

# The analysis of the association between the copy numbers of survival motor neuron gene 2 and neuronal apoptosis inhibitory protein genes and the clinical phenotypes in 40 patients with spinal muscular atrophy

## Observational study

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### Abstract

In this article, the correlation between the copy number of survival motor neuron 2 (*SMN2*) gene, neuronal apoptosis inhibitory protein (*NAIP*), and the phenotype of spinal muscular atrophy patients were analyzed. Forty patients with spinal muscular atrophy (SMA) were included in the study at the Department of Medical Genetics of the First People's Hospital and the Department of Neurology of the Second People's Hospital in Yunnan Province from January 2012 to September 2018. Multiplex ligation-dependent probe amplification assay was performed to determine the copy numbers of *SMN2* and *NAIP* genes. Statistical analysis was performed to determine the correlation between copy numbers of the *SMN2* and *NAIP* genes and the clinical phenotypes of SMA. Our results show that among the 40 SMA patients, there were 13 type I cases, 16 type II cases and 11 type III cases. A total of 37 patients possessed a homozygous deletion of *SMN1* exons 7 and 8, while the other 3 SMA patients possessed a single copy of *SMN1* exon 8. There was no correlation between SMA subtypes and the deletion types of *SMN1* exon 7 and 8 ( $P = .611$ ). The percentage of 2, 3, and 4 copies of *SMN2* exon 7 was 25.0%, 62.5%, and 12.5%, respectively. The percentage of 0, 1, and 2 copies of *NAIP* exon 5 was 10%, 57.5%, and 32.5%, respectively. The distributions of *SMN2* and *NAIP* copy numbers among various SMA types were significantly different (all  $P < .05$ ). Five combined *SMN1*-*SMN2*-*NAIP* genotypes were detected, of which 0-3-1 genotype had the highest proportion than the others, accounting for 42.5%. The copy number of *SMN2* and *NAIP* gene had synergistic effect on SMA phenotype. The combined *SMN1*-*SMN2*-*NAIP* genotypes with fewer copies were associated with earlier onset age, higher mortality, and smaller average age at death in SMA patients.

Therefore, we conclude that the copy number variance of *SMN2* and *NAIP* is correlated with the SMA phenotype. Analysis of the copy number structure of the *SMN1*-*SMN2*-*NAIP* gene is helpful for SMA typing, disease prognosis prediction, and genetic counseling.

**Abbreviations:** *CORO1C* = coronin-like actin-binding protein 1C, *GTF2H2* = general transcription factor IIH polypeptide 2 gene, *H4F5* = human 4F5 gene, MLPA = multiplex ligation-dependent probe amplification, *NAIP* = neuronal apoptosis inhibitory protein, *NCALD* = neurocalcin delta, *PLS3* = plastin 3, SMA = spinal muscular atrophy, *SMN* = survival motor neuron gene.

**Keywords:** clinical phenotype and genotype, multiplex ligation-dependent probe amplification (MLPA), neuronal apoptosis inhibitory protein, spinal muscular atrophy, survival motor neuron gene

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## 1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease characterized by symmetrical proximal muscle weakness and atrophy due to degeneration of the anterior horn cells of the spinal cord. It affects approximately 1 in 6000 to 10,000 white newborns and is the second most common fatal autosomal recessive disorder after cystic fibrosis.<sup>[1]</sup> The population carrying rate is about 1/54 in the world,<sup>[2,3]</sup> and the frequency of SMA carriers in the Chinese population is about 1/42 to 1/62.<sup>[4–6]</sup>

SMA is classified into types I through IV depending on the age of onset and the clinical course. SMA type I, also known as infantile SMA or Werdnig–Hoffmann disease, is the most common and severe type of SMA. SMA type I manifests as severe muscle weakness and hypotonia with onset in early infancy, and fatal respiratory failure usually before 2 years of age. SMA type II (intermediate form) occurs between 6 and 18 months of age, characterized by an ability to sit but not to walk unaided, and survival beyond 4 years of age. SMA type III (Kugelberg–Wielander disease) is typically present with signs of weakness at or after 18 months of age and progresses to a chronic course. SMA type IV is a mild late (adult) onset form that is usually present in the second or third decade of life and has a normal life expectancy similar to SMA type III.<sup>[7,8]</sup>

SMA is caused by a deletion or mutation in the survival motor neuron 1 (*SMN1*) gene. *SMN1* was discovered in 1995 and is located on chromosome 5q11-q13.1.<sup>[9]</sup> The *SMN* gene comprises 9 exons, with a stop codon is present near the end of exon 7. Two almost identical *SMN* genes are present at 5q13: the telomeric or *SMN1* gene and centromeric or *SMN2* gene. The coding sequence of *SMN2* differs from that of *SMN1* by a single nucleotide (840C>T), which does not alter the amino acid but has been shown to be important in splicing.<sup>[10]</sup> *SMN2* copy numbers are a demonstrated phenotypic modifier in the SMA population, in which milder forms of the disease are associated with increased copy numbers of *SMN2*.<sup>[11–14]</sup>

Both copies of the *SMN1* exon 7 are absent in approximately 95% of affected SMA patients. Although SMA patients lack *SMN1*, they always carry at least 1 copy of *SMN2*, which is partially functional but unable to compensate for the absence of *SMN1*. The remaining 5% of affected cases are compound heterozygotes for *SMN1* exon 7 deletion and small intragenic mutations.<sup>[15]</sup> Type IV patients show only 20% to 30% homozygous deletion of *SMN1* gene.<sup>[16,17]</sup>

*NAIP*, a regulatory gene, shows homology with a baculoviral inhibitor of apoptosis and inhibits apoptosis in mammalian cells.<sup>[18]</sup> The *NAIP* gene, spanning 56 kb of genomic sequence with 6.1 kb transcript, consists of 17 exons and is present in multiple and variable copy number on chromosome 5. Deletion of intact *NAIP* gene is associated with SMA. Homozygous deletion of *NAIP* exon 5 is more commonly observed in the severe and acute form of SMA than mild or chronic forms. Deletion of *NAIP* is found in approximately 45% of type I SMA and 18% of type II and III SMA.<sup>[19]</sup>

The *NAIP* gene is located in the functional domain near the *SMN1* gene and is a potentially better SMA candidate regulatory gene. However, its functional role in the pathogenesis of SMA has not been fully elucidated. Therefore, to elucidate the relationship between *SMN2* and *NAIP* copy number variation and SMA phenotype is important for clinical classification, disease

progression, prognosis evaluation, and genetic counseling of SMA patients in this region.

## 2. Methods

From January 2012 to September 2018, 40 patients with SMA who were admitted to the Department of Medical Genetics of the First Affiliated Hospital of Yunnan Province and the Department of Neurology of the Second People's Hospital of Yunnan Province were selected for clinical classification according to the criteria defined by the 1992 International SMA Association,<sup>[20]</sup> including 22 males and 18 females. The patient's age ranged from 3 months to 24 years, and the median age was 3 years. The age of onset of patients ranged from 3 months to 6.5 years, with a median age of 3 years. Thirteen patients suffered from type I SMA, 16 patients from type II SMA, and 11 patients from type III SMA. The study was reviewed and approved by the Ethics Review Committee of the First People's Hospital of Yunnan Province (batch number: 2014YXLH055), and all patients and their legal guardians signed the informed consent form for the genetic diagnosis.

The experiments were performed as follows:

- (1) Reagents and instruments: The instruments included QIAamp DNA Blood Mini Genomic DNA Extraction Kit (Qiagen, Germany), ABI2720 PCR Amplifier (ABI Applied Biosystems, Foster City, Calif., USA), SALSA MLPA probemix P021-A2 SMA Kit (MRC-Holland, The Netherlands) and ABI-3130 Genetic Analyzer (ABI), POP4 Gel (ABI). The primers were obtained from Bioengineering (Shanghai, China) Co, Ltd.
- Extraction of genomic DNA: 2 mL of venous blood was taken from the subject, and EDTA-K2 was anticoagulated. The genomic DNA of the test subject was extracted with QIAamp DNA Blood Mini reagent, and the DNA concentration was measured by an ultraviolet spectrophotometer. The DNA concentration was then adjusted to 100 ng/μL with sterilized double-distilled water and stored at −20°C.
- (3) MLPA technology amplification and detection: SALSA MLPA probemix P021-A2 SMA kit (MRC) was used, strictly in accordance with the SMA-MLPA kit operating instructions. Samples with 2 copies of *SMN1*, *SMN2*, and *NAIP* genes were selected as negative controls, and reference samples were randomly distributed among all samples.
- (4) Analysis of MLPA results: CoffalyserNET software (MRC, www.mlpa.com-MLPA) was used to analyze the experimental results. According to the kit instructions, the copy number of exons 7, 8 of *SMN1* and *SMN2* genes and the copy number of exon 5 of *NAIP* gene were estimated. The parameters used to determine the copy numbers were listed in Table 1.
- (5) Patient survival follow-up: Data collection was performed on the patient's morbidity status and current living status through a combination of telephone, home visit, and inpatient medical case analysis.
- (6) Statistical analysis: All data were analyzed using the SPSS software package (version 17, SPSS Inc., Chicago, IL). The measurement data were expressed as mean ± standard deviation, the count data were expressed as the rate, and the comparison was performed by the  $\chi^2$  test (Fisher exact test). *P*-value of <.05 was considered statistically significant.

**Table 1****The relationship between the relative ratio and the copy number.**

Relative ratio	Copy number
0	0
0 < ratio value < 0.7	1
0.7 ≤ ratio value < 1.3	2
1.3 ≤ ratio value < 1.7	3
1.7 ≤ ratio value < 2.3	4

### 3. Results

#### 3.1. Forty cases of SMA patients with SMN1 gene mutation

The samples taken from forty patients with SMA were analyzed by MLPA and 37 (92.5%) patients had homozygous deletion of exon 7 and 8 of *SMN1*. Only 3 patients with exon 7 deletion were present, accounting for 7.5% (3/40) of patients. There was no correlation between different SMA types and the 7th and 8th exon deletion types of *SMN1* gene ( $P = .611$ ). See Table 2.

#### 3.2. The relationship between SMN2 and NAIP copy number and SMA clinical phenotype

Among the 40 patients with SMA, the copy number 2, 3, and 4 of exon 7 of the *SMN2* gene accounted for 25.0% (10/40), 62.5% (25/40), and 12.5% (5/40) of patients, respectively. The copy number 0, 1, and 2 of the 5th exon of the *NAIP* gene accounted for 10% (4/40), 57.5% (23/40), and 32.5% (13/40) of patients, respectively. The average age of onset for those with 2 copies of *SMN2* gene was lower than that of patients with 3 or 4 copies of *SMN2*. The mortality rate of patients with 2 copies of *SMN2* gene was 50% (5/10), which was 6.2 times higher than that of patients with 3 copies of the *SMN2* gene, and the average death age of patients with 2 copies of *SMN2* gene was less than that with 3 copies of *SMN2* gene. There were no deaths in those with 4 copies.

The average age of onset of the *NAIP* gene in patients with 0 copies was less than that of patients with 1 and 2 copies, and the death toll was 75.0% (3/4), which was 4.3 times higher than that of patients with 1 copy of *NAIP* gene. The average age of patients with 0 copies was less than that with 1 copy, and there were no deaths in patients with 2 copies of *NAIP* gene. The results showed that there was a significant difference in the distribution of *SMN2* and *NAIP* gene copy numbers among different types of SMA ( $P$  all < .05), and the copy number is related to the age of SMA onset. The smaller the copy number, the earlier the age of onset, the

**Table 2****MLPA technology to detect the deletion of exon 7 and exon 8 in the SMN1 gene.**

Type	Case	Deletion of Exon 7 and Exon 8	
		Deletion of E7 and E8N (%)	Deletion of E7N (%)
I	13	13 (100.0)	0
II	16	14 (87.5)	2 (12.5)
III	11	10 (90.9)	1 (9.1)
Total	40	37 (92.5)	3 (7.5)

E7 = the 7th exon of the *SMN1* gene, E8 = the 8th exon of the *SMN1* gene, MLPA = multiplex ligation-dependent probe amplification, N = number, *SMN1* = survival motor neuron gene 1.

higher the death rate, and the smaller the average age at death. See Table 3.

#### 3.3. Relationship between SMN1-SMN2-NAIP genotype and clinical phenotype of SMA

Five *SMN1-SMN2-NAIP* genotypes were detected in 40 patients with SMA, of which 0-3-1 was the highest, accounting for 42.5% (17/40) of patients (Table 4). Type 0-2-0 and 0-2-1 were the most common type I patients, accounting for 30.8% (4/13) of patients. Types 0-3-1 were the most common in type II and type III patients, accounting for 56.3% (9/16) and 45.5% (5/11), respectively. However, the proportion of 0-3-2 and 0-4-2 in type III patients was higher than that in type I and type II patients, accounting for 27.3% (3/13) of patients. There was a significant difference in the distribution of *SMN1-SMN2-NAIP* genotypes between different types of SMA ( $P = .026$ ). *SMN2* and *NAIP* genes had synergistic modification effects on SMA phenotype. The smaller the copy number, the earlier the patient's onset age, the higher the death rate, and the smaller the average age at death.

### 4. Discussion

SMA is a more common autosomal recessive disorder in childhood. The *SMN1* gene located on chromosome 5q11.2-q13.2 is mainly associated with development of SMA, and about 80% to 95% of children with SMA have homozygous *SMN1* gene. Deletion of a small number (~5%) of complex heterozygous mutations may ultimately lead to a decrease in the expression of *SMN* protein.<sup>[7]</sup> The modified *SMN2* gene of SMA is highly homologous to the *SMN1* gene, with 5 base differences, 1 in intron 6, 1 in exon 7, 2 in intron 7, and 1 in exon 8.<sup>[4]</sup> The most pivotal gene modification is located at the 6th base in the 7th exon of *SMN1* gene is C, while that of *SMN2* gene is T. Because the large variations in the region of splicing enhancer in the *SMN2* gene exon, about 90% of the exon 7 in *SMN2* pre-mRNA is incorrectly spliced out, and finally encoded an unstable, easily degraded truncated protein (*SMNΔ7*).<sup>[21,22]</sup> However, the *SMN2* gene can still express 10% to 20% of full-length functional *SMN* proteins. The more gene copy number, the greater the production of full-length transcripts, which can partially compensate for the deficiency of *SMN* protein and reduce the severity of SMA phenotype. The *SMN2* gene copy number is inversely related to the severity of SMA.<sup>[7]</sup>

In addition to the *SMN* gene, the *NAIP* gene is also located in the functional domain near chromosome 5q13.2 and the distribution of the *NAIP* domain in the central nervous system is closely related to the degradation of SMA neural selection. The *NAIP* gene is a closely-associated SMA gene, and its copy number variation is related to the degree of SMA disease. When the copy number of *NAIP* gene is smaller, and the patient's condition is more serious.<sup>[23,24]</sup> The molecular genetic basis of SMA pathogenesis is potentially due to the deletion of exon 5. Therefore, clarifying the copy number and gene sequence structure of *SMN1*, *SMN2*, and *NAIP* genes is of great significance for analyzing the molecular mechanism and clinical diagnosis of SMA pathogenesis.<sup>[14]</sup>

MLPA technology is a relatively quantitative analysis technique. The concentration of DNA template used does not need to be accurately quantified. There are multiple internal reference probes for different chromosomes in the system, which constitute an internal control system of the detection system,

**Table 3**  
Relationship between *SMN2* and *NAIP* gene copy number and clinical phenotype of SMA.

Gene	Copy number	Case number (%)	Age of onset, mo	Clinical classification (%)			Clinical outcome		
				Type I	Type II	Type II	Death (%)	Age at death, mo	
<i>SMN2</i>	Exon 7	2	10 (25.0)	6.1 ± 3.3	8 (61.5)	2 (12.5)	0 (0)	5 (50.0)	5.2 ± 3.8
		3	25 (62.5)	17.8 ± 13.8	5 (38.5)	12 (75.0)	8 (72.7)	2 (8.0)	10.5 ± 2.1
		4	5 (12.5)	31.4 ± 25.8	0 (0)	2 (12.5)	3 (27.3)	0	/
<i>NAIP</i>		0	4 (10.0)	4.0 ± 1.4	4 (30.8)	0 (0)	0 (0)	3 (75.0)	3.3 ± 0.6
		1	23 (57.5)	15.6 ± 13.9	7 (53.8)	11 (68.8)	5 (45.5)	4 (17.4)	9.2 ± 3.8
		2	13 (32.5)	22.2 ± 18.8	2 (15.4)	5 (31.2)	6 (54.5)	0	/
Total		40	16.6 ± 15.6	13	16	11	7	6.7 ± 4.2	

mo=month, *NAIP*=neuronal apoptosis inhibitory protein, *SMA*=spinal muscular atrophy, *SMN2*=survival motor neuron gene 2.

effectively avoiding false positive and false negative results. In recent years, MLPA technology has been widely used in the diagnosis of SMA, especially its 1-time detection, which can simultaneously analyze the copy number variation of multiple genes, providing important support for SMA typing, disease prognosis, prenatal diagnosis, and genetic counseling.<sup>[4,5,25,26]</sup> In this study, we used MLPA technique to analyze *SMN1* gene deletion in 40 patients with SMA and detected 37 cases of simultaneous deletion of exon 7 and exon 8 of *SMN1*, accounting for 92.5% (37/40) of patients, and 3 cases of a single copy of *SMN1* exon 8, accounting for 7.5% (3/40) of patients. There was no correlation between SMA subtypes and the deletion types of *SMN1* exon 7 and 8 ( $P = .611$ ), indicating that the *SMN1* gene is the molecular genetic basis of pathogenesis, with no correlation of SMA phenotype.<sup>[13]</sup>

Several studies have shown that the copy number of *SMN2* and *NAIP* is related to the disease type. Wathiyati et al<sup>[27]</sup> found that 25% of type I patients carried 1 copy of *SMN2*, while the remaining 71% carried 2 copies. Among the type II and type III SMA patients, 29% of cases carried 2 copies of the gene, while 71% carried 3 or 4 copies of *SMN2*. Deletion analysis of *NAIP* showed that 50% of type I SMA patients had a homozygous deletion of exon 5 of this gene and that only 10% of type II SMA cases carried a homozygous deletion, while all type III patients carried intact copies of the *NAIP* gene. Qu et al<sup>[13]</sup> found that in 232 Chinese patients with SMA, the copy number of exon 7 of *SMN2* gene was 2, 3, and 4, accounting for 28.5% (66/232), 65.9% (153/232), and 5.6% (13/232) of patients, respectively, but 0% in those with 0 or 1 copy. The copy number of exon 5 of *NAIP* gene was 0, 1, and 2 copies, accounted for 15.1% (35/232), 62.5% (145/232), and 22.4% (52/232), respectively. Fang et al<sup>[14]</sup> found that in 42 Chinese patients with SMA, the copy

number 1, 2, 3, and 4 of exon 7 of *SMN2* gene accounted for 4.8% (2/42), 33.3% (14/42), 57.1% (24/42), and 4.8% (2/42) of patients, respectively, with none in those with 0 copies. The copy number 0, 1, and 2 of exon 5 of *NAIP* gene accounted for 9.5% (4/42), 61.9% (26/42), and 28.6% (12/42) of patients respectively. Ahn et al<sup>[24]</sup> found that 6 of 33 SMA patients had *NAIP* deletions and showed a more-severe phenotype of SMA. The proportion of patients who had died or received ventilator support was also higher in those with an *NAIP* deletion. Similar to the above research results, in 40 patients with SMA in this study, the copy number of exon 7 of *SMN2* gene was 2, 3, and 4 copies, accounting for 25.0% (10/40), 62.5% (25/40), and 12.5% (5/40) of patients, respectively. The copy number of exon 5 of *NAIP* gene was 0, 1, and 2 copies, accounting for 10.0% (4/40), 57.5% (23/40), and 32.5% (13/40) of patients, respectively. The above results indicate that the *SMN2* gene of Chinese SMA patients is mostly 2 or 3 copies, and the *NAIP* gene is mostly 1 copy. The data of this group indicated that the distribution of *SMN2* and *NAIP* gene copy number was significantly different among different types of SMA ( $P < .05$ ), and the copy number was correlated with the age of SMA. The smaller the copy number, the earlier the onset, the higher the proportion of death. It can be seen from Table 3 that the proportion of 3 copies of *SMN2* gene in patients with type I SMA is 38.5%, while the proportion of 0 copies of *NAIP* gene is 30.8%, lower than that reported by others.<sup>[27,28]</sup> The higher proportions of 3 copies of *SMN2* gene and lower proportions of 0 copies of *NAIP* gene indicate that the survival status of Chinese patients with type I SMA is better than that of Caucasians. This finding is similar to that reported by Qu et al.<sup>[13]</sup>

In this study, 5 *SMN1*-*SMN2*-*NAIP* genotypes were detected in 40 patients with SMA, of which 0-3-1 was the highest,

**Table 4**  
Relationship between *SMN1*-*SMN2*-*NAIP* genotype and clinical phenotype of SMA.

Genotype	Case numbers (%)	Age of onset, mo	Clinical classification (%)			Clinical outcome	
			Type I	Type II	Type III	Death rate (%)	Age at death, mo
0-2-0	4 (10.0)	4.0 ± 1.4	4 (30.8)	0 (0)	0 (0)	3 (75.0)	3.3 ± 0.6
0-2-1	6 (15.0)	7.5 ± 3.6	4 (30.8)	2 (12.5)	0 (0)	2 (33.3)	7.5 ± 6.4
0-3-1	17 (42.5)	18.4 ± 15.1	3 (23.1)	9 (56.3)	5 (45.5)	2 (11.8)	10.5 ± 2.1
0-3-2	8 (20.0)	16.4 ± 11.3	2 (15.4)	3 (18.8)	3 (27.3)	0	/
0-4-2	5 (12.5)	31.4 ± 25.8	0 (0)	2 (12.5)	3 (27.3)	0	/
Total	40	16.6 ± 15.6	13	16	11	7	6.7 ± 4.2

mo=month, *NAIP*=neuronal apoptosis inhibitory protein, *SMA*=spinal muscular atrophy, *SMN1*=survival motor neuron gene 1, *SMN2*=survival motor neuron gene 2.



accounting for 42.5% (17/40) of patients. Type 0-2-0 and 0-2-1 patients were the most common type I patients, accounting for 30.8% (4/13). There was a significant difference in the distribution of *SMN1-SMN2-NAIP* genotypes among different types of SMA ( $P < .05$ ). *SMN2* and *NAIP* genes had synergistic modification effects on SMA phenotype. The smaller the copy number was, the earlier the age of onset. In addition, the higher the death rate was, the smaller the average age at death. A recent report suggests that the dose effect of *SMN2* is much higher than that of *NAIP* in terms of the age, mortality and survival rate of SMA patients.<sup>[13]</sup> As can be seen from Table 3 and Table 4, the patient mortality rate was 17.4% (4/23) when the *NAIP* gene was 1 copy, and the patient mortality rate increased to 33.3% (2/6) when the 0-2-1 genotype was used. The 0-3-1 genotype was 11.8% (2/17), which further supports the above conclusions. Our combined *SMN1-SMN2-NAIP* genotype analyses implied that the phenotype-modifier effect was more significant when the copy number increased for both *SMN2* and *NAIP* genes than when there was an increase in either individual gene. Thus, identification of only *SMN2* copies would be incomplete for genetic counseling and prognosis assessment for a SMA family, as *NAIP* copy number also has a certain modifier effect on the survival status of the SMA patients.<sup>[13]</sup> Furthermore, our data revealed that only confirming the gene copy numbers might not be sufficient to the clinical diagnosis, prenatal diagnosis and phenotype evaluation of SMA. The abnormal gene structures should be considered in the clinical diagnosis of SMA. Therefore, the analyses of SMA-related gene structures were also important to the molecular diagnostics of SMA patients.<sup>[14]</sup>

Due to the small sample size, the conclusions of this study have certain limitations. For example, the limited number of cases sometimes may affect the conclusion for the disease outcome of children with SMA due to larger standard deviation of the statistical results. In addition, the interactions of SMA with the regulatory genes may influence the phenotype of SMA. There are also several modified genes such as human 4F5 gene (*H4F5*), general transcription factor IIIH polypeptide 2 gene (*GTF2H2*) and plastin 3 (*PLS3*) in the vicinity of the *SMN1* gene in the 5q13 region.<sup>[29–32]</sup> Because of their proximity to the *SMN1* gene, *H4F5*, and *GTF2H2* are thought to be disease-modifying factors. In previous studies, the absence of *H4F5* or *GTF2H2* was highly associated with the severe form of SMA type I, although no direct association between these gene deficits and SMA phenotypes has been observed.<sup>[33]</sup> Beyond the region 5q13, the effect of *PLS3*, coronin-like actin-binding protein 1C (*CORO1C*) and neurocalcin delta (*NCALD*) on SMA have been confirmed *in vivo* and *in vitro* recently. Through upregulation of *PLS3* and *CORO1C* genes and downregulation of *NCALD* gene, endocytosis of cells damaged by *SMN* gene defect can be repaired, synaptic function can be restored, which affects SMA prognosis.<sup>[29,31–34]</sup> As a result, the *SMN1* gene conversion, *SMN1* subtle mutations, *SMN2* copy number, the extent of deletion in the 5q13 region, and the other non-5q13 molecular modifiers should all be considered in the genotype-phenotype analysis of SMA.

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