European Journal of Human Genetics (2003) 11, 275–277

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SHORT REPORT

# Inverse correlation between *SMN1* and *SMN2* copy numbers: evidence for gene conversion from *SMN2* to *SMN1*

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**Most carriers of autosomal recessive spinal muscular atrophy (SMA) have only one copy of *SMN1* because of *SMN1* gene deletions or gene conversions from *SMN1* to *SMN2*, which has only one base difference in coding sequence from *SMN1*. Using *SMN* gene dosage analysis, we determined the copy numbers of *SMN1* and *SMN2* in the general population as well as in SMA patients and carriers. Increased *SMN1* copy number is associated with decreased *SMN2* copy number in the general population; that is, *SMN2* copy number was decreased to one or zero copies in 11 of 13 individuals with three or four copies of *SMN1*, whereas only 71 of 164 individuals with two copies of *SMN1* had one or zero copies of *SMN2* ($P<0.01$). *SMN2* copy number was increased to three or four in a subset of *SMN1* deletion/conversion carriers, and in most SMA patients with a milder phenotype. In conclusion, our data provide evidence that gene conversion from *SMN2* to *SMN1* occurs, and that *SMN1* converted from *SMN2* is present in the general population.**

*European Journal of Human Genetics* (2003) **11,** 275–277. doi:10.1038/sj.ejhg.5200957

**Keywords:** spinal muscular atrophy; *SMN1*; *SMN2*; copy number; gene conversion; SMN

## Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disorder caused by mutations in the *SMN1* gene (MIM# 600354).[1] *SMN2* (MIM# 601627), an *SMN1* homologue, typically differs from *SMN1* by five nucleotides (in intron 6, exon 7, intron 7, and noncoding exon 8).[2] Only one of these nucleotide changes (840C→T) is in the coding sequence, and it is translationally silent.[2] Approximately 94% of clinically typical SMA patients lack both copies of *SMN1* exon 7,[3] and most carriers have only one copy of *SMN1* exon 7, as determined by *SMN* gene dosage analysis.[4,5] In addition to large deletions that include the entire *SMN1* gene, loss of *SMN1* exon 7 can occur by gene conversion from *SMN1* to *SMN2*.[6] SMA type III patients have, on average, more *SMN2* copies than SMA type II or type I patients,[7–9] and hence more copies of *SMN2* derived by conversion from *SMN1*. The copy number of *SMN2* correlates with longer survival and inversely with disease severity.[8,9] Although gene conversion from *SMN1* to *SMN2* had been demonstrated, gene conversion from *SMN2* to *SMN1* has not. We show herein that increased *SMN1* copy number is associated with decreased *SMN2* copy number in the general population, which provides evidence that gene conversion from *SMN2* to *SMN1* occurs, and that *SMN1* converted from *SMN2* is present in the general population.

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**Received 23 September 2002; revised 16 December 2002; accepted 20 December 2002**

## Materials and methods

### Sample collection and DNA extraction

Using Puregene reagents (Gentra Systems, Minneapolis, MN, USA), DNA was extracted from peripheral blood specimens that were received with informed consent by the Molecular Pathology Laboratory of the Hospital of the University of Pennsylvania for *SMN* analyses on a clinical basis. We used results from all available individuals with no family history of SMA, (N=180)[10] and 107 parents of an SMA patient; either parents with only one copy of *SMN1*, or parents of an SMA child who lacked *SMN1* or had only one copy of *SMN1* with a high clinical index of suspicion for *SMN1*-related SMA. Results were anonymized and used for our study. We also randomly selected and anonymized samples from 32 SMA patients with a known clinical type for our study.

### *SMN1* and *SMN2* copy number assay (*SMN* gene dosage analysis)

A nonradioisotopic *SMN* gene dosage assay,[7] a modification of the original method of McAndrew *et al*,[4] was modified further, and validated for the determination of *SMN1* and *SMN2* copy numbers.[11,12] *SMN1* exon 7 copy number is used as a surrogate for *SMN1* gene copy number. Briefly, *SMN1* exon 7, *SMN2* exon 7, a genomic two-copy reference (*CFTR* exon 4), and *SMN* and *CFTR* internal standards were coamplified in a single reaction. The PCR products were incubated with *Dra*I, which digests only the *SMN2* products, but not the *SMN1* products. Copy number of *SMN1* or *SMN2* per cell (or more precisely, per diploid genome) was determined by quantification of the digested PCR products, followed by normalization utilizing the genomic standard, the internal standards, and five control samples with known *SMN1* and *SMN2* copy numbers. The quantitative accuracy of this method was validated as described.[7,11,12] All test samples were analyzed in duplicate.

## Results

Distributions of *SMN* genotypes in unaffected individuals are shown in Table 1. *SMN2* copy number and *SMN1* copy number correlated inversely in the general population. *SMN2* copy number was decreased to one or zero copies in 11 of 13 individuals with three or four copies of *SMN1*, whereas only 71 (43%) of 164 individuals with 2 *SMN1* copies had one or zero *SMN2* copies ($P < 0.01$; $\chi^2$ test).

There was a notable tendency for SMA type I carriers to have fewer *SMN2* copies, compared to type II and type III carriers (Table 1). While only three (1.7%) of 177 noncarriers (in the column indicated by 'No FH' in Table 1) with two or more *SMN1* copies had three *SMN2* copies, *SMN2* copy number was increased to three or four copies in 19 (17%) of 109 carriers with *SMN1* deletion/conversion mutations ($P < 0.001$; $\chi^2$ test).

The distribution of *SMN* genotype in 32 SMA patients is as follows (with genotype expressed as '(*SMN1* copy number):(*SMN2* copy number)'): Among the 16 type patients, three were 0:1 and 13 were 0:2; the single type I-II patient was 0:2; among the eight type II patients, one was 0:2 and seven were 0:3 and one was 0.4; and one patient with very mild adult-onset SMA (which may be called type IV) was 0:4. The *SMN2* copy number correlated inversely with disease severity.

## Discussion

Using *SMN* gene dosage analysis, three or more copies of *SMN1* were detected in some individuals in the general

**Table 1** *SMN* genotype distributions in unaffected individuals

| | *Parents of a child affected with SMA* | | | | |
|---|---|---|---|---|---|
| *SMN1:SMN2 Genotype*[a] | *No FH*[b] | *Type I* | *Type II (III*[c]*)* | *Unknown type* | *Subtotal* |
| 4:0 | 1 | 0 | 0 | 0 | 0 |
| 4:1 | 1 | 0 | 0 | 0 | 0 |
| 3:1 | 9 | 0 | 0 | 0 | 0 |
| 3:2 | 2 | 0 | 0 | 0 | 0 |
| 2:0 | 10 | 0 | 0 | 0 | 0 |
| 2:1 | 61 | 0 | 0 | 0 | 0 |
| 2:2 | 90 | 0 | 0 | 3[d] | 3 |
| 2:3 | 3 | 1[e] | 1[e] | 0 | 2 |
| 1:0 | 1 | 0 | 0 | 0 | 0 |
| 1:1 | 1 | 23 | 3 | 9 | 35 |
| 1:2 | 1 | 30 | 8 | 12 | 50 |
| 1:3 | 0 | 8 | 7[c] | 2 | 17 |
| Total | 180 | 62 | 19 | 26 | 107 |

[a]Genotype is expressed as '(*SMN1* copy number):(*SMN2* copy number)'. [b]Individuals with no family history. [c]Includes two parents of an SMA type III child. [d]Includes two parents with two *SMN1* copies on one chromosome 5 and a deletion mutation on the other chromosome 5 (also referred to as the '2 + 0 genotype'), and one parent of an SMA child with one copy of *SMN1*. [e]One parent each showed the '2 + 0 genotype'.

population, indicating the presence of chromosome 5s with two copies of *SMN1*.[4,10] We meta-analyzed published data,[3,4,8,10,13,14] and updated deduced *SMN1* allele frequencies[5] as follows: 'zero-copy allele' (chromosome 5 lacking *SMN1* exon 7), $9.83 \times 10^{-3}$; 'one-copy allele', $9.57 \times 10^{-1}$; 'two-copy allele' (chromosome 5 with two copies of *SMN1* exon 7), $3.27 \times 10^{-2}$; and '$1^{D}$ allele' (chromosome 5 with a small intragenic mutation in *SMN1*), $1.80 \times 10^{-4}$.

One hypothesis to explain the presence of two copies of *SMN1* on one chromosome 5 is unequal crossing over between homologous chromosomes during meiosis. Because of the presence of a large inverted repeat in the 5q13 region, and multiple smaller repeats contained therein, the *SMN* locus is considered highly susceptible to recombination. And, in fact, studies have shown that unequal crossing over at the *SMN1* locus can cause *de novo* deletions of *SMN1*.[7,15]

An alternative hypothesis is that gene conversion from *SMN2* to *SMN1* can result in two *SMN1* copies on one chromosome 5. In this scenario, *SMN2* copy number would decrease after the gene conversion, in contrast to most unequal crossover events at the *SMN1* locus. Our *SMN1*- and *SMN2*-copy-number data in the general population support this hypothesis. Our *SMN2*-copy-number data among SMA carriers and patients also support the hypothesis of the gene conversion from *SMN1* to *SMN2*. Previous studies indicated that increased *SMN2* copy number due to gene conversion from *SMN1* to *SMN2* is associated with a milder SMA phenotype.[6–9]

van der Steege *et al*[16] studied three nucleotide differences in intron 6, exon 7, and exon 8, and found a hybrid *SMN1/SMN2* gene (*SMN1* intron 6/exon 7-*SMN2* exon 8; intron 7 unknown) in one of their controls. Hahnen *et al*[17] studied five nucleotide differences in intron 6, exon 7, intron 7, and exon 8, and found a hybrid gene (*SMN1* intron 6/exon 7/intron 7-*SMN2* exon 8) in one of their controls. The presence of these rare hybrid genes may support the hypothesis of gene conversions in either direction between *SMN1* and *SMN2*, since a vast majority of hybrid genes present in SMA patients (presumably due to *SMN1*-to-*SMN2* gene conversion[6]) had only *SMN2* sequences except for one nucleotide in exon 8 (*SMN2* intron 6/exon 7/intron 7-*SMN1* exon 8).[17] Other minor hybrid gene variants have also been described.[17–19]

In conclusion, our data provide population-based evidence of gene conversion from *SMN2* to *SMN1*. Additional studies are necessary to confirm the hypothesis of gene conversion from *SMN2* to *SMN1*.

**Acknowledgements**

*We thank the following individuals for supporting various aspects of our work: PE McAndrew, TW Prior, H Rennert, K-L Chen, H-J Dong, C Turino, and T Smith.*

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