ABSTRACT

Copy number analysis of the SMN (Survival Motor Neuron) genes in healthy individuals with no history of spinal muscular atrophy (SMA) is important to assess carrier frequency, the frequency of patients with two copies of SMN1 on one chromosome (a factor that could lead to a false-negative result when testing for SMA carriers) and the mechanisms responsible for these chromosomes with two copies of SMN1. We retrospectively analyzed the copy number of SMN1 and SMN2 genes detected in blood samples of 119 gamete donors by Multiplex Ligation-Dependent Probe Amplification (MLPA) assay during the last two years. The number of donors with a heterozygous deletion of exon 7 of SMN1 was 1 in 59 samples (1.7%). It was estimated that 5.4% of the studied alleles presented two SMN1 copies. The percentage of individuals with one or zero copies of SMN2 was not statistically different among individuals with two copies of SMN1 (48.1%) and those with three or four SMN1 copies (63.6%) (p= 0.327). The frequency of individuals with one copy of SMN1 is low and consistent with previously reported data; therefore, universal screening is not cost-effective, although it could be so in gamete donors. Our results are not in agreement with the hypothesis that the occurrence of gene conversion from SMN2 to SMN1 results in two SMN1 copies on one chromosome.

Key words: SMN genes, SMA, carrier, gene conversion.

RESUMEN

El análisis del número de copias de los genes SMN (de sus siglas en inglés Survival Motor Neuron) en población sana sin historia de atrofia muscular espinal (AME) es importante para establecer la frecuencia de portadores, la frecuencia de pacientes con dos copias de SMN1 en un cromosoma (un factor que puede dar lugar a falsos negativos cuando se realizan estudios de portadores) y los mecanismos responsables de estos cromosomas con dos copias de SMN1. Hemos analizado retrospectivamente el número de copias de los genes SMN1 y SMN2 detectadas por MLPA (amplificación de sondas dependiente de ligandos múltiples, de sus siglas en inglés Multiplex Ligation-dependent Probe Amplification) en muestras de sangre de 119 donantes de gametos durante los dos últimos años. El número de donantes con una delección en heterocigosis del exón 7 del SMN1 fue de uno cada 59 muestras (1,7%). La estimación del porcentaje de alelos estudiados con dos copias de SMN1 fue de 5,4 % y el porcentaje de individuos con una o ninguna copia de SMN2 no fue estadísticamente significativo entre los individuos con dos copias de SMN1 (48,1 %) y aquellos con tres o cuatro copias (63,6 %) (p=0,327). La frecuencia de individuos con una copia de SMN1 es baja y consistente con datos anteriores publicados, por lo que el tamizado universal no es efectivo en términos de costo, aunque podría serlo en donantes de gametos. Nuestros resultados no están de acuerdo con la hipótesis de que la conversión de SMN2 a SMN1 da lugar a cromosomas con dos copias de SMN1.

Palabras clave: genes SMN, AME, portador, conversión génica.
INTRODUCTION

Spinal muscular atrophy (SMA) is the second most common lethal autosomal recessive disorder after cystic fibrosis in the western hemisphere (Ogino et al., 2002). The carrier frequency is between 1/40 and 1/60 in diverse populations, affecting 1 in 10,000 live births (Cusin et al., 2003; Feldkott et al., 2002). This disorder is characterized by the degeneration of the lower motor neurons of the spinal cord, leading to symmetrical muscle weakness, atrophy and, in the majority of cases, premature death.

SMA is caused by mutation in the survival motor neuron 1 (SMN1) gene (Lefebvre et al., 1995). A 96 % of the SMA patients present a homozygous deletion of at least SMN1 exon 7. The remaining 4 % of cases are caused by a variety of molecular lesions, including point mutations, small deletions and small insertions (Wirth, 2000; Monani et al., 1999).

Survival motor neuron 2 (SMN2) gene is highly similar to SMN1. It differs from SMN1 in only five nucleotides: two in exonic positions (exon 7 and exon 8) and three in intronic positions (one in intron 6 and two in intron 7) (Wirth et al., 1999). The nucleotide change in exon 7 of SMN2 alters an exonic splicing enhancer. As a result, most SMN2 transcripts lack exon 7 and are not functional, being more than 90 % of the functional SMN1 protein due only to the action of SMN1 gene (Lorson et al., 1999). Therefore, it is SMN1 copy number, and not SMN2, which determines the patient status. SMN2 acts only as a disease-modifying gene: the more SMN2 copies a patient has, the milder the SMA phenotype is.

Copy number analysis of the SMN1 gene in healthy individuals with no history of SMA is important to assess carrier frequency in each population and to be able to recommend universal screening. Hendrickson et al. (2009) reported that the frequency of SMA carrier is different among different ethnic groups. Moreover, analysis of the SMA locus may result useful to evaluate the frequency of patients with two copies of SMN1 on one chromosome, an important factor that can modify the rate of being SMA carrier in a population. An assessment of SMN2 copy number may help to explain the presence of two copies of SMN1 on one chromosome.

METHODS

In the study reported herein, we retrospectively analyzed the copy number of SMN1 and SMN2 genes that had been detected in blood samples of 119 gamete donors by MLPA assay during 2012 and 2013. All subjects studied were born in the Andalusia region and did not have a family history of SMA.

MLPA was performed using Kit P060 version B2 (MRC Holland, Netherland). This MLPA probe set detects copy number changes of exon 7 and 8 of the SMN1 and SMN2 genes. Analysis of PCR products were done in a 3130AB1 sequencer (Applied Biosystems, USA). Data was further studied with Genemapper® software v4.1.

RESULTS

The number and the percentage of different SMN1/SMN2 genotypes observed in our study are shown in Table 1, and the frequencies of the SMN1 alleles are listed in Table 2.

Two SMN1 copies were the more prevalent genotype (89.1 %), whereas the frequency of individuals with only one SMN1 copy (and, therefore, carriers of SMA) was low, 1 in 119 samples (1.7 %).

Nine and two of the 119 patients studied presented three and four copies of SMN1, respectively. Hence, 13 alleles of the 238 studied was estimated to be two copies-alleles (5.4 %). Consequently, it is possible that there were more SMA carriers among those individuals in which two copies of SMN1 were detected.

Previously published data have shown that increased SMN1 copy number is associated with decreased SMN2 copy number due probably to gene conversion from SMN2 to SMN1 (Ogino et al., 2003). Nevertheless, in our study the percentage of individuals with one or zero copies of SMN2 was statistically non-significant among individuals with two copies of SMN1 (48.1 %) and those with three or four SMN1 copies (63.6 %) (p=0.887, X² test).

<table>
<thead>
<tr>
<th>Copy relations</th>
<th>SMN1: SMN2</th>
<th>N° of subjects</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:3</td>
<td>2</td>
<td>SMA carrier</td>
<td></td>
</tr>
<tr>
<td>2:0</td>
<td>9</td>
<td>non-carrier</td>
<td></td>
</tr>
<tr>
<td>2:1</td>
<td>42</td>
<td>non-carrier</td>
<td></td>
</tr>
<tr>
<td>2:2</td>
<td>52</td>
<td>non-carrier</td>
<td></td>
</tr>
<tr>
<td>2:3</td>
<td>3</td>
<td>non-carrier</td>
<td></td>
</tr>
<tr>
<td>3:0</td>
<td>1</td>
<td>non-carrier</td>
<td></td>
</tr>
<tr>
<td>3:1</td>
<td>4</td>
<td>non-carrier</td>
<td></td>
</tr>
<tr>
<td>3:2</td>
<td>4</td>
<td>non-carrier</td>
<td></td>
</tr>
<tr>
<td>4:0</td>
<td>1</td>
<td>non-carrier</td>
<td></td>
</tr>
<tr>
<td>4:1</td>
<td>1</td>
<td>non-carrier</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. SMN1 and SMN2 copy numbers in a sample of 119 gamete donors from southern Spain

DISCUSSION

Using SMN gene dosage analysis, we studied SMN1 and SMN2 copy numbers in a sample of a healthy population from Andalusia. To our knowledge, it is the first study of these characteristics performed in the above mentioned area.

The frequency of individuals with a heterogeneous deletion of exon 7 of SMN1 gene in our cohort is consistent with the prevalence of SMA carriers in previously reported data. Nevertheless, there may be a more carriers in the sample studied because the MLPA assay does not allow both the detection of some types of uncommon mutations and the identification of carriers with two SMN1 copies on one chromosome and zero in the other of the pair.

The number of alleles with two, three and four copies of SMN1 genes also agrees with published data from other authors in other populations (Ogino et al., 2004; Anhaf et al., 2003).

The fact that three or more copies of SMN1 were detected in some individuals, indicates the presence of alleles with two copies of SMN1 on one chromosome. This is a fundamental factor that could lead to a false-negative result when testing for SMA carriers and two copies are identified. Calculations for estimating the probability of being an SMA carrier in an individual without a family history of SMA and two copies of SMN1, take into consideration the estimated frequency of alleles with two copies of SMN1. In Caucasian populations and for an estimated frequency of two SMN1 copy similar to detected by us (5.4 %), the adjusted risk is approximately 1:632 (Smith et al., 2007). Rare mutations, undetectable by MLPA, are also taken into account for these calculations.

Therefore, it is important to determine how many SMN1 alleles present more than two copies in order to obtain an estimated frequency of 2-copy alleles in each population and to calculate the probability of being an SMA carrier even though two SMN1 copies had been identified.

The mechanisms responsible for the prevalence of two copies of SMN1 on one chromosome remain unknown. Ogino et al. (2003) studied 13 individuals with three or four copies of SMN1, and speculated that the occurrence of gene conversion from SMN2 to SMN1 results in two SMN1 copies on one chromosome. We evaluated a similar number of this type of patients. Our results are in disagreement with Ogino et al. (2003) observation because if this were so, increased SMN1 copy number would be associated with decreased SMN2 copy number. In the sample studied, copies of SMN2 are similar among individuals with two copies of SMN1 and those with three or four SMN1 copies. Additional investigations with a higher number of patients with three and four copies of the SMN1 gene are necessary to ascertain if the hypothesis of gene conversion from SMN2 to SMN1 should be accepted.
Although guidelines from the American College of Medical Genetics (ACMG) published in 2008 recommend universal carrier screening for SMA, this screening is not cost-effective, as has yet been studied elsewhere (Little et al., 2010). However, in gamete donors, and given that these gametes may be used by several recipients, there is an increased risk of autosomal recessive disease in the offspring of multiple pairings over that of a single couple. Hence, it is reasonable to perform screening of potential gamete donors for common and severe disorders such as SMA.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES


