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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.

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 μL reaction contained 50 ng DNA, 5 μL of 500 mM KCl, 100 mM Tris–HCl (pH 8.4), 1–2 μl of 50 mM MgCl2, and 2 μL of 10 mM dNTP, 5 U SMQ-*Taq* DNA polymerase and 0.4 μL 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 sofware (Raymond and Rousset, 1995). The matching probability (pM), power of discrimination (*PD*) and exclusion (*PE*), polymorphism information content (*PIC*) and typical paternity index (*PItypical*) 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 disquilibrium (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 (*PItypical*) were determined. As presented in Table 1, a total of 15 different alleles for the markers were observed including six for D5S351 and 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).

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	
*H*obs	51%	52%
*H*exp	55%	63%
Forensic statistics		
pM	0.132	0.060
PIC	0.68	0.82
PD	0.868	0.940
Paternity statistics		
PE	0.398	0.418
PIT	1.56	1.63

*H*obs, observed heterozygosity; *H*exp, expected heterozygosity; pM, matching probability; PIC, polymorphism information content; PD, power of discrimination; PE, power of exclusion; PIT, typical paternity index.

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

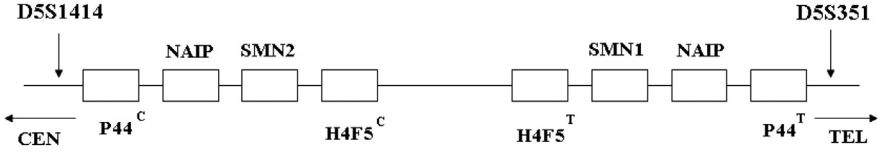

Fig. 1. Diagrammatic representation of the location of D5S351 and D5S1414 markers at the SMA critical region. CEN, centromer; TEL, telomere; NIAP, Neural inhibitory apoptosis protein.

Table 2
The frequency of D5S351–D5S1414 haplotypes estimated by means of FBAT and PHASE programs in the Iranian population.

D5S351–D5S1414 haplotype	FBAT frequency	PHASE frequency
19–17	0.130000	0.141453
19–16	0.085000	0.087432
17–14	0.080000	0.081057
17–17	0.070000	0.062256
17–18	0.070000	0.057010
19–20	0.050000	0.065244
21–14	0.045000	0.037837
19–14	0.045000	0.031105
17–20	0.040000	0.034700
19–19	0.035000	0.035859
21–17	0.030000	0.029241
19–15	0.030000	0.024416
22–17	0.030000	0.054047
21–19	0.030000	0.015131
17–13	0.030000	0.022134
21–15	0.025000	0.022569
17–16	0.025000	0.011085
18–17	0.020000	0.029670
17–19	0.015000	0.024010
21–20	0.010000	0.016722
18–19	0.010000	0.013381
22–19	0.010000	0.011619
17–12	0.010000	0.008333
19–18	0.010000	0.015974
18–16	0.010000	0.008333
20–16	0.010000	0.008333
21–16	0.010000	0.018150
17–15	0.010000	0.016081
18–13	0.005000	0.004348
18–15	0.005000	0.002600
19–13	0.005000	0.006851
21–18	0.010000	0.002016
22–15	0.000000	0.001001

the alleles with 20 and 12 CA repeats had the lowest frequency, respectively (Table 1). The heterozygosity of the D5S351 and D5S1414 markers were almost 50%, indicating these two markers were suitable in linkage analysis status in SMA families (Table 1). The discrimination power of these markers was very high. Therefore, these markers could also be used to distinguish between two unrelated people, suggesting that these markers could be applicable in forensic testing and individual identification in the Iranian population. Although, the markers have not been reported to be used in forensic testing in other populations yet, however, in view of the present data, they could be suggested to be examined as possible informative markers for other populations. Obviously, their power of discrimination needs to be tested in other populations before their application as useful markers in forensic testing.

Among 54 estimated possible haplotypes for D5S351–D5S1414, thirty three haplotypes was found in the studied population. Six haplotypes, including 19–17, 19–16, 17–14, 17–17, 17–18 and 19–20, showed the frequency >0.05 in the studied population. These haplotypes were introduced as informative haplotypes for D5S351–D5S1414 in the Iranian population. In contrast, haplotypes such as 22–17, 21–19, 17–16 and 21–18 which showed different frequency using FBAT and PHASE with at least in one program <0.05, could not be considered as informative. The estimation of D′ coefficient and χ^2 value of D5S351–D5S1414 revealed the presence of linkage disequilibrium between the two STR markers. However, these markers were not in complete linkage disequilibrium. The obtained data from D5S351–D5S1414 haplotypes study could be utilized as advantageous tools in analysis of all transmission in molecular diagnosis SMA disease.

In this study, the investigation of D5S351 and D5S1414 polymorphic markers revealed that these two markers could be used as informative markers in molecular diagnosis of SMA disease in families with an affected child. Moreover, our results indicated that these markers are suitable in personal identification for legal purposes among the Iranian population.

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References

Brzustowicz, L.M., Lehner, T., Castilla, L.H., Penchaszadeh, G.K., Wilhelmsen, K.C., Daniels, R., Davies, K.E., Leppert, M., Ziter, F., Wood, D., Dubowitz, V., Zerres, K., Hausmanowa-Petrusewicz, I., Ott, J., Munsat, T.L., Gilliam, T.C., 1990. Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q11.2–13.3. Nature 344, 540–541.

Burglen, L., Seroz, T., Miniou, P., Lefevbre, S., Burlet, P., Munnich, A., Pequignot, E.V., Egly, J., Melki, J., 1997. The gene encoding p44, a subunit of the transcription factor TFIIH, is involved in large-scale deletions associated with Werdnig–Hoffmann disease. Am. J. Hum. Genet. 60 (1), 72–79.

Chen, K.-L., Wang, Y.L., Rennert, H., Joshi, I., Mills, J.K., Leonard, D.G.B., Wilson, R.B., 1999. Duplications and de novo deletions of the SMNt gene demonstrated by fluorescence-based carrier testing for spinal muscular atrophy. Am. J. Med. Genet. 85, 463–469.

Chen, W.J., Wu, Z.Y., Lin, M.T., Su, J.F., Lin, Y., Murong, S.X., Wang, N., 2007. Molecular analysis and prenatal prediction of spinal muscular atrophy in Chinese patients by the combination of restriction fragment length polymorphism analysis, denaturing high-performance liquid chromatography, and linkage analysis. Arch. Neurol. 64 (2), 225–231.

Dubowitz, V., 2009. Ramblings in the history of spinal muscular atrophy. Neuromuscul. Disord. 19, 69–73.

Feldkötter, M., Schwarzer, V., Wirth, R., Wienker, T.F., Wirth, B., 2002. Quantitative analyses of SMN1 and SMN2 based on real-time LightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. Am. J. Hum. Genet. 70, 358–368.

Hasanzad, M., Azad, M., Kahrizi, K., Saffar, B.S., Nafisi, S., Keyhanidoust, Z., Azimian, M., Refah, A.A., Also, E., Urtizberea, J.A., Tizzano, E.F., Najmabadi, H., 2010. Carrier frequency of SMA by quantitative analysis of the SMN1 deletion in the Iranian population. Eur. J. Neurol. 17, 160–162.

He, J., Zhang, Q.J., Lin, Q.F., Chen, Y.F., Lin, X.Z., Lin, M.T., Murong, S.X., Wang, N., Chen, W.J., 2013. Molecular analysis of SMN1, SMN2, NAIP, GTF2H2, and H4F5 genes in 157 Chinese patients with spinal muscular atrophy. Gene 518 (2), 325–329.

Lefebvre, S., Burglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., Le Paslier, D., Frézal, J., Cohen, D., Weissenbach, J., Munnich, A., Melki, J., 1995. Identification and characterization of spinal muscular atrophy-determining gene. Cell 80 (1), 155–165.

Marchini, J., Cutler, D., Patterson, N., Stephens, M., Eskin, E., Lin, S., Qin, Z.S., Munro, H.M., Abecasis, G.R., Donnelly, P., International HapMap Consortium, 2006. A comparison of phasing algorithms for trios and unrelated individuals. Am. J. Hum. Genet. 78, 437–450.

McAndrew, P.E., Parsons, D.W., Simard, L.R., Rochette, C., Ray, P.N., Mendell, J.R., Prior, T.W., Burghes, A.H., 1997. Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. Am. J. Hum. Genet. 60 (6), 1411–1422.

Melki, J., Sheth, P., Abdelhak, S., Burlet, P., Bachelot, M.F., Lathrop, M.G., Frezal, J., Munnich, A., 1990a. Mapping of acute (type I) spinal muscular atrophy to chromosome 5q12-14. Lancet 336, 271–273.

Melki, J., Abdelhak, S., Sheth, P., Bachelot, M.F., Burlet, P., Marcadet, A., Aicardi, J., Barois, A., Carriere, J.P., Fardeau, M., Fontan, D., Ponsot, G., Billette, T., Angelini, C., Barbosa, C., Ferrier, G., Lanzi, G., Ottolini, A., Babron, M.C., Cohen, D., Hanauer, A., Clerget-Darpoux, F., Lathrop, M.G., Munnich, A., Frezal, J., 1990b. Gene for chronic proximal spinal muscular atrophies maps to chromosome 5q. Nature 344, 767–768.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

Ogino, S., Leonard, D.G., Rennert, H., Ewens, W.J., Wilson, R.B., 2002. Genetic risk assessment in carrier testing for spinal muscular atrophy. Am. J. Med. Genet. 110, 301–307.

Prior, T.W., Snyder, P.J., Rink, B.D., Pearl, D.K., Pyatt, R.E., Mihal, D.C., Conlan, T., Schmalz, B., Montgomery, L., Ziegler, K., Noonan, C., Hashimoto, S., Garner, S., 2010. Newborn and carrier screening for spinal muscular atrophy. Am. J. Med. Genet. A 152A, 1605–1607.

Rabinowitz, L., 2000. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. Hum. Hered. 50, 211–223.

Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.

Roy, N., Mahadevan, M.S., Mclean, M., Shutler, G., Yaraghi, Z., Farahani, R., Baird, S., Besner-Johnston, A., Lefebvre, C., Kang, X., Salih, M., Aubry, H., Tamai, K., Guan, X., Ioannou, P., Crawford, T.O., de Jong, P.J., Surh, L., Ikeda, J., Korneluk, R.G., MacKenzie, A., 1995. The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with SMA. Cell 80 (1), 167–178.

Scharf, J.M., Endrizzi, M.G., Wetter, A., Huang, S., Thompson, T.G., Zerres, K., Dietrich, W.F., Wirth, B., Kunkel, L.M., 1998. Identification of a candidate modifying gene for spinal muscular atrophy by comparative genomics. Nat. Genet. 20, 83–86.

Tsai, C.H., Jong, Y.J., Hu, C.J., Chen, C.M., Shih, M.C., Chang, C.P., Chang, J.G., 2001. Molecular analysis of SMN, NAIP and P44 genes of SMA patients and their families. J. Neurol. Sci. 190, 35–40.

Velasco, E., Valero, C., Valero, A., Moreno, F., Hernandez-Chico, C., 1996. Molecular analysis of the SMN and NAIP genes in Spanish spinal muscular atrophy (SMA) families and correlation between number of copies of cBCD541 and SMA phenotype. Hum. Mol. Genet. 5 (2), 257–263.

Wirth, B., 2000. An update of the mutation spectrum of the survival motor neuron gene (SMN1) in autosomal recessive spinal muscular atrophy (SMA). Hum. Mutat. 15 (3), 228–237.

Wirth, B., Voosen, B., Röhrig, D., Knapp, M., Piechaczek, B., Rudnik-Schöneborn, S., Zerres, K., 1993. Fine mapping and narrowing of the genetic interval of the spinal muscular atrophy region by linkage studies. Genomics 15 (1), 113–118.

Wirth, B., Pick, E., Leutner, A., Dadze, A., Voosen, B., Knapp, M., Piechaczek-Wappenschmidt, B., Rudnik-Schöneborn, S., Schönling, J., Cox, S., Spurr, N.K., Zerres, K., 1994. Large linkage analysis in 100 families with autosomal recessive spinal muscular atrophy (SMA) and 11 CEPH families using 15 polymorphic loci in the region 5q11.2-q13.3. Genomics 20 (1), 84–93.

Zapletalová, E., Hedvicáková, P., Kozák, L., Vondrácek, P., Gaillyová, R., Maríková, T., Kalina, Z., Jüttnerová, V., Fajkus, J., Fajkusová, L., 2007. Analysis of point mutations in the SMN1 gene in SMA patients bearing a single SMN1 copy. Neuromuscul. Disord. 17 (6), 476–481.

Zeesman, S., Whelan, D.T., Carson, N., McGowan-Jordan, J., Stockley, T.L., Ray, P.N., Prior, T.W., 2002. Parents of children with spinal muscular atrophy are not obligate carriers: carrier testing is important for reproductive decision-making. Am. J. Med. Genet. 107, 247–249.

Zhao, J.H., 2004. 2LD, GENECOUNTING and HAP: computer programs for linkage disequilibrium analysis. Bioinformatics 20, 1325–1326.