Transmission characteristics of *SMN* from 227 spinal muscular atrophy core families in China

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Abstract

To define the relationship between the survival motor neuron 1 gene (*SMN1*) and *SMN2*, and explore the variability of these two genes within the generations, *SMN1* and *SMN2* copy numbers were determined for 227 SMA families. The association analysis indicated that there was a negative correlation between the copy number of *SMN1* and *SMN2* (Spearman = -0.472 , $P < 0.001$) in 227 SMA children and 454 of their parents. The average *SMN* copies from father and mother in each SMA family were used to represent the copy number in the parent's generation. Subsequently, *SMN* transmission analysis showed that the similar distribution trend of *SMN1* and *SMN2* copy number was not only in the SMA children and their parents' generation but also in the non-SMA families. Moreover, when the *SMN2* copy number was one in the parent's generation, 75% of their SMA children had type I and 25% of them had type II/III. However, when the *SMN2* copies were three in the parent's generation, all of their SMA children were type II/III. Therefore, the diversity of *SMN* copies was mostly inherited and the *SMN2* copy number in the parent's generation could predict the disease severity of SMA children to some extent.

Introduction

Spinal muscular atrophy (SMA) is caused by deficiency of the survival motor neuron (SMN) protein, resulting in degeneration of anterior horn cells of the spinal cord and characterized by symmetrically proximal muscle weakness and atrophy of lower limbs, and can involve multiple systems. SMA is a leading genetic cause of infant mortality, with an estimated incidence of 1/10 000 live births and a carrier frequency from 1/35 to 1/60 worldwide [1, 2]. Based on age at onset and the highest level of motor function achieved, childhood SMA can be classified into three groups: severe type I (MIM 253300), intermediate type II (MIM 253550), and mild type III (MIM 253400).

Survival motor neuron 1 gene (*SMN1*) is the disease-causing gene, and another nearly identical gene, *SMN2*, is the

phenotype-modified gene in a copy-dependent manner. Most often, SMA is inherited from carrier parents in an autosomal recessive pattern. However, ~2% of cases result from de novo variant [3]. In SMA patients, about 95% of cases carry a homozygous loss of *SMN1*, caused by gene deletion or conversion of *SMN1* to *SMN2*. And the remaining 5% are compound heterozygous variant in *SMN1* [4].

SMA-related genes are located on chromosome 5q13, a complex genomic structure region, which is predisposed to gene duplication, deletion, and rearrangement. Therefore, the *SMN* copies varied in different individuals and different populations (controls, SMA carriers, and patients) [5]. However, it is unclear how the *SMN* copy number changes between the generations. In this study, we analyzed the copy number in *SMN1* and *SMN2* from 227 SMA core families to define the relationship between *SMN1* and *SMN2* and explore the variability in *SMN* copy number within the generations.

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Materials and methods

Subjects

In brief, 227 SMA core families registered in the “National rare diseases registry system of China” (<https://www.nrdrs.org.cn>)

from the Department of Medical Genetics of Capital Institute of Pediatrics were recruited for the study, including 227 unrelated SMA children and their parents ($n = 454$) between March 2017 and July 2019. Molecular diagnosis was conducted for all SMA children. Genomic DNA was extracted from the peripheral blood using the EasyPure Blood Genomic DNA Kit (Transgen, Beijing, China). All participants signed informed consent, and this study was approved by the Ethics Committee of Capital Institute of Pediatrics.

Quantitative analysis of *SMN* and *SMN1* variant screening

SMN1 and *SMN2* copy numbers were determined by multiplex ligation-dependent probe amplification (MLPA) using the P060-B2 SMA kit (MRC-Holland, Amsterdam, Netherlands). For SMA children with heterozygous deletion in *SMN1*, Sanger sequencing strategy [6] was used for the subtle variant screening.

Linkage analysis

For the suspected '2 + 0' carriers, further linkage analysis was conducted to define *SMN1* copy number on each allele. A total of 12 polymorphic markers (UHM (upstream haplotype marker) 2, UHM3, UHM4, UHM5, UHM7, UHM8; DHM (downstream haplotype marker) 1, DHM2, DHM4, DHM6, DHM7, and DHM8) within 2 Mb of *SMN2* upstream and *SMN1* downstream were used for linkage analysis, which was completed by Shanghai Chromosky Medical Research Co., Ltd.

Statistical analyses

The average copy numbers of *SMN1* and *SMN2* in two groups (e.g., SMA and carriers) or multiple groups (e.g., SMA I, II, and III) were compared using the t test and ANOVA test, respectively. The rank correlation analysis was used to assess the association between the copy number of *SMN1* and *SMN2*. Contingency table χ^2 test was carried out to evaluate the distribution differences of SMA types among different *SMN2* copies. Statistical analyses were performed using the software package SPSS 23 for Window (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

Results

The 227 SMA children included 64 patients with SMA type I, 101 patients with SMA type II, and 62 patients with SMA type III. The distribution of copy numbers of *SMN1* and

Table 1 *SMN* copy numbers in 227 SMA families

Items	Children; N (%)	Parents; N (%)
<i>SMN1</i> copy number		
0	210 (92.5)	0
1	17 (7.5)	411 (90.5)
2	0	43 (9.5) ^a
Average (mean $\pm$ SEM) ^b	0.07 $\pm$ 0.018	1.09 $\pm$ 0.014
<i>SMN2</i> copy number		
1	1 (0.4)	68 (15.0)
2	51 (22.5)	238 (52.4)
3	169 (74.4)	144 (31.7)
4	6 (2.6)	3 (0.7)
5	0	1 (0.2)
Average (mean $\pm$ SEM) ^b	2.79 $\pm$ 0.032	2.19 $\pm$ 0.032

^aIncluding 17 parents with *SMN1* subtle variant, 8 with '2 + 0' genotype, and 18 suspected with '2 + 0' genotype

^b $P < 0.001$ analyzed by t test

SMN2 was shown in Table 1. In 227 SMA, 210 cases carried *SMN1* homozygous deletions and 17 cases carried compound heterozygous variants in *SMN1*; 169 had three copies of *SMN2*, which accounted for 74.4% of patients. In 454 SMA parents (obligate SMA carriers), as expected, 411 individuals had one copy of *SMN1*. However, 43 parents had two copies of *SMN1*, which included 17 parents with *SMN1* subtle variant in one allele. The remaining 26 parents from 26 different families were suspected with the '2 + 0' genotype, which was characterized by that one chromosome carried two copies of *SMN1* in *cis* while the other chromosome carried none. Eight of them (8/26; 30.77%) were willing to accept further family analysis. Combined *SMN1* copy number and linkage analyses indicated that the eight parents had two *SMN1* copies on one allele, confirming the '2 + 0' genotype.

In our study, the average copies of *SMN1* in SMA were 0.07 ± 0.018 (mean $\pm$ standard error mean, SEM), which were significantly lower than those in parents (1.09 ± 0.014 ; $P < 0.001$). In contrast, the average copies of *SMN2* in SMA were significantly higher than the parents (2.79 ± 0.032 vs. 2.19 ± 0.032 ; $P < 0.001$). Among 227 SMA and 454 parents, when the *SMN1* copy number was 0, 1, and 2, the average copies of *SMN2* were 2.82 ± 0.031 , 2.24 ± 0.033 , and 1.72 ± 0.077 ($P < 0.001$), respectively. As seen in Fig. 1a, there was a negative correlation (Spearman = -0.472 , $P < 0.001$). Subsequently, we further analyzed the correlation between average *SMN2* copies and *SMN1* genotypes. When the *SMN1* genotype was '0 + 0' (*SMN1* copy number = 0), '0 + 1^D' (*SMN1* copy number = 1), '1 + 0' (*SMN1* copy number = 1), '1 + 1^D' (*SMN1* copy number = 2) and '2 + 0' (*SMN1* copy number = 2), the average copies of *SMN2* were 2.82 ± 0.031 , 2.41 ± 0.15 , 2.24 ± 0.034 , 1.82 ± 0.095 , and

Fig. 1 The correlation between *SMN* in 227 SMA families. **a** The average of *SMN2* copies and *SMN1* copies; **b** The average of *SMN2* copies and *SMN1* genotypes. The data were expressed as mean $\pm$ SEM. The genotype of '0 + 0' represents *SMN1* homozygous deletion,

'0 + 1^D' represented *SMN1* compound heterozygous mutation, '1 + 0' represented classical carrier, '1 + 1^D' represented one wild-type allele and the other allele with a mutation on each chromosome, and '2 + 0' represented two *SMN1* alleles on the same chromosome

Fig. 2 The average copies of *SMN* in the parent's generation and SMA children among SMA types. **a** The average of *SMN1* copies. **b** The average of *SMN2* copies. The data were expressed as mean $\pm$ SEM. The copies in the parent's generation was the average of *SMN* copies from father and mother in each family ((father + mother)/2). * $P < 0.001$ compared with type I

1.65 ± 0.11 ($P < 0.001$), respectively (Fig. 1b). Similarly, accompanied by the increased number of *SMN1* copies, the average copy number of *SMN2* decreased gradually (Spearman = -0.471 ; $P < 0.001$). Interestingly, for individuals with *SMN1* genotype '0 + 1^D' and '1 + 0', although the copy number of *SMN1* was both one, there was a trend that the *SMN2* average copy number of the former was higher than that of the latter (2.41 ± 0.15 vs. 2.24 ± 0.034); the same trend was found in individuals with *SMN1* genotype of '1 + 1^D' and '2 + 0' (1.82 ± 0.095 vs. 1.65 ± 0.11), although they all carried the same two copies of *SMN1*.

If the variation of *SMN* copies comes from inheritance, the *SMN* copy number of children is determined by the *SMN* copy number of their father and mother. Therefore, we used the average *SMN* copies from the parents in each SMA family, namely, (1/2 *SMN1* copies from father + 1/2 *SMN1* copies from mother) and (1/2 *SMN2* copies from father + 1/2 *SMN2* copies from mother) to represent the copy number in the parent's generation, and then we compared *SMN* copy numbers between the parent's generation and SMA children to explore whether the variation of *SMN* copy number originated from inheritance or de novo in generations. The average number of *SMN1* copies was significantly higher

in the parent's generation than those in SMA regardless of SMA types (all $P < 0.001$) (Fig. 2a). By contrast, the average number of *SMN2* copies was lower in the parent's generation than those of SMA (all $P < 0.001$) (Fig. 2b). Moreover, the average *SMN1* copy number in the parent's generation whose SMA offspring was type I, type II, and type III was 1.11 ± 0.026 , 1.09 ± 0.019 , and 1.09 ± 0.024 , respectively, and there was no difference among the three types of SMA ($P = 0.801$) (Fig. 2a). However, type II and type III SMA parent's generation had more copies of *SMN2* than type I SMA parent's generation (both $P < 0.001$) (Fig. 2b). Similarly, no difference was found in 227 SMA children carrying the average of *SMN1* copies of 0.078 ± 0.034 for type I, 0.079 ± 0.027 for type II and 0.065 ± 0.031 for type III ($P = 0.936$) (Fig. 2a) and more *SMN2* copies were observed in type II (2.97 ± 0.017) and III (2.97 ± 0.061) than type I (2.34 ± 0.064) SMA (both $P < 0.001$) (Fig. 2b). The correlation analysis showed there was a significantly positive correlation for *SMN* copies between the parent's generation and SMA children (Spearman = 0.589 ; $P < 0.001$ for *SMN1* and Spearman = 0.486 ; $P < 0.001$ for *SMN2*) (Supplementary Table 3). These data mentioned above suggested that the distribution trend of *SMN1* and *SMN2* copy number was consistent between the parent's generation and SMA children.

As the most important phenotype-modified gene, *SMN2*, the copy number of this gene was negatively related to the severity of SMA. In order to determine whether the *SMN2* copy number of the parent's generation could predict the SMA severity of the offspring, we calculated the proportion of the offspring suffering from heavy SMA (type I) and relatively light SMA (type II / III) when the *SMN2* copy number of the parent's generation was fixed. For example, when the *SMN2* copy number was one in the parent's generation, 75% of their SMA offspring had type I and 25% of them had type II/III. However, when the parent's generation carried three copies of *SMN2*, all of their SMA offspring were type II/III (Fig. 3). The distribution of type I and type II/III was significantly different in different *SMN2* copies ($\chi^2 = 37.061$, $P < 0.001$).

Discussion

In this study, we analyzed the copy numbers of *SMN* from 227 SMA children and 454 their parents using MLPA combined with Sanger sequencing strategy. The results showed that 92.5% of SMA children had zero copy of *SMN1* and 7.5% had one copy of *SMN1* with the *SMN2* copies ranged from one to four. The proportion of *SMN1* compound heterozygous variants (7.5%) in this study was higher than the previously reported ~5% [4]. In the parents, 90.5% had one copy of *SMN1* and 9.5% had two copies of *SMN1* with the *SMN2* copies ranged from one to five. For the parents with two *SMN1* copies, except for 17 parents carry one normal allele and a pathogenic variant in the other allele, eight of 26 parents were confirmed '2 + 0' genotype in *SMN1* by dosage and linkage analysis. The remaining 18 parents with two copies of *SMN1* could not be distinguished the '2 + 0' carriers or noncarriers with '1 + 1' genotype. However, considered their SMA children and spouses were all homozygous deletions and heterozygous deletion in *SMN1*, respectively, the '2 + 0' genotype was the most likely.

Fig. 3 *SMN2* copies in the parent's generation and the disease severity of their SMA children. *SMN2* copy number was the average *SMN2* copies from parents in each family ((father + mother)/2)

Reduction in copy number of *SMN1* can be caused by either gene conversion or deletion [7]. There was bidirectional gene conversion between *SMN1* and *SMN2* in different population groups [8]. As early as 1997, the physical evidence was presented for conversion of *SMN1*-to-*SMN2* in SMA patients and carriers [9, 10]. However, the gene conversion in the opposite direction, *SMN2*-to-*SMN1* was found in general population [10]. As shown in Fig. 1, in our 227 SMA families, the *SMN1* copies were negatively correlated with the *SMN2* copies, suggesting that there might be gene conversion. Subsequently, we analyzed the relationship between *SMN2* copies and *SMN1* genotype and found that with the increase in *SMN1* copies, *SMN2* copy number gradually decreased, presenting an inverse correlation between them. Moreover, there was a notable tendency for the individuals with function impaired alleles in *SMN1* to have more *SMN2* copies, compared with the alleles with nonimpaired function: '0 + 1^D' (2.41 ± 0.15) vs. '1 + 0' (2.24 ± 0.034) (Fig. 1b) and '1 + 1^D' (1.82 ± 0.095) vs. '1 + 1' (1.56 ± 0.076) (Fig. 1b; Supplementary Table 1), although the copy number of *SMN1* was the same. This suggested that the dose compensation of *SMN2* was related to not only the *SMN1* copies but also the *SMN1* function. When the copy number of *SMN1* is the same, the individual with severely damaged function in *SMN1* allele tends to have more *SMN2* copies to compensate the deficiency of SMN protein. Besides the pathogenic variant will affect the function of *SMN1*, some studies also had shown that gene conversion was common in mild SMA, while deletion was relatively common in severe SMA, indicating that the function of *SMN1* might differ as a result of conversion and deletion [11].

SMN1 and *SMN2* are all located in a region of chromosome 5q13, which includes many homologous and mirror-aligned genes. The complexity of this region has made it unstable and prone to deletion or conversion. We analyzed the copy number of *SMN1* and *SMN2* in SMA children and their parents' generation to determine whether the variation in *SMN* copies resulted from inheritance or de novo. The results showed that the variation trend of *SMN1* and *SMN2* copy number was the same in SMA children and their parents' generation regardless the SMA types. And there was a positive correlation for *SMN* copies between these two generations.

In addition, we also analyzed the copy number of *SMN* in 27 phenylketonuria (PKU) core families, a common genetic metabolic disease, which caused by variants in *PAH*. The results showed that the distribution of both the copy number of *SMN1* and *SMN2* had no significant difference between the PKU children and their parents ($P = 0.607$ for *SMN1* and $P = 0.326$ for *SMN2*; contingency table χ^2 test) (Supplementary Table 2). And there was a positive correlation between PKU children and their parents' generation both in the copy number *SMN1* and *SMN2* (Spearman = 0.75,

$P < 0.001$ for *SMN1*; Spearman = 0.696, $P < 0.001$ for *SMN2*). All the results from SMA families and PKU families suggested that the variation of *SMN* copy number was largely inherited and could be steadily transmitted. Therefore, we can infer that the *SMN2* copy number in the parent's generation has an effect on those of SMA children and then determine the disease severity to some extent.

Molecular characteristics of the *SMN* region determined that an individual might have gene deletion or conversion at a certain time. Under the founder effect, two populations with special variation in *SMN* copy number, SMA carriers and SMA patients, were gradually formed. This phenomenon had both occasionality and universality. Occasionality may be the initiating factor of gene deletion or conversion. Some studies have shown that unequal crossing-over of *SMN1* loci in meiosis might be the cause [12]; however, a certain universality may be due to the fact that the population carriers and morbidity of SMA are not rare.

In conclusion, our study showed an inverse correlation of the copy number between *SMN1* and *SMN2* in SMA children and their parents, suggesting the *SMN1*-to-*SMN2* gene conversion; the variation of *SMN* copy number was resulted from inheritance and therefore, the disease severity of the SMA children was determined by their parent's generation to some extent.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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