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Identification of bidirectional gene conversion between *SMN1* and *SMN2* by simultaneous analysis of *SMN* dosage and hybrid genes in a Chinese population ☆

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ABSTRACT

Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by programmed motoneuron death. The survival motor neuron 1 (*SMN1*) gene is an SMA-determining gene and *SMN2* represents an SMA-modifying gene. Here, we applied capillary electrophoresis to quantify the *SMN* gene dosage in 163 normal individuals, 94 SMA patients and 138 of their parents. We further quantified exons 7 and 8 in *SMN1* and *SMN2*. We found that the SMA patients carried the highest *SMN2* copies, which was inversely correlated with disease severity among its three subtypes. Increased *SMN1* was significantly associated with decreased *SMN2* in the normal group. We also observed that parents of type I SMA patients had significantly fewer *SMN2* copies than those of types II and III patients. The hybrid *SMN* genes were detected in two normal individuals and one patient and her mother. These results imply that increased *SMN2* copies in SMA patient group might be derived from *SMN1*-to-*SMN2* conversion, whereas the trend that normal individuals with higher *SMN1* copies simultaneously carry fewer *SMN2* copies suggested a reverse conversion, *SMN2*-to-*SMN1*. Together with the identification of hybrid *SMN* genes, our data provided additional evidence to support that *SMN1* and *SMN2* gene loci are interchangeable between population groups.

1. Introduction

With the incidence of 1 in 10,000 live births, autosomal-recessive spinal muscular atrophy (SMA) accounts for the leading genetic cause of infant mortality [1]. According to the age of onset and the highest level of motor function achieved, SMA is classified into: severe type I (onset before 6 months old with no ability to sit unaided), intermediate type II (onset before 18 months old with the ability to sit but not walking independently), and mild type III (onset after 18 months old with the ability to walk) [2]. SMA is caused by deficiency of the survival motor neuron (SMN) protein, which is encoded by two highly homologous genes on chromosome 5q13, telomeric *SMN* (*SMN1*) and centromeric *SMN* (*SMN2*) [3]. *SMN1* is the SMA-determining gene deleted homozygously in more than 95% of patients. *SMN2* differs from *SMN1* by five nucleotides, in which one critical C-to-T transition of exon 7 resulting in alternative splicing during transcription. Consequently, in contrast to *SMN1*, *SMN2* genes produce primarily truncated, unstable SMN protein [4].

Loss of the *SMN1* gene can occur either by gene deletion or by gene conversion [5]. Although *SMN2* gene might be dispensable in normal individuals [3], it is vital for SMA patients because at least one *SMN2* gene is retained in all SMA patients [6]. Furthermore, it has been suggested that the *SMN2* gene can attenuate disease severity in SMA patients [7], and this hypothesis has been verified by the SMA-like mouse model [8]. However, this genotype–phenotype correlation is not absolute, and thus it implies that the *SMN2* gene might not be functionally equivalent in all subjects and that other modifying factors may exist [9,10]. On the other hand, the efficiency of *SMN1*-to-*SMN2* gene conversion has also been proposed to determine the clinical severity [11,12].

Because the determination of the *SMN* gene dosage is crucial in genetic counseling and prognostic evaluation, several different quantitative methods have been applied. Recently, we have developed a

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capillary electrophoresis (CE) assay to analyze *SMN* gene dosages [13,14]. In this study, we quantified *SMN1* and *SMN2* gene dosage in three Chinese population groups to investigate the role of *SMN* gene conversion in the equilibrium of these two highly homologous genes. Furthermore, we simultaneously resolved the *SMN1*/*SMN2* genes in exons 7 and 8 to explain the molecular basis of the conversion events. Our study could provide additional evidence that may lead to better understanding of these two genes in homeostasis among different populations and patients with three SMA subtypes.

2. Materials and methods

2.1. Subjects

This study was approved by the institutional review board at Kaohsiung Medical University Hospital (*KMUH-IRB*-980125). We enrolled 94 SMA patients whose genotypes report homozygous deletion without intragenic mutation of the *SMN1* gene. Of these, 10 patients were type I, 44 were type II, and 40 were type III. We also enrolled 138 SMA carriers, who were the parents of the aforementioned homozygous *SMN1* deletion SMA patients. Besides, we enrolled 163 normal individuals with no family history of SMA, who were designated as the control group.

2.2. Assay for quantification of SMN1 and SMN2 copy number

Genomic DNA extracted from the peripheral blood was further processed by GFX™ Genomic Blood DNA Purification kit according to the manufacturer's instructions. We used a universal multiplex PCR and CE to calculate the copy number of the *SMN1* and *SMN2* genes. Briefly, exon 7 of *SMN1*/*SMN2* was amplified via a multiplex PCR method for determining the relative gene dosage of *SMN1* and *SMN2* within the genome [14]. Amplification of the *KRIT1* and *CYBB* genes was used as endogenous controls for comparison. Quantification of the copy number of *SMN1* and *SMN2* genes was accomplished by CE (Beckman P/ACE MDQ system; Fullerton, CA, USA), and the quantitative accuracy of this method was also validated by another method, multiplex ligation-dependent probe amplification (MLPA), as previously described [13,14]. All test samples were analyzed in triplicate.

2.3. Detection of SMN conversion events by simultaneous quantification of exons 7 and 8 in SMN1 and SMN2

We further designed two additional pairs of primers to simultaneous amplify the *SMN1*/*SMN2* genes in exons 7 and 8 for detection of gene conversion events between these two genes. The primer sequences for the forward primer, containing a universal section, and the reverse primer are as follows: ATAAGTGACGTACTAGCAACGTCGAACTCCTGAGCTCAGGT and AAAAGTAAGATTCACTTTCA, respectively, for exon 7; and ATAAGTGACGTACTAGCAACGGAACATTAAAAAGTTCAGATGTTA and TTTAAGACACTCTAACACTT, respectively, for exon 8. The universal section (ATAAGTGACGTACTAGCAACG) is a non-human sequence and is utilized for the labeling of the amplicon with a fluorescent tag by a universal FAM primer. The method principle was conducted as described in a recent study [15]. Similarly, exon 7 and exon 8 belonging to *SMN1* and *SMN2*, respectively, were simultaneously amplified and then analyzed by the same CE system with the use of *β-globin* and *KRIT1* genes for internal controls to detect the relative gene dosage. All the detected hybrid *SMN* genes with regard to conversion events were further confirmed by sequencing analysis. All samples were also assayed in triplicate.

2.4. Identification of SMN2 c.859G>C variant in SMA patients

In this study, we attempted to investigate a previously reported positive disease modifier, *SMN2* c.859G>C substitution, in a certain portion of our patient group. By direct sequencing using primers and methodology reported previously [9], we analyzed c.859G>C variant in exon 7 of *SMN2* for all our type II or type III SMA patients with only two *SMN2* copies.

2.5. Statistical analysis of SMN gene distribution

The copy numbers of the *SMN1* and *SMN2* genes in normal individuals, carriers and different subtypes of SMA patients were compared by analysis of variance (ANOVA) and the Tukey test for post-hoc comparison. Student's *t*-test was applied when two independent groups were compared. The frequency, reported as a distribution, of *SMN1* compared to *SMN2* copy in the control group was also analyzed by the chi-square (x^2) test. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Gene dosage of SMN1 and SMN2 in the different population groups

The copy numbers of *SMN* genes in three groups are shown in Table 1. The average copy number of *SMN1* gene of normal individuals was significantly higher than the carriers and SMA patients ($p<0.01$). In contrast, the average copy number of *SMN2* gene was significantly higher in SMA patients than those of the carriers and normal controls (both $p<0.01$). Each of the average copy numbers of *SMN2* in patients with SMA type II and type III was significantly higher than of the average copy number in type I patients (both $p<0.01$). If the patients with SMA type II and type III were combined into one group, named type II–III, we still found a higher number of *SMN2* copies in this combined group (3.13 ± 0.71; mean ± SD) than in type I patients ($p<0.01$; *t*-test).

The distribution of the *SMN2* copy number in 3 subgroups of SMA patients is shown in Table 2. No significant correlation was observed between the distribution of *SMN2* copies and gender in SMA patients. Two *SMN2* copies were found in 7 of 10 type I patients (70%), compared to only 9 of 44 type II patients (20.5%) and 6 of 40 type III patients (15%). However, we did not identify any *SMN2* c.859G>C substitutions in aforementioned 15 types II and III SMA patients with two *SMN2* copies (as shown in supplementary data). Three or more *SMN2* copies were found in 69 of 84 (82.1%) types II and III SMA patients, whereas 3 of 10 (30%) type I patients possessed three *SMN2* copies.

The distribution of *SMN* genotypes in normal individuals is shown in Table 3. Four of 163 controls (2.5%) carried one *SMN1* copy and were therefore identified as SMA carriers. The majority of normal individuals (139/163; 85.3%) carried two *SMN1* copies, and their *SMN2* gene copy number varied between zero and three. Homozygous absence of the *SMN2* gene was observed in 10 of 163 (6.1%) normal individuals. We found that increased *SMN1* copies was significantly associated with reduced *SMN2* copies; that is, all the normal individuals with ≥3 copies of *SMN1* typically had zero or one *SMN2*

Table 1
SMN1/*SMN2* gene copies in different groups of population.

Subject	Numbers	M/F	Average *SMN1* copy number (mean ± SD)	Average *SMN2* copy number (mean ± SD)
Controls	163	54/109	2.12 ± 0.46[a]	1.48 ± 0.63
Carriers	138	63/75	1.01 ± 0.12	2.33 ± 0.77
SMA patients:	94	56/38	0	3.04 ± 0.73[b]
Type I	10	4/6	0	2.33 ± 0.50[c]
Type II	44	28/16	0	3.05 ± 0.68
Type III	40	24/16	0	3.23 ± 0.73

M: male; F: female.
[a] Significantly higher in controls than in carriers and SMA patients ($p<0.01$).
[b] Significantly higher in SMA patients than in controls and carriers ($p<0.01$).
[c] Significantly lower in type I than in types II and III SMA subgroups ($p<0.01$).