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Original article

Combination of *SMN2* copy number and *NAIP* deletion predicts disease severity in spinal muscular atrophy

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Abstract

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the *SMN1* gene. The *SMN2* gene is highly homologous to *SMN1* and has been reported to be correlated with severity of the disease. The clinical presentation of SMA varies from severe to mild, with three clinical subtypes (type I, type II, and type III) that are assigned according to age of onset and severity of the disease. Here, we aim to investigate the potential association between the number of copies of *SMN2* and the deletion in the *NAIP* gene with the clinical severity of SMA in patients of Malaysian origin. Forty-two SMA patients (14 of type I, 20 type II, and 8 type III) carrying deletions of the *SMN1* gene were enrolled in this study. *SMN2* copy number was determined by fluorescence-based quantitative polymerase chain reaction assay. Twenty-nine percent of type I patients carried one copy of *SMN2*, while the remaining 71% carried two copies. Among the type II and type III SMA patients, 29% of cases carried two copies of the gene, while 71% carried three or four 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. We conclude that there exists a close relationship between *SMN2* copy number and SMA disease severity, suggesting that the determination of *SMN2* copy number may be a good predictor of SMA disease type. Furthermore, *NAIP* gene deletion was found to be associated with SMA severity. In conclusion, combining the analysis of deletion of *NAIP* with the assessment of *SMN2* copy number increases the value of this tool in predicting the severity of SMA.

Keywords: Spinal muscular atrophy; Survival motor neuron; Neuronal apoptosis inhibitory protein; Copy number; Disease severity

1. Introduction

Spinal muscular atrophy (SMA) is a neuromuscular disease characterized by degeneration of the motor neuron in the spinal cord. SMA is a common autosomal recessive disease with a prevalence of 1 in 10,000 newborns [1]. The survival motor neuron gene (*SMN*, present in two highly homologous copies, *SMN1* and *SMN2*) and the neuronal apoptosis inhibitory protein gene (*NAIP*, adjacent to *SMN*) are two candidate genes for proximal SMA [2,3]. Manifestation of the disease

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requires mutation of both alleles of *SMN1*, located on chromosome 5. The homozygous deletion of *SMN1* is responsible for SMA in 95% of SMA patients, while other intragenic mutations cause the disease in the remaining 5% of patients [2].

The disease is classified into three clinical subtypes (type I, type II, and type III) depending on age of onset, motor development milestones, and severity of clinical course [1]. Type I (Werdnig–Hoffmann disease) is the most severe form, while type III (Kugelberg–Welander disease) is the mildest form. The differences in severity among SMA patients have prompted researchers to investigate the genomic variations that contribute to these phenotypes.

Previous studies showed that variation in the number of copies of the *SMN2* gene contributes to the severity of SMA [4,5]. Furthermore, given that *SMN2* is known to be transcribed, a difference in *SMN2* copy number would also translate into a variation in the amount of functional protein produced. This hypothesis is supported by the demonstration of a correlation between disease severity and SMN protein levels and by the finding of a higher ratio of *SMN2*/*SMN1* gene dosage in the parents of SMA type II and III patients, compared with the parents of type I patients [6]. In addition, Coovert et al. reported a correlation between disease severity and the amount of nuclear SMN protein located in structures called gems [7].

The *NAIP* gene was reported to be deleted in most patients suffering from severe SMA [3]; however, a direct effect of *NAIP* on SMA disease severity has not been established. Here, we investigate the clinical and molecular characteristic of 42 SMA patients in an attempt to measure the combined effect of *SMN2* copy number and *NAIP* deletion on clinical severity in SMA patients.

2. Materials and methods

2.1. Patients

This study was approved by the Research and Ethics Committee of the School of Medical Sciences, Universiti Sains Malaysia. Forty-two patients fulfilled the diagnostic criteria defined by the International SMA Consortium. Of these, 14 cases were type I, 20 were type II, and 8 type III. The subjects included 32 Malays, 6 Chinese, 2 Indians, and 2 with mixed parentage. The analysis of *SMN1* gene deletion was performed prior to the *SMN2* gene analysis. To avoid a clinical bias in the selection of patients, we performed quantification analyses exclusively in patients carrying homozygous deletion of the *SMN1* gene.

2.2. SMN2 copy number analysis

A total of 42 SMA samples with a deletion of *SMN1* confirmed by the polymerase chain reaction restriction enzyme (PCR-RE) approach were further analyzed by quantification of *SMN2* copy number. Real-time PCR using SYBR® Green 1 was used to quantify the number of copies of this gene. Exon 4 of *CFTR* (used as a reference gene) was amplified using the following primer set: forward 5′-AGTCACCAAAGCAGTACAGC-3′ and reverse 5′-GGGCCTGTGCAAGGAAGTATTA-3′ [8]. The amplification of *SMN2* was performed using the following primer set: cen*SMN*ex7forw 5′-TTTAT TTTCCTTACAGGGTTTTA-3′ and cen*SMN*int7rev 5′-GTGAAAGTATGTTTCTTCCACGCA-3′ [9]. The quantification was based on the ratio of *SMN2* to *CFTR*.

2.3. NAIP gene deletion analysis

Deletion analysis of the *NAIP* gene was performed by PCR amplification of exon 5 using the following primer set: 1863 5′-CTCTCAGCCTGCTCTTCAGAT-3′ and 1864 5′-AAAGCCTCTGACGAGAGGATC-3′, according to a method described by Roy et al. [3]. The PCR products were electrophoresed on 2% agarose gel to verify the presence or absence of exon 5 of *NAIP*.

2.4. Statistical analysis

The number of *SMN2* copies in the clinical subtypes and *NAIP* genotypes of SMA patients was compared by chi-square test and Fisher's exact test. When there was less than 4 number in data, Fisher's exact test was used. A p-value of less than 0.05 was considered to indicate a significant difference.

3. Results

The copy number of *SMN2* in the analyzed patients is shown in Table 1. Among type I patients, 71% of cases carried two copies of the gene, while 29% had one copy. In patients suffering from milder forms of the disease (types II and III), 29% of the cases carried two *SMN2* copies, 57% presented with three copies, and 14% with four. This means that 71% of the type II and III patients were shown to carry three or more copies of the SMN2 gene, while type I patients had either one or two copies of the gene.

Deletion analysis of exon 5 of the *NAIP* gene is shown in Table 2. Combined analysis of *SMN2* gene copy number and *NAIP* gene deletion in Malaysian spinal muscular atrophy (SMA) patients showed 50% of type I and 7% of type II and III SMA patients carried a homozygous deletion of this gene (Table 3).

We then sub-grouped the SMA patients that were shown to carry two copies of the *SMN2* gene (18 patients in total) into those who had a deletion of *NAIP* (5 cases) and those without (13 patients), and ascertained their clinical presentation. All patients carrying