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*Original Article*

# *Survival motor neuron 1* (*SMN1*) gene acts as a promising prognostic biomarker for potential spinal muscular atrophy in the Chinese population

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**Abstract:** Spinal muscular atrophy (SMA) is a group of neuromuscular disorders characterized by degeneration of the anterior horn cells of the spinal cord. This study aimed to investigate the prognostic role of the *SMN1* gene in the pathogenesis of SMA. A total of 1648 peripheral blood samples were obtained from volunteers at The Third Affiliated Hospital of Guangzhou Medical University. Genotype of SMA patients and *SMN1* deletion status of 252 SMA patients' parents were investigated using multiplex ligation-dependent probe amplification (MLPA). Universal primer multiplex PCR was used to simultaneously amplify fragments of the *SMN1*, β-globin, and *KRIT1* genes. We used logistic regression to compare SMA risk and calculated odds ratios with 95% confidence intervals. Results indicated that there were 457 SMA patients with homozygous *SMN1* deletions (94.8% of patients examined), and 25 SMA patients with heterozygous *SMN1* deletions (5.2% of patients examined). *SMN1*-1 in both father and mother accounted for 95.6% (241 cases), and SMN1 gene on 1 allele (SMN1-1) or SMN1 gene on 2 alleles (SM*N1*-2) in either father or mother accounted for 4.4% (11 cases). Among the 217 participants, there were 101 individuals carrying the *SMN1*-1 copy (accounting for 46.5%) and only 9 individuals carrying *SMN1*-2 copy (accounting for 2.1%). The inheritance risk of SMA is 25% when both parents are *SMN1*-1 carriers. The risk ratio of SMA is $5.2\times10^{-3}$ when only the father or mother is an *SMN1*-1 carrier. In conclusion, the *SMN1* gene acts as a prognostic biomarker for SMA, and provides information for genetic counseling and aristogenesis fine rearing.

**Keywords:** Spinal muscular atrophy, survival motor neuron 1, genotype, biomarker

## Introduction

Spinal muscular atrophy (SMA) is a group of neuromuscular disorders characterized by degeneration of the anterior horn cells of the spinal cord, leading to progressive proximal muscle weakness and atrophy affecting the upper and lower limbs [1, 2]. SMA is subdivided into four types according to phenotype and age of onset: type I is an infantile acute form and often results in death in early childhood; type II is an infantile chronic form with intermediate severity; type III is a childhood and adolescent form and shows mild severity; and type IV is an adult form [3].

SMA is one of the most common autosomal recessive diseases with an incidence of approximately 1 in 10,000 live births and a carrier frequency of 1 in 35-117 [4, 5]. There are no significant differences among Chinese, Caucasian, Korean, Australian, American, and African populations, which illustrates that there are no racial or regional differences with respect to SMA in general populations [6]. Approximately 94% of SMA cases are caused by absence of the *SMN1* gene. The *SMN2* gene differs from *SMN1* by five nucleotides in exon 7, which results in decreased transcription and deficiency of the normal stable SMN protein [7]. Therefore, quantification of *SMN1* in exon 7 is a good strategy for estimating *SMN1* deletion or *SMN1* to *SMN2* gene conversion. Carrier (heterozygous deletion of *SMN1* exon 7) prevalence was found to be 2.39% in the general Chinese population [6].

Therefore, in this study we aimed to analyze the genetic characteristics of the *SMN1* gene in SMA patients and their family members, and to

**Table 1.** Probe sequences for the linkage analysis

| Probes | Sequences (5'-3') |
|---|---|
| D5S435-F | CACCGCAGGCAGGAGATTA |
| D5S435-R | AGACTGGTCCTTAGATAGGGTTGAT |
| D5S629-F | GTCCACCCCACCTACTAATCAG |
| D5S629-R | GACAGGAGAATCGCTTGAACC |
| D5S1413-F | AAAATAGGCTTGTGAAACCAACGC |
| D5S1413-R | GCTACAGGCCAGATGAGGGAAATAG |
| D5S610-F | TGTCCTGTTTTTAGGTTCATTGATCT |
| D5S610-R | GTCCTCAAGTGACCCTCCCA |

evaluate genotype/deletion frequencies of the *SMN1* gene in a Chinese population.

## Materials and methods

### *Participants*

A total of 1648 peripheral blood samples were obtained from volunteers at the Third Affiliated Hospital of Guangzhou Medical University. Anonymously coded peripheral blood specimens were used from samples submitted for SMA testing from 2010 to 2015 with written consent. This study was approved by the Institutional Review Board of Third Affiliated Hospital of Guangzhou Medical University.

### *DNA Isolation*

DNA samples from SMA patients, carriers, and normal individuals were obtained at the Third Affiliated Hospital of Guangzhou Medical University. Genomic DNA was collected from peripheral whole blood using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany) as described by the manufacturer. A total of 1648 DNA samples were analyzed in this study, including those from 482 patients with SMA, 519 family members of the patients, and 647 control individuals from the general population.

### *MLPA analysis*

The MLPA assays were performed using the SALSA MLPA kit P060-B1 (MRC Holland, Amsterdam, the Netherlands) following the manufacturer's directions. The TaqMan MGB5'-labeled VIC probes for the SMN1 and SMN2 exon 7 locus used in this study were listed in **Table 1**. The MLPA products were detected using an Applied Biosystems 3100 Genetic Analyzer (Life Technologies, Foster City, CA) and analyzed using a combination of Gene-Mapper Analysis Software (Life Technologies) and Coffalyser Software. The Coffalyser MLPA analysis module, P060-B2, was downloaded from www.coffalyser.net.

### *Multiplex real-time PCR*

In this experiment, multiplex real-time PCR was used to simultaneously amplify the fragments of SMN1, β-globin, and KRIT1 genes (primers shown in **Table 2**). The amplicons of the β-globin and KRIT1 genes served as controls for determining the relative gene dose of exons 7 and 8 of SMN1. These primers were designed to have similar melting temperatures and different PCR product lengths, which is optimal for the universal-primer multiplex PCR system. The final reaction volume was 25 μL and contained 100 ng of genomic DNA, the proper concentration of each primer (**Table 1**), 200 μM dNTPs, 1.0 unit of TaKaRa Taq™ enzyme (TaKaRa Biotechnology, Japan), and 2.5 μL of 10× PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) as provided by the manufacturer. The PCR amplification was performed in a Px2 thermocycler (Thermo Electron Corp, Burlington, Ontario, Canada) with an initial denaturing step at 95°C for 10 min, followed by three cycles of denaturing at 95°C for 45 s, annealing and elongation at 60°C for 2 min, and then 25 cycles consisting of denaturation at 95°C for 45 s, annealing at 50°C for 90 s, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. After PCR cycling was completed, the product was diluted to one quarter concentration by adding 3 volumes of double-distilled water, electrokinetically injected into the capillary, and subsequently analyzed by the CE instrument.

### *Statistical analysis*

Statistical analysis was performed using SPSS 20.0 statistical package (IBM Corporation, Armonk, NY, USA). We used logistic regression analysis to compare SMA risk, and calculated odds ratios with 95% confidence intervals. The risk ratio (RR) of the SMA occurrence was calculated using the OR value as described previously [8].

## Results

### *Diagnostic flow for SMA patients*

The diagnostic flow chart for this study is illustrated in **Figure 1**. Sixteen hundred and forty-eight participants were recruited into the fol-

**Table 2.** Primers for the amplification of SMN, β-globin and KRIT1 genes

| Gene | Primers | Sequences | DNA length (bp) |
|---|---|---|---|
| SMN1 (exon 7) | Forwards | ATAAGTGACGTACTAGCAACGTCGAACT | 540 |
| | Reverse | AAAAGTAAGATTCACTTTCA | |
| KRIT1 | Forwards | ATAAGTGACGTACTAGCAACGTTCGAAT | 343 |
| | Reverse | AAAACGTCTTTTAAATCAGAGC | |
| β-globin | Forwards | ATAAGTGACGTACTABDAACGGAACATT | 260 |
| | Reverse | TTTAAGACACTCTAACACTT | |

lowing categories 1) 482 clinically diagnosed SMA patients; and 2) 252 pairs of parents of those SMA patients. Recruitment was also extended to grandparents and siblings (11 such pairs were identified, and 6 extended families were recruited) when only one of the two parents showed a 1+0 genotype [9] 647 healthy subjects, including 217 with a family history of SMA and 430 with no family history. This protocol included a single PCR to generate amplified segments containing exon 7 of SMN1/SMN2 followed by DHPLC quantitative analysis.

*Genotypes and phenotypes in SMA patients*

Genotypes of 482 SMA patients were examined using the MLPA method. Results indicated that there were 457 SMA patients with homozygous deletions of *SMN1* (accounting for 94. 8% of participants), and 25 SMA patients with heterozygous *SMN1* deletions (accounting for 5.2% of participants) (**Table 2**). Results also showed that Type II SMA was the most prevalent phenotype in *SMN1* deletion homozygotes (339 cases) and heterozygotes (14 cases) (**Table 3**).

*MLPA for parents of 252 SMN1 homozygous deletion patients*

We selected 252 SMA patients with homozygous SMN1 deletions to investigate the SMN1 deletion status of their parents using MLPA. Results showed that the copy of SMN1 gene on 1 allele (SMN1-1) in both father and mother accounted for 95.6% (241 cases), and the copy of SMN1-1 or SMN1 gene on 2 alleles (SMN1-2) in either father or mother accounted for 4.4% (11 cases) (**Table 4**).

*Effects of family history on the SMA occurrence*

Two hundred and seventeen SMA individuals with a family history of SMA (father or mother) were also genotyped to identify SMN1 copy number using MLPA. Results indicated that among the 217 individuals, there were 101 individuals carrying the SMN1-1 copy, which accounted for 46.5% of participants. Furthermore, we examined SMN1 copy distribution of the 430 SMA individuals without a family history of SMA. Results indicated that there were only 9 individuals carrying the SMN1-2 copy, which accounted for 2.1%.

*SMA risk evaluation according to family history (parents)*

Risk ratio (RR) of SMA was calculated according to family history. Results indicated that the risk ratio of SMA is 25% when both parents are SMN1-1 carriers, and that the risk ratio of SMA is $5.2\times10^{-3}$ when only the father or mother is an SMN1-1 carrier. The risk ratio of SMA is $2.3\times10^{-3}$ when only the father or mother is an SMN1-1 carrier and with a positive family history for SMA. The risk ratio of SMA is $1.1\times10^{-4}$ when neither parent is an SMN1-1 carrier and without any family history of SMA.

## Discussion

Clinically, SMA patients do not always present with symptoms of a primary beta-oxidation defect, and usually present with the obvious abnormalities of elevated fatty acid metabolites and decreased levels of serum and muscle concentrations of carnitine [10, 11]. The neural effects of SMA may arise from the down-regulation of fatty acid oxidation due to deficiency of SMN protein, or due to the potential decrease or loss of a gene contiguous to SMN [12, 13]. Tein et al. reported that in 15 patients with clinical SMA diagnoses, there was an obvious difference among SMA phenotypes [14]. Observations in the current study also identified that there were 4 SMA phenotypes among study participants, the most prevalent of which was Type II.

In this study, copy number of *SMN1* was evaluated using MLPA. Results showed that the presence of *SMN1*-1 in both father and mother accounts for 95.6% (241 cases), and the presence of *SMN1*-1 or *SMN1*-2 in either father or

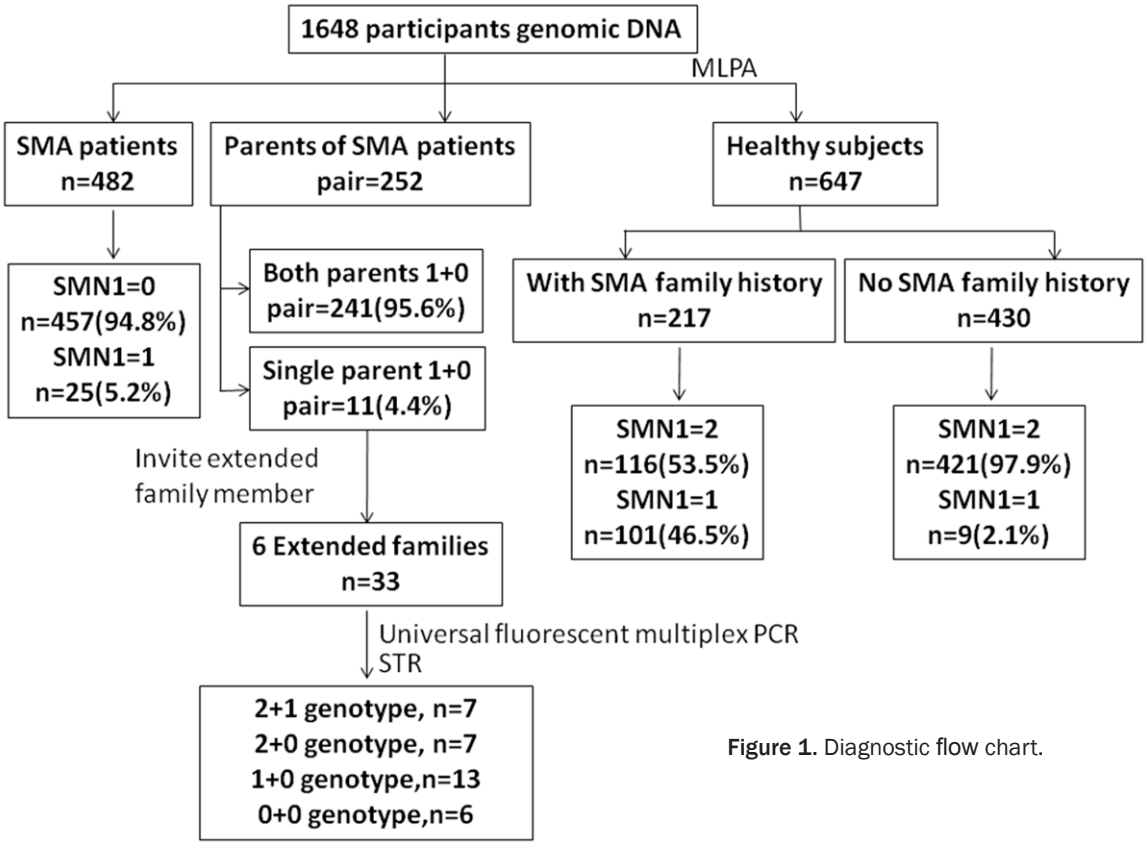


**Figure 1.** Diagnostic flow chart.

**Table 3.** Genotypes and corresponding phenotypes in patients

| | I | II | III | IV | Total |
|---|---|---|---|---|---|
| [a]Homozygotes | 67 | 339 | 46 | 5 | 457 |
| [b]Heterozygotes | 9 | 14 | 2 | 0 | 25 |
| Total | 76 | 353 | 48 | 5 | 482 |

[a]SMA patients with absence of exon 7 of the SMN1 gene on 1 allele only. [b]SMA patients with absence of exon 7 of the SMN1 gene on 2 alleles.

mother accounts for 4.4% (11 cases) (**Table 3**). For this disease, *SMN1*-1 plays an important role in the prognosis of the SMA in the children. Amold et al. cites that several promising therapeutics are being tested in early-phase clinical trials for SMA [15]. Qian et al. found that the psychosocial effects of coping with SMA are substantial and wide ranging both for the individuals living with the condition and family members of affected individuals [16]. This observation may indicate that the parents play critical role in children's SMA occurrence both by genetic factors and environmental factors. Also, family history affects SMA occurrence, which is consistent with the previous study. Liyanage et al. presented identical clinical and electrophysiological data of two brothers with type IV SMA associated with a unique form of non-progressive myoclonic epilepsy without any classical features, and suggested that further genetic investigations be performed [17].

In this study, we also discovered that *SMN1*-1 plays an important role in the pathogenesis of the SMA. Our results indicated that among the 217 individuals, there were 101 individuals carrying *SMN1*-1, which accounts for 46.5%. Jedrzejowska et al. also reported notable carrier risks for individuals having two copies of *SMN1* in SMA families with 2-copy alleles (SMN1-1) [18]. Yamamoto et al. revealed that the intragenic mutations in *SMN1* may contribute more significantly to clinical severity than *SMN2* copy number in SMA patients [19]. The above studies suggest that *SMN1* could develop as a prognostic biomarker for the SMA diagnosis.

Based on the above results, we analyzed the risk ratio (RR) of SMA according to family history by using the *SMN1*-1 genotype distribution. Results suggest that the risk ratio of SMA could be as high as 25% when both parents are *SMN1*-1 carriers. However, the risk ratio of SMA

**Table 4.** Analysis of extended family members in 6 families

| | Genotype | D5S435 | D5S629 | D5S1413 | D5S610 |
|---|---|---|---|---|---|
| Family446 | | | | | |
| Proband | 0/0 | 184/190 | 299/297 | 124/122 | 251/241 |
| Mother | 0/1 | 184/190 | 299/297 | 124/120 | 251/241 |
| Father | 0/2 | 190/186 | 297/307 | 122/132 | 241/241 |
| Grandma (P) | 0/1 | 190/186 | 297/299 | 122/114 | 241/239 |
| Grandpa (P) | 1/2 | 190/186 | 297/307 | 122/132 | 239/241 |
| Family479 | | | | | |
| Proband | 0/0 | 190/186 | 303/299 | 122/122 | 247/249 |
| Mother | 0/2 | 190/178 | 303/301 | 122/122 | 247/251 |
| Father | 0/1 | 186/190 | 299/299 | 122/122 | 249/241 |
| Grandma (M) | 0/1 | 190/190 | 303/299 | 122/124 | 247/239 |
| Grandpa (M) | 1/2 | 190/178 | 299/301 | 122/122 | 241/251 |
| Sister | 0/1 | 190/190 | 303/299 | 122/122 | 247/241 |
| Family659 | | | | | |
| Proband | 0/0 | 178/190 | 299/299 | 122/122 | 241/249 |
| Mother | 1/0 | 186/178 | 299/299 | 120/122 | 241/241 |
| Father | 0/2 | 190/190 | 299/299 | 122/124 | 249/239 |
| Grandma (P) | 0/1 | 190/186 | 299/299 | 122/114 | 249/239 |
| Grandpa (P) | 1/2 | 190/190 | 297/299 | 122/124 | 239/239 |
| Uncle (P) | 0/2 | 190/190 | 299/299 | 122/124 | 249/239 |
| Family723 | | | | | |
| Proband | 0/0 | 190/190 | 299/297 | 122/124 | 241/239 |
| Mother | 0/2 | 190/190 | 299/299 | 122/124 | 241/239 |
| Father | 0/1 | 190/178 | 297/297 | 124/122 | 239/241 |
| Grandma (M) | 1/2 | 190/190 | 297/299 | 122/124 | 239/239 |
| Grandpa (M) | 0/1 | 190/186 | 299/299 | 122/114 | 241/239 |
| Family778 | | | | | |
| Proband | 0/0 | 188/192 | 303/307 | 122/122 | 251/253 |
| Mother | 0/2 | 188/178 | 303/297 | 122/120 | 251/255 |
| Father | 0/1 | 192/186 | 307/297 | 122/122 | 253/255 |
| Grandma (M) | 0/1 | 188/190 | 303/297 | 122/124 | 251/251 |
| Grandpa (M) | 2/1 | 178/178 | 297/309 | 120/122 | 255/245 |
| Family817 | | | | | |
| Proband | 0/0 | 178/190 | 299/303 | 122/114 | 241/231 |
| Mother | 0/1 | 178/186 | 299/297 | 122/120 | 241/243 |
| Father | 2/0 | 190/190 | 299/303 | 120/114 | 245/231 |
| Grandma (P) | 1/0 | 186/190 | 299/303 | 122/114 | 241/231 |
| Grandpa (P) | 1/2 | 186/190 | 297/299 | 120/120 | 243/245 |
| Brother | 2/1 | 190/186 | 299/297 | 120/120 | 245/243 |

is very low when one or neither parent is an *SMN1* deletion carrier. Although prior studies have explored the role of *SMN1*-1 in SMA patients, calculation of the risk ratio of SMA using the *SMN1*-1 allele has not been previously performed.

In conclusion, the present study analyzed the characteristics of the *SMN1* gene in SMA patients and their families, and evaluated the frequency of the *SMN1* deletion in the Chinese population. The *SMN1* gene acts as a prognostic biomarker in SMA, and provided information for the genetic counseling and aristogenesis fine rearing.

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## Disclosure of conflict of interest

None.

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