Malvern, Pa., USA) at 4 °C, followed by autoradiography. DNA sequence analysis was performed using subcloned PCR fragments as templates. In both SMA patients the PCR fragments were obtained by reverse transcription-PCR using total RNA isolated from the lymphoblastoid cell lines and primers flanking exons *6* and *8* of the *SMN* gene. The PCR fragments were subcloned into pNoTA/T7 vector and propagated in competent hosts using Primer PCR CLONER cloning system (5 Prime –>3 Prime, Boulder, Colo., USA). Colony PCR was performed using primers flanking exon *8* followed by restriction enzyme digestion [17] to screen for the clones containing the *SMN*^{T} copy. Similar procedures were used for both parents, except that genomic DNA was used as the PCR templates and the *SMN*^{T} copies were selected by restriction enzyme digestion utilizing a T/C base-pair polymorphism on exon *7* to distinguish the *SMN*^{T} from *SMN*^{C} [17]. Manual sequencing was carried out using USB sequencing kits (Amersham Life Science, Cleveland, Ohio, USA).

RESULTS AND DISCUSSION

Initial SSCP assays of exon *7* revealed an aberrant band on one of the affected patients (7236) and her father (7234) (band A, Fig. 1A). This band is not seen in her

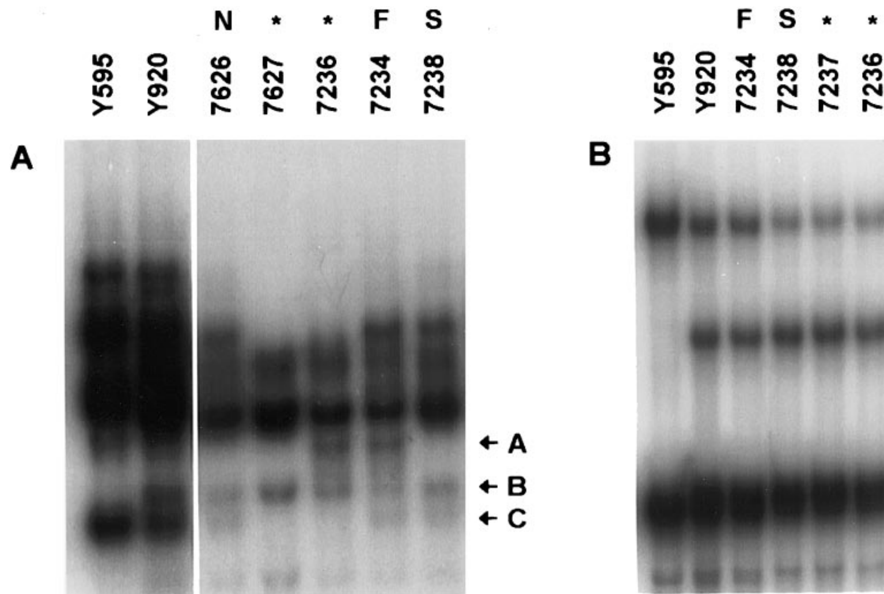

Fig. 1 Single-strand conformation polymorphism (SSCP) analysis identifies an aberrant band in a spinal muscular atrophy (SMA) patient and her father. **A** SSCP assays for *SMN* gene exon *7*. Primers flanking exon *7* (R111 and 541C770) were selected from previous report [1]. Two control yeast artificial chromosome (YAC) contigs are included for comparison. YAC595C11 contains only an SMN^{T} copy (*band C*). YAC920C9 contains both SMN^{C} and SMN^{T} copies (*bands B* and *C*). The SMA patient 7236 and her father 7234 both contain an aberrant *band A*. The patient contains no intact SMN^{T} copy (*band C*), while the father retains both *band A* and *C*. An unrelated SMA patient 7627 is missing an SMN^{T} copy. Two unaffected persons (7626 and the patient's sister 7238) retain two normal *bands B* and *C*. **B** SSCP assays for *SMN* gene exon *8*. All family members and the affected siblings retain at least an intact copy of the SMN^{T} and SMN^{C}

unaffected sister (7238), a normal individual (7626), or an unrelated SMA patient (7627). The same aberrant band is seen in her affected brother (7237), but not in the mother (data not shown). The patient 7236 is missing a normal SMN^{T} exon *7* copy (band C, Fig. 1A), as is her affected brother 7237 (data not shown). The father's DNA retains both bands A and C suggesting a heterozygous mutation. Both affected siblings retain at least one copy of the intact exon *8* of the SMN^{T} gene (Fig. 1B). Sequence analysis of the subcloned exon *7* PCR fragments revealed a G to T transversion in both affected siblings and their father, but not in the mother (Fig. 2). The substitution occurs at the first base-pair of exon *7*, codon 279, nucleotide position 868. The G to T transversion results in an amino acid change from glycine (GGT) to cysteine (TGT). The father was heterozygous for the mutation, containing a normal and a mutant sequence, while the mother's DNA contains only a normal SMN^{T} sequence. Combined PCR and restriction enzyme digestion of exon *7* of the *SMN* gene using maternal DNA does show a much-weaker telomeric band compared with that of the centromeric band, suggesting that the mother is a hemizygous carrier lacking a copy of the SMN^{T} gene (data not shown). This mutation is not seen in 100 non-SMA chromosomes (data not shown).

Since both affected siblings retain no intact copy of SMN^{T} but only a mutant copy on the paternal chromosome, we propose that the maternal SMA chromosome harbors a deletion or gene conversion [3, 5, 18] of the SMN^{T} gene. This hypothesis is supported by the fact that we did not see the mutation on the maternal chromosomes either by SSCP or by direct sequencing, and the fact that a much-weaker band of the SMN^{T} copy was observed when compared with that of her SMN^{C} copy (data not shown).

The *SMN* gene encodes a novel protein that is decreased in both SMA lymphocytes and spinal cord compared with those of normal tissues [19, 20]. The SMN protein plays an essential role in the biogenesis of spliceosomal small nuclear ribonucleoproteins (snRNPs) [21, 22]. One of the snRNPs, Sm B, binds to the domains of the SMN protein encoded by the exon *6* and *7* regions of the gene [21]. These reports indicate that the C-terminus of the *SMN* protein is essential for its function in RNA metabolism. Recently another SMN^{T} missense mutation, G279V, has been reported on nucleotide position 869 [13]. The mutation reported here corresponds to the same codon but on position 868. This nucleotide substitution causes an amino acid change from glycine to cysteine and may alter the SMN protein structure significantly due to the introduction of a disulfide bond. The fact that two missense mutations have been found on the same codon and that both result in individuals with SMA suggests a functional significance of this amino acid.

Identification of a heterozygous SMN^{T} mutation combining a deletion or gene conversion that resulted in SMA, such as reported here, poses a cautionary point for the clinical diagnostic screening using restriction enzyme digestion. Base-pair mutations like this are

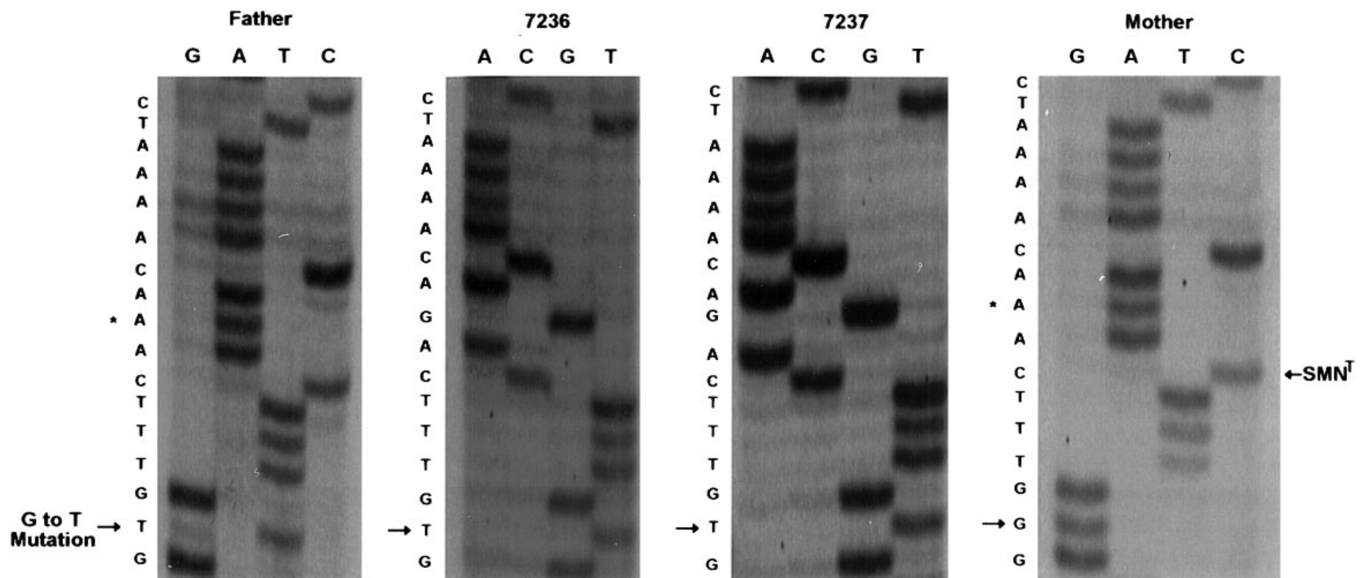

Fig. 2 Sequence analysis reveals a G to T transversion at the first nucleotide of exon *7* of the SMN^T gene. Subcloned polymerase chain reaction (PCR) fragments containing exon *7* of the *SMN* gene were used as sequencing templates. A G to T transversion was observed in both patients and their father, but not in their mother. The sequence is derived from the SMN^T copy, as shown by the C nucleotide at the sixth position of exon *7* (5 base pairs above the mutation) which is specific for the SMN^T copy. The parents' sequences also contain a nucleotide substitution (G to A), indicated by an *asterisk*. This was introduced by a PCR primer mismatch designed to create a *Dra*I restriction site for selection of the SMN^T copy [17]

likely missed by this method. In a compelling situation, screening for point mutations using SSCP or direct sequencing should be used, particularly when a decrease in gene dosage is suspected [3].

Acknowledgement This study is supported by a NINDS Clinical Investigator Development Award NS01576.

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