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Detection of Spinal Muscular Atrophy Carriers in a Sample of the Brazilian Population

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Key Words
Spinal muscular atrophy · *SMN1* gene · Denaturing high-performance liquid chromatography

Abstract

Background: Spinal muscular atrophy is a common autosomal recessive neuromuscular disorder caused by mutations in the *SMN1* gene. Identification of spinal muscular atrophy carriers has important implications for individuals with a family history of the disorder and for genetic counseling. The aim of this study was to determine the frequency of carriers in a sample of the nonconsanguineous Brazilian population by denaturing high-performance liquid chromatography (DHPLC). ***Methods:*** To validate the method, we initially determined the relative quantification of DHPLC in 28 affected patients (DHPLC values: 0.00) and 65 parents (DHPLC values: 0.49–0.69). Following quantification, we studied 150 unrelated nonconsanguineous healthy individuals from the general population. ***Results:*** Four of the 150 healthy individuals tested (with no family history of a neuromuscular disorder) presented a DHPLC value in the range of heterozygous carriers (0.6–0.68). ***Conclusions:*** Based on these results, we estimated there is a carrier frequency of 2.7% in the nonconsanguineous Brazilian population, which is very similar to other areas of the world where consanguineous marriage is not common. This should be considered in the process of genetic counseling and risk calculations.

Introduction

Spinal muscular atrophy (SMA) is the 2nd most common lethal autosomal recessive disease. SMA affects approximately 1/6,000 to 1/10,000 live births [1–3] with a carrier frequency of 1/35 to 1/50 [3–6]. The disease is characterized by progressive muscle weakness and atrophy due to degeneration of motor neurons in the anterior horn of the spinal cord.

The SMA-determining gene, called the survival motor neuron gene (SMN), is present on chromosome 5q13 and has two homologous copies: *SMN1* and *SMN2*. More than 90% of SMA patients are associated with a homozygous deletion in the *SMN1* gene [7–10]. The copy number of both *SMN* genes varies greatly in the population. The majority of affected individuals has no copies of *SMN1*; most carriers have 1 copy of *SMN1*, and noncarriers have 2–4 copies of the gene.

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SMA is considered to be a panethnic disorder, although most published studies to date involve small and heterogeneous sample sets of restricted populations [4, 5, 11–14]. If the carrier frequency in a population is high, the carrier test for SMA is an important part of the process of genetic counseling. The American College of Medical Genetics has recently recommended population carrier screening for SMA [15].

Reports of carrier frequencies in populations vary: there are low frequencies in African Americans (1/91) [14] and in the geographic regions of Spain (1/125) [14], Northeast England (1/80) [16], Warsaw (1/70) [17], South China (1/63) [18] and Israel (1/62) [19]; intermediate frequencies in Ashkenazi Jews (1/46), Caucasians (1/37) [14], Chinese (1/42) [20] and in the regions of Italy (1/57) [21], Asia (1/56) [14], Korea (1/47–1/50) [6, 22], North Dakota (1/41) [23], Philadelphia (1/40) [24], Poland and Germany (1/35) [3, 4], and France (1/34) [5]; and high frequencies in Saudi Arabia and Iran (1/20) [25, 26].

Considering the importance of SMA and the lack of population data in Brazil, this study was carried out to determine the carrier frequency of *SMN1* deletions in a sample of the nonconsanguineous Brazilian population using denaturing high-performance liquid chromatography (DHPLC).

Subjects and Methods

Genomic DNA was isolated according to a standard protocol [27]. To amplify specifically the *SMN1* gene in the polymerase chain reactions (PCR), the 3′ ends of the primers were designed to finish on the *SMN1*-specific sequence in exon 7 at position 6 and intron 7 at position +2: the SMN1 forward primer is 5′-CCTTTTATTTTCCTTACAGGGTTTC-3′ and the SMN1 reverse primer is 5′-GATTGTTTTACATTAACCTTTCAACTTTT-3′. As the reference gene, we used exon 12 of the human serum albumin (ALB). Its forward primer is 5′-AGCTATCCGTGGTCCTGAAC-3′, and the reverse primer is 5′-TTCTCAGAAAGTGTGCATATATCTG-3′. Primer sequences were according to Lee et al. [6]. PCR were carried out in 50 μl reaction volume containing 5 μl dNTP (200 mM), 2 μl of each SMN1 primer (10 pM), 2.5 μl of each ALB primer (5 pM), 3 μl $MgCl_2$ (50 mM), 2.5 units Taq (Platinum®), 5 μl 10× buffer (50 mM) and 2 μl genomic DNA (100 ng), with the following cycling profile: 5 min denaturation at 95°C and 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, followed by 10 min of final extension step at 72°C. Reactions were carried out on a Gene Amp® PCR System 9700 (Applied Biosystems). Amplicons were checked by 2% agarose gel electrophoresis before DHPLC analysis.

For quantitative analysis, we initially determined the relative quantification of DHPLC in 28 affected patients and 65 parents with at least 1 child that possessed the homozygous deletion of exon 7. Following quantification, we studied 150 unrelated nonconsanguineous healthy individuals from the general population, aged between 23 and 68, who had no family history of neuromuscular disorders. Informed consent for DNA analysis was obtained from each subject. This study was approved by the ethical committee of the University Hospital of the School of Medicine of Ribeirão Preto, University of São Paulo (Ethical Committee document HCMRP 3079/2004).

All data were analyzed directly by DHPLC using the D.WAVE® 4.500 DNA-fragment analysis system (Transgenomic, San José, Calif., USA). We used ALB as a calibration sample. All tested samples were analyzed with the calibrator sample on every assay plate. Under nondenaturing conditions (50.8°C), the PCR products were eluted at 4.6 and 4.8 min, respectively. The elution profile of each amplicon is usually composed of 2 biphasic peaks. If no signal was detected for the PCR control (a sample without DNA), the data were analyzed. The results were expressed as the percentage of peak area for the *SMN1* and *ALB* genes as calculated by WAVEMAKER software. The mean *SMN1/ALB* ratio derived from controls was used to normalize the percentage of peak area for the *SMN1/ALB* ratios of each individual. This calculation was performed according to the formula: (*SMN1/ALB*) test sample/(mean *SMN1/ALB*) controls.

Table 1. Copy number of the *SMN1* gene in affected and carrier individuals

Subjects	*SMN1* copy number	RQ range
Patients (n = 28)	0	0.00–0.00 (0.00)
Parents (n = 65)	1	0.49–0.69 (0.60)

Relative quantification (RQ) values calculated by WAVEMAKER software. Figures in parentheses show means.

Results

The percentage of peak area, which is proportional to the amount of PCR product in the reaction, was calculated automatically. In the linear phase of the PCR amplification, we expected *SMN1/ALB* gene ratios to be approximately 1.0 for normal controls, 0.5 for parents of SMA patients, and 0.0 for SMA patients with homozygous deletion of the *SMN1* gene. The range of measured relative quantification in patients and carriers is shown in table 1. To estimate the frequency of carriers in the general population, we tested 150 nonconsanguineous healthy individuals, mostly from the southeast region of Brazil (table 2).

To validate the analysis, we determined the range of carrier gene dosage in 28 affected patients (0.00–0.00) and 65 parents (0.49–0.69) of SMA patients in whom the homozygous deletion of the *SMN1* gene was identified previ-

Table 2. Results of the estimation of *SMN1* copy number in 150 normal Brazilian individuals

SMN1 copies	RQ range	Subjects	Frequency, %
1	0.6–0.68 (0.66)	4	2.7
2	0.85–1.14 (0.97)	143	95.3
3	1.21–1.3 (1.25)	3	2
4	0.00–0.00	0	0
Total	–	150	100

Relative quantification (RQ) values calculated by WAVEMAKER software. Figures in parentheses show means.

ously. Among the 150 healthy individuals tested, who had no known family history of genetic or neuromuscular disease, we detected 143 individuals who showed values compatible with 2 *SMN1* gene copies (mean: 0.97; range: 0.85–1.14). Three individuals also showed values compatible with 3 *SMN1* gene copies (mean: 1.25; range: 1.21–1.3), and 4 individuals showed values compatible with 1 *SMN1* gene copy (mean: 0.66; range: 0.6–0.68). Therefore, the screening of 150 nonconsanguineous healthy individuals predicts a carrier frequency for *SMN1* deletion of approximately 1 in 37 Brazilians (2.7%).

Discussion

Identification of the heterozygous carriers of the *SMN1* deletion is important in genetic counseling for SMA, the second most lethal autosomal recessive disease [16].

In Brazil, to the best of our knowledge, there are no studies estimating the frequency of SMA carriers in the general population. Kim et al. [28] characterized the genotype of Brazilian patients with SMA, but they did not evaluate the carrier frequency of the deleted *SMN1* gene in nonrelated and healthy Brazilians with no family history of SMA.

To determine the carrier frequency in a sample of the Brazilian population using DHPLC, we initially determined the range of the carrier gene dosage in 65 parents of SMA patients (0.49–0.69). The homozygous deletion of the *SMN1* gene in these patients had been previously identified by PCR restriction fragment length polymorphism and was confirmed in this study by DHPLC (table 1). Next, we screened 150 nonconsanguineous, healthy, normal individuals with no known family history of genetic or neuromuscular disease and found 4 individuals with a single *SMN1* gene, resulting in a carrier frequency of 2.7% (1/37) for the tested population. This frequency is similar to populations in North Dakota (1/41) [23], Philadelphia (1/40) [24], Poland and Germany (1/35) [3, 4] and France (1/34) [5], and probably reflects the European origin of the population tested. This is the first report of SMA carrier frequency in a nonconsanguineous Brazilian population, and will help with genetic counseling for SMA. However, Brazil is a large country with many different ethnic origins, and the obtained data need to be confirmed in other regions of the country where the population may have different characteristics.

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