MOLECULAR ANALYSES OF SMN2 AND NAIP GENES FOR PROGNOSIS PREDICTION OF SPINAL MUSCULAR ATROPHY IN VIETNAMESE AND MALAYSIAN PATIENTS

Teguh Haryo Sasongko1, Watihayati Mohd. Shamsuddin2, Tran Van Khan3, Hisahide Nishio4, Zilfalil Bin Alwi1

1Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian Kelantan, Malaysia
2Center for Chemical Biology, Universiti Sains Malaysia, Minden, Penang, Malaysia
3Institute of Biotechnology, Vietnamese Academy of Science and Technology, Hanoi, Vietnam
4Department of Genetic Epidemiology, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan
teguhhs@kk.usm.my

Background

Progress in the research of spinal muscular atrophy and investigations of SMN2 and NAIP genes has provided important milestones in understanding the pathogenesis of the disorder. This understanding leads to the advances in diagnosis as well as possible future therapeutics approaches. This study aims at elaborating two consecutive studies involving Vietnamese and Malaysian SMA patients, studying the copy number analyses of SMN2 and NAIP genes and providing prognosis information for SMA patients.

Method:

Seventy-six Vietnamese and Malaysian SMA patients of different clinical severity with deletion of SMN1 gene were enrolled in this study. Copy number analysis of SMN2 was performed by real-time quantitative PCR using LightCycler Instrument. Analysis of NAIP was performed either by conventional PCR or real-time quantitative PCR.

Results:

The SMN2 copy number in the severe-type SMA was significantly lower than that in patients with less severe types. NAIP deletion was commonly found in patients with severe-type of SMA.

Conclusions:

SMN2 and NAIP were found to be related with SMA severity in Vietnamese and Malaysian SMA patients. Such analyses will be beneficial not only to help clinicians provide prognosis information to the patients, but also to predict the severity of affected fetuses and to provide essential information for future therapeutic studies.

Keywords: SMN2, NAIP, spinal muscular atrophy, copy number, prognosis, real time quantitative PCR

Introduction

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder characterized by degeneration of anterior horn cells (motor neurons) in the spinal cord, resulting in weakness of the proximal limb and trunk muscles. According to the disease severity, childhood-onset SMA is classified into three subtypes: type I (severe form, unable to sit unsupported), type II (intermediate form, unable to stand or walk unsupported) and type III (mild form, able to stand or walk unsupported) (Zerres and Davies, 1999).

All three clinical subtypes were mapped to chromosome 5q11.2-13.3 using linkage analysis, 2 – 4 and the SMN1, SMN2 and NAIP genes were subsequently identified in this SMA-related region. The three genes are located next to each other within the SMA locus of chromosome 5q (Lefebvre et al., 1995; Roy et al., 1995). SMN1 and SMN2 are almost identical genes that encode the same protein, SMN (Lefebvre, 1995).
Homozygous deletion of \textit{SMN1} is responsible for SMA in 95% of SMA patients, while other intragenic mutations cause the disease in the remaining 5\% (Brzustowics \textit{et al.}, 1990). \textit{SMN2} gene has been characterized as a modifying factor of the clinical severity of SMA (Feldkotter \textit{et al.}, 2002). Given that \textit{SMN2} is known to be transcribed, a difference in \textit{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 \textit{SMN2}/\textit{SMN1} gene dosage in the parents of SMA type II and III patients, compared with the parents of type I patients (Lefebvre \textit{et al.}, 1997).

Homologous \textit{NAIP} deletion has been more frequently observed in type I patients than in type II – III patients (Roy \textit{et al.}, 1995). Taylor \textit{et al.} (1998), however, found no differences in the age of onset and length of survival between type I patients lacking of or retaining the \textit{NAIP} gene, and concluded that the \textit{NAIP} gene may not have an effect on the clinical severity. Despite the aforementioned observations, the contribution of \textit{NAIP} to the pathogenesis of SMA remains unclear.

There have been no studies that simultaneously address the three genes within SMA locus (\textit{SMN1}, \textit{SMN2} and \textit{NAIP}) in relation to SMA severity. This study aimed to evaluate the contribution of various genotypic pattern of the three genes to prognosis prediction of spinal muscular atrophy among Vietnamese and Malaysian patients.

\section*{Patients and Methods}

\subsection*{Patients}
Seventy-six patients fulfilled the diagnostic criteria as defined by the 1998 International SMA Consortium (Zerres and Davies, 1999) and showed \textit{SMN1} deletion according to the method of van der Steege (van der Steege \textit{et al.}, 1995). Thirty-four were Vietnamese and 42 were Malaysians. All patients gave their informed consent prior to blood taking. Genomic DNA was extracted from whole blood using commercially available kit.

\subsection*{Quantitative real time PCR of \textit{SMN2} and analysis of \textit{NAIP} genes}
To determine the copy numbers of \textit{SMN2}, we employed a calibrator-normalized relative quantification method using real-time PCR of LightCycler Instruments (Roche Diagnostics, Mannheim, Germany) (Tran \textit{et al.}, 2008, Watihayati \textit{et al.}, 2009). We used previously described primers for quantifications of \textit{SMN2} (Feldkotter, 2002). We used \textit{CFTR} as reference gene with previously described primers (McAndrew, 1997). Analysis of \textit{NAIP} gene used either the same copy number analysis method using previously published primers (Tran \textit{et al.}, 2008) or \textit{NAIP} deletion test (Roy \textit{et al.}, 1995).

\section*{Results and Discussion}

We found remarkable differences in the genotypic pattern of SMA locus between patients with severe type and patients with milder type (Table 1).

All patients with 1 \textit{SMN2} copy presented with severe clinical features, regardless of \textit{NAIP} deletion status. However, among 33 patients with 2 \textit{SMN2} copies, variable severity was observed. In this regard, patients with 2 copies of \textit{SMN2} and 0 copies of \textit{NAIP} always presented with severe clinical features, while only 50\% of patients with 2 copies of \textit{SMN2} and
>0 copies of NAIP presented with severe clinical features. Patients with more than 2 copies of SMN2 almost always (95%) presented with milder clinical features.

These data suggests that in the presence of single SMN2 copy, NAIP gene may not be an important factor in severity prediction. This is also the case for patients with more than 2 copies of SMN2. However, in the presence of 2 copies of SMN2, NAIP is an important factor for severity prediction. These facts were reasonable since higher copy number of SMN2 would be translated into higher level of functional SMN protein. Furthermore, a previous report that NAIP protein could prevent motor neuron degeneration after sciatic nerve axotomy in rats (Perrelet et al., 2000) may support NAIP’s role in modifying SMA severity.

However, it is interesting to note that two of our patients with more than 2 copies of SMN2 and presence of NAIP showed severe clinical features. This may be caused by the presence of a pathogenic mutation within the existing copies of SMN2 which hamper the production of functional SMN protein.

Table 1. Distribution of genotypic patterns of SMA locus among SMA patients with different clinical severity

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient number</th>
<th>Patient number in different clinical severity</th>
<th>Severe-Type I (%)</th>
<th>Milder-Type II/III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMN1-SMN2-NAIP</td>
<td>0 - 1 - 0</td>
<td>2</td>
<td>2(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>0 - 1 - &gt;0</td>
<td>2</td>
<td>2(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>0 - 2 - 0</td>
<td>9</td>
<td>9(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>0 - 2 - &gt;0</td>
<td>24</td>
<td>12(50)</td>
<td>12(50)</td>
</tr>
<tr>
<td></td>
<td>0 - &gt;2 - 0</td>
<td>3</td>
<td>0(0)</td>
<td>3(100)</td>
</tr>
<tr>
<td></td>
<td>0 - &gt;2 - &gt;0</td>
<td>36</td>
<td>2(5)</td>
<td>34(95)</td>
</tr>
</tbody>
</table>

The presence and absence of NAIP possibly reflects the extent of deletion, in which a large deletion involves both SMN1 and NAIP and a small deletion only involves SMN1. This idea is highly plausible since SMN1 and NAIP are adjacent to each other. A small deletion, i.e. NAIP retention, may be associated with gene conversion of SMN1 to SMN2, which would lead to a seemingly apparent deletion of SMN1. However, we also revealed that gene conversion can occur on chromosomes with a large deletion, i.e. NAIP deletion. In the present study, three of our type III patients lacking SMN1 showed 3 copies of SMN2 and 0 copies of NAIP (Table 1), and there is another case reported elsewhere of a type III patient with 0 copies of SMN1, 4 copies of SMN2 and 0 copies of NAIP (Yamashita et al., 2004). In these cases, gene conversion of SMN1 to SMN2 and deletion of NAIP seem to have occurred together.

This report indicated significant progress in predicting the severity of SMA patients. Severity prediction is important for clinicians in providing patients with prognosis information. The information will be useful not only in the context of patient management, but also to provide prediction on the birth outcome of SMA-affected fetuses. It is also noteworthy, that this finding may contribute to designing efficient SMA therapeutic study. Patients or cell lines with genotypically high copies of SMN2 and presence of NAIP will be expected to respond better to substances being tested, compared to those with low copies of SMN2 and absence of NAIP.
Conclusion

SMN2 and NAIP were found to be related with SMA severity in Vietnamese and Malaysian SMA patients. Such analyses will be beneficial not only to help clinicians in providing prognosis information to the patients, but also to predict the severity of affected fetuses after birth and to provide essential information for future therapeutic studies.

References
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