DETERMINATION OF SURVIVAL MOTOR NEURON (SMN) GENES COPY NUMBERS IN A SAMPLE OF HEALTHY POPULATION FROM SOUTHERN SPAIN

DETERMINACIÓN DEL NÚMERO DE COPIAS DE GENES DE SUPERVIVENCIA DE LA NEURONA MOTORA (SMN) EN UNA MUESTRA DE UNA POBLACIÓN SANA DEL SUR DE ESPAÑA

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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 deleción 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.

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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; Feldkötter *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 SMN 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.

The copy number of *SMN1* ranges from zero to four. Two, three or four copies may be detected in healthy subjects. Nevertheless, in the vast? majority of SMA carriers only one copy of *SMN1* is identified, although some SMA carriers are characterized by the presence of two *SMN1* copies on only one chromosome of the pair. No copies of the *SMN1* exon 7 are present in most SMA patients.

Most screening methods for carriers, including the multiplex ligation-dependent probe amplification (MLPA) assay, typically perform a quantitative analysis of *SMN1* exon 7 copy number. These methods do not allow the detection of either point mutations, small deletions or small insertions. Moreover, it is not possible to distinguish carriers with two copies on one chromosome from "wild type" patients, with one copy on each chromosome of the pair.

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, Netherlands). This MLPA probemix detects copy number changes of exon 7 and 8 of the *SMN1* and *SMN2* genes. Analysis of PCR products was done in a 3130ABI 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 59 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 copy-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=0.387, χ^2 test).

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

Copy relations SMN1: SMN2	N° of subjects	Status
1:3	2	SMA carrier
2:0	9	non-carrier
2:1	42	non-carrier
2:2	52	non-carrier
2:3	3	non-carrier
3:0	1	non-carrier
3:1	4	non-carrier
3:2	4	non-carrier
4:0	1	non-carrier
4:1	1	non-carrier

Table 2. *SMN1* copies observed in the 119 gamete donors studied

Number of allele copies	Number of donors (%)
1	2 (1.7)
2	106 (89.1)
3	9 (7.5)
4	2 (1.7)

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 heterozygous 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 few 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 *SMN* genes also agrees with published data from other authors in other populations? (Ogino *et al.*, 2004; Anhuf *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 presence of two copies of *SMN*1 on one chromosome 5 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´s 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.

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