



D5S351 and D5S1414 located at the spinal muscular atrophy critical region represent novel informative markers in the Iranian population

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ABSTRACT

Spinal muscular atrophy (SMA) is a degenerative neuromuscular disease associated with progressive symmetric weakness and atrophy of the limb muscles. In view of the involvement of numerous point mutations and deletions associated with the disease, the application of polymorphic markers flanking the SMA critical region could be valuable in molecular diagnosis of the disease. In the present study, D5S351 and D5S1414 polymorphic markers located at the SMA critical region in the Iranian populations were characterized. Genotyping of the markers indicated the presence of six and nine different alleles for D5S351 and D5S1414, respectively. Haplotype frequency estimation in 25 trios families and 75 unrelated individuals indicated the presence of six informative haplotypes with frequency higher than 0.05 in the studied population. Furthermore, the D' coefficient and the χ^2 value for D5S351 and D5S1414 markers revealed the presence of linkage disequilibrium between the two markers in the Iranians. These data suggested that D5S351 and D5S1414 could be suggested as informative markers for linkage analysis and molecular diagnosis of SMA in the Iranian population.

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1. Introduction

Spinal muscular atrophy (SMA) is a severe neuromuscular disease characterized by degeneration of the alpha motor neurons of the spinal cord associated with progressive symmetric weakness and atrophy of the limb muscles (Dubowitz, 2009). Different phenotypes identified in this disease were usually classified into three groups according to the age of onset and severity of the clinical symptoms, i) acute Werdnig–Hoffmann (type I), ii) intermediate Werdnig–Hoffmann (type II), and iii) Kugelberg–Welander disease (type III) (Chen et al., 1999). Molecular investigations had indicated that all these three types of disease were linked to the SMA critical region on chromosome 5q13 (Brzustowicz et al., 1990; Melki et al., 1990a; Melki et al., 1990b). A large duplication in this region contains two centromeric and telomeric homologous copies of at least 4 genes including survival motor neuron (SMN), neuronal apoptosis inhibitory protein (NAIP), *p44* gene, a subunit of transcription factor *TFIIH* and *H4F5* (Lefebvre et al., 1995; Roy et al., 1995; Burglen et al., 1997; Scharf et al., 1998). Deletions of telomeric copy of *SMN* (*SMNt*) have been observed in approximately 95% of the SMA patients (Zeesman et al., 2002). However, deletions of other genes of SMA critical region have been found mainly in the severe forms of the disease (Velasco et al., 1996; McAndrew et al., 1997;

Scharf et al., 1998; Tsai et al., 2001; Feldkötter et al., 2002). Moreover, several subtle mutations which cause rare cases of the SMA disease have been reported in different populations (He et al., 2013; Zapletalová et al., 2007; Wirth, 2000).

The SMA disease seems to be among the most common genetic diseases worldwide with an estimation of 1 in 6000 to 1 in 10,000 live births and the carrier frequency from 1:40 to 1:60 (Ogino et al., 2002; Prior et al., 2010). The frequency of the disease has been reported to be relatively high in the Iranian population. A recent report indicated a very high incidence of the disease with carrier frequency of 1 in 20 in the Iranian population (Hasanzad et al., 2010). This indicated the high demand and the necessity of carrier detection and prenatal diagnosis of the SMA disease in the Iranian population.

The SMA gene region contains several polymorphic genetic markers including restriction fragment length polymorphisms (RFLPs) and short tandem repeats (STRs) (Wirth et al., 1993; Wirth et al., 1994; Chen et al., 2007). Polymorphic markers usually show various frequency and heterozygosity in different populations. Among the STRs present in the SMA gene region, D5S351 and D5S1414, which flank the critical region, could be used in molecular diagnosis of the SMA disease. However, no investigation has been performed on heterozygosity and allele frequency of these markers in the Iranian population. In the present study, we evaluated the polymorphism level and haplotype frequency of these two polymorphic markers in the Iranian population.

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2. Material and methods

2.1. DNA samples

Blood samples were collected from 75 healthy unrelated healthy individuals and 25 Trios families (150 individuals). The Trios families included unrelated parents and at least one child. All the individuals were from the Iranian population. Genomic DNA was extracted from peripheral blood lymphocytes by standard salting out procedure (Miller et al., 1988).

2.2. Genotyping

D5S351 (UniSTS: 8886) and D5S1414 (UniSTS: 149791) markers located on the SMA critical region were genotyped. The genomic position of these two markers is represented in Fig. 1. Amplification condition for D5S1414 marker was as follows: 5 cycles consisting of primary denaturation at 94 °C for 9 min, denaturation at 94 °C for 15 s, annealing at 60 °C for 15 s, and extension at 72 °C for 30 s followed by 20 cycles consisting of denaturation at 94 °C for 15 s, annealing at 55 °C for 15 s, and extension at 72 °C for 30 s, followed by an extension period of 5 min at 72 °C. Temperature cycling conditions for D5S351 marker were as follows: primary denaturation 5 min, 94 °C; denaturation 15 s, 94 °C; annealing 15 s, 59 °C; extension 30 s, 70 °C; 23 cycles; final extension 5 min, 72 °C. Each 50 μ L reaction contained 50 ng DNA, 5 μ L of 500 mM KCl, 100 mM Tris-HCl (pH 8.4), 1–2 μ L of 50 mM MgCl₂, and 2 μ L of 10 mM dNTP, 5 U SMQ-*Taq* DNA polymerase and 0.4 μ L of 10 pM each primer.

The amplified alleles were resolved by vertical gel electrophoresis through 10% non-denaturing polyacrylamide gel. The alleles were isolated and sequenced on an ABI 737 sequencer (Perkin Elmer/ABI, Life Technologies, Germany).

2.3. Statistical analysis

Allele frequency, observed and expected heterozygosity were calculated with GENEPOPE software (Raymond and Rousset, 1995). The matching probability (pM), power of discrimination (PD) and exclusion (PE), polymorphism information content (PIC) and typical paternity index (P_{typical}) were calculated by use of the PowerStats Microsoft Excel workbook template provided by promega cooperation (<http://www.promega.com/geneticidtools/powerstats/>).

The genotype data obtained from 25 trios families and 75 unrelated individuals were used to estimate the haplotype frequency by use of FBAT and PHASE programs, respectively (Rabinowitz, 2000; Marchini et al., 2006). We also used 2LD program for the estimation of linkage disequilibrium (LD) between D5S351 and D5S1414 markers in the studied population (Zhao, 2004).

3. Results

In the present study, two STR markers including D5S351 and D5S1414 located in the SMA critical region were genotyped. The allele frequency distributions and heterozygosity of the markers were determined. Moreover, several statistical values including matching probability (pM), power of discrimination (PD) and exclusion (PE), polymorphism information content (PIC) and typical paternity index (P_{typical}) were determined. As presented in Table 1, a total of 15 different alleles for the markers were observed including six for D5S351 and

Table 1

Allele frequency and statistical values estimated for D5S351 and D5S1414 markers in the Iranian population.

Allele (the number of repeats)	D5S351	D5S1414
	Freq	
12		0.007
13		0.040
14		0.153
15		0.087
16		0.127
17	0.307	0.280
18	0.053	0.073
19	0.393	0.113
20	0.027	0.120
21	0.120	
22	0.100	
Hobs	51%	52%
Hexp	55%	63%
<i>Forensic statistics</i>		
pM	0.132	0.060
PIC	0.68	0.82
PD	0.868	0.940
<i>Paternity statistics</i>		
PE	0.398	0.418
PIT	1.56	1.63

Hobs, observed heterozygosity; Hexp, expected heterozygosity; pM, matching probability; PIC, polymorphism information content; PD, power of discrimination; PE, power of exclusion; PIT, typical paternity index.

nine for D5S1414 marker. The allele frequencies ranged from 0.007 to 0.393. The observed heterozygosity of D5S351 and D5S1414 were 51 and 52%, respectively. The highest PIC and PD was observed in D5S1414 marker (PIC: 0.82; PD: 0.940).

The haplotype frequency of haplotype D5S351–D5S1414 estimated by FBAT and PHASE programs is shown in Table 2. The haplotypes 19–17, 19–16, 17–14, 17–17, 17–18 and 19–20 were shown the frequency >0.05, indicating these haplotypes could be introduced as informative haplotypes of D5S351–D5S1414 in the Iranian population.

The D' coefficient for haplotype D5S351–D5S1414 was estimated 0.396. The χ^2 value for this haplotype obtained by means 2LD program was 82.43 (df = 40), revealing that the estimated χ^2 value was higher than χ^2 value obtained by using χ^2 table ($p < 0.05$). The obtained results were supported from partially linkage between these two markers in the Iranian population.

4. Discussion

The purpose of the present study was to investigate the allele frequency and haplotype estimation of two short tandem repeat (STR) markers, D5S351 and D5S1414 in the Iranian population. The data indicated that two studied STRs could suggest as informative markers in linkage analysis and molecular diagnosis of SMA mutations in the Iranian population.

The data showed the presence of six different alleles for the D5S351 marker in the studied population. The identified alleles were corresponded to 17, 18, 19, 20, 21 and 22 repeats of CA sequence (Table 1). Also, nine alleles were identified for D5S1414 marker including 12, 13, 14, 15, 16, 17, 18, 19, 20 repeats of the CA sequence. The most common allele for D5S351 and D5S1414 marker were 19 and 17, and

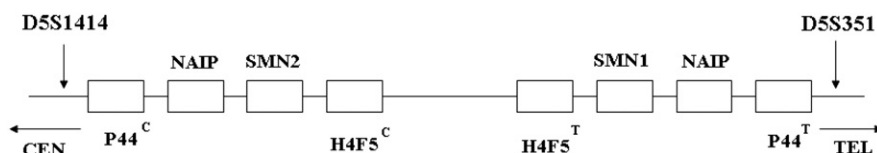


Fig. 1. Diagrammatic representation of the location of D5S351 and D5S1414 markers at the SMA critical region. CEN, centromere; TEL, telomere; NAIP, Neural inhibitory apoptosis protein.

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