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ALLELE DISTRIBUTION OF D5S125, MAP1B5' AND D5S679 MICROSATELLITE MARKERS IN TURKISH SPINAL MUSCULAR ATROPHY FAMILIES*

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SUMMARY: Erdem H, Pehlivan S, Topaloğlu H, Togan İ, Özgüç M. (Department of Medical Biology and Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Allele distribution of D5S125, MAP1B5' and D5S679 microsatellite markers in Turkish spinal muscular atrophy families. Turk J Pediatr 1997; 39: 447-452.

Spinal muscular atrophy (SMA) is an autosomal recessive disease and one of the most common genetic causes of death in childhood. The gene for SMA has been mapped to chromosome 5q11.2-13.3. Chromosomal distribution of the alleles of D5S125, MAP1B5' and D5S679 polymorphic microsatellite markers in 14 unrelated Turkish SMA families have been determined. It is observed that the A9 allele of D5S679 has a significant (χ^2: 3.41 p: 0.065) non-random association with mutant chromosomes.
Key words: spinal muscular atrophy, microsatellite, polymorphism, non-random association.

Spinal muscular atrophy (SMA) is the second most common lethal, autosomal recessive disease in Caucasians that affects the α-motor neurons, leading to symmetrical weakness and wasting of voluntary muscles. There are three childhood onset forms of SMA: acute Werdnig-Hoffmann disease (type I), intermediate SMA (type II), and Kugelberg-Welander (type III)[1,2]. It has been shown that all three forms of the disease are due to mutations in the same region (5q11.2-q13.3)[3-6].

Recent studies have shown that this region contains multiple copies of genes and pseudogenes[7,8]. Two candidate genes, neuronal apoptosis inhibitory protein (NAIP) and survival motor neuron (SMN), have been reported as the cause of childhood SMA, and these genes frequently show homozygous deletions in SMA patients. The SMN gene is present in two copies within the SMA-candidate region. Single base substitutions in exons 7 and 8 distinguish the telomeric and the centromeric copy of SMN. The telomeric copy has been found to be deleted

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in 95-98 percent of all forms of the disease[9-13]. The NAIP gene shows deletions in about 50 percent of type I SMA patients and 18 percent of type II and III patients[14, 15].

On the other hand, several multicopy dinucleotide markers which show strong linkage disequilibrium with the disease have been identified and used for molecular typing of SMA families[16-20]. Genetic linkage analyses have demonstrated that with respect to CA-repeat markers, the SMA gene lies within the interval (4cM) between D5S125 and MAP1B5'. Microtubule-associated protein (MAP1B5') locus has two CA-repeat regions at 5' and 3' direction of the gene[21-23]. Microsatellites corresponding to these loci exhibit variability in their length because of the differences in their number of repeat units. D5S125, MAP1B5' and D5S679 have 2, 6 and 10 different alleles, respectively[24, 25].

Material and Methods

In 14 families, SMA was diagnosed according to the telomeric SMN gene (exons 7 and 8). Deletions and patients were classified by the standard criteria presented by the international SMA consortium[26]. The clinical classification of the 14 SMA families was of type I and II. These families were unrelated. To determine the haplotypes of the normal and mutant chromosomes with respect to D5S125, MAP1B5' and D5S679 loci, 10 ml blood samples were taken under consent from the parents and children. DNA samples from leukocytes were obtained by salt precipitation method[27].

Dinucleotide repeat polymorphisms were analyzed by polymerase chain reaction. Each genomic DNA sample (200 ng) was amplified in a 50 µl reaction consisting of 10 mM Tris, 200 µM dGTP, 200 µM dCTP, 200 µM dTTP, 200 µM dATP, 1.5 mM $MgCl_2$, 50 mM KCl, 110 pmol of each primer, and 2.5 units of Taq DNA Polymerase (Promega). After an initial denaturation at 94 °C for five minutes, the samples were cycled through 94 °C 1 min, 62 °C 1 min (D5S125 and MAP1B5'), 53 °C 1 min (D5S679) and 72 °C 1 min D5S125 and D5S679 PCRs were cycled 30 times and MAP1B5' PCRs were cycled 23 times. After cycling, the reactions were incubated for 7 min at 72 °C to ensure complete elongation of all products.

The primer sequences used for amplifications are:

D5S125:
F: AAG AGG ACT CCC ATG CTT GTT G
R: TAG AAA ATA CTA CAG TCT TCT TGG G

MAP1B5':
F: TTC CTT CTT CCA AAA CCA GGG TGA AGC CTC
R: AAA TTT CTA GGA TGC TTG CGG GAT CTC TTC

D5S679:
F: TTG CAT TTG GGA AAG AG
R: GCA ACT TCT TTA CTC TC

PCR products of D5S125 and MAP1B5' were analyzed on 12 percent acrylamide (19:1) nondenaturing gels (7h, 340 V) and D5S679 was analyzed on 6 percent acrylamide (19:1) denaturing gel (5h, 36 W). The results were visible with silver staining technique[28]. Gels are fixed by incubation in 10 percent ethanol, 0.5 percent acetic acid for three minutes twice, stained in 0.1 percent aqueous solution of silver nitrate for 15 minutes. After incubating the gel in 1.5 percent NaOH and 0.1 percent formaldehyde solution for 20 minutes, it is fixed with 0.75 percent solution of Na_2CO_3.

To detect the possible significant association between the mutant and normal chromosomes, a chi-square test was carried out[29].

Results

Consanguinity was observed in 64 percent of the families. This was taken into consideration in normalizing the allele counts. Normal and mutant chromosomes were analyzed with respect to D5S125, MAP1B5' and D5S679 markers. The haplotype of each individual was determined by the CA-repeat length patterns that each carries. As an example: in Figure 1, genetic structures of the individuals based on D5S679 locus is shown. The chromosomal distributions of the allele types of the markers and the results of the chi-square test are presented in Table I.

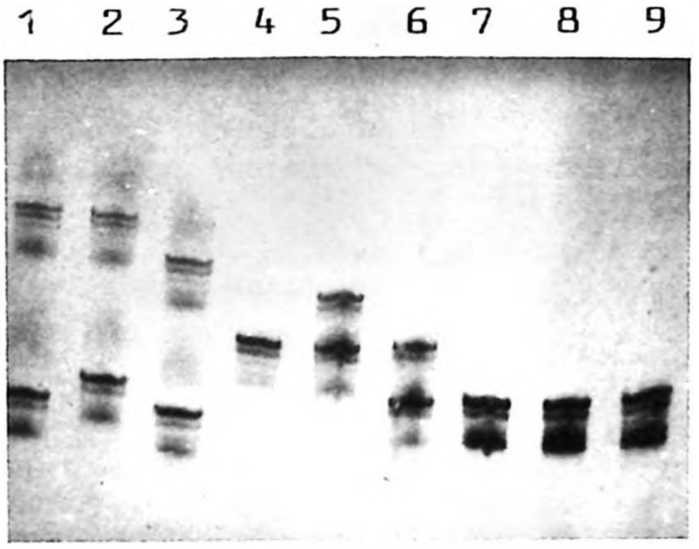

Fig. 1: Segregation of D5S679 polymorphism in 3 families with SMA.
Lane 1 (Patient 1): 4/10
Lane 2 (Father 1) : 4/9
Lane 3 (Mother 1): 5/10
Lane 4 (Patient 2): 7/7
Lane 5 (Father 2) : 6/7
Lane 6 (Mother 2): 7/9
Lane 7 (Patient 3): 9/9
Lane 8 (Father 3) : 9/9
Lane 9 (Mother 3): 9/9

Table I: Chromosomal Distribution of Microsatellite Allele Types and χ^2 Values Obtained by Chi-Square Test

Microsatellite Marker	Alleles	Allele Size (bp)	Normal Chromosomes	Mutant Chromosomes	χ^2 - Values
D5S125					
	A1	143	21	18	
	A2	147	7	3	0.083
MAP1B5'					
	A1	139	1	–	
	A2	137	4	3	
	A3	135	3	7	
	A4	133	9	7	
	A5	131	9	10	
	A6	129	1	1	1.05
D5S679					
	A1	222	–	–	
	A2	220	2	–	
	A3	212	3	–	
	A4	210	3	–	
	A5	208	–	–	
	A6	198	1	–	
	A7	196	4	4	
	A8	190	–	1	
	A9	184	11	15	
	A10	180	3	2	3.41*

*: Statistically significant (χ^2: 3.41 p: 0.065).

Eleven of the 14 families were informative for all three markers simultaneously. In Table II, distribution of the alleles of these three markers on normal and mutant chromosomes are given.

Table II: Haplotype Determination Using D5S679, D5S125 and MAP1B5' Markers

Family No.	Normal Chromosome	Mutant Chromosome
1	3-2-5	9-1-2
	9-1-1	9-1-5
2	9-1-5	9-1-3
	9-1-5	9-1-4
3	6-2-5	7-1-4
	9-2-3	7-1-2
4	7-1-4	7-1-5
	3-2-2	7-1-5
5	9-1-4	9-2-3
	7-1-4	9-1-5
6	4-1-5	9-1-5
	4-1-4	3-1-3
7	2-1-4	9-1-2
	9-2-2	9-1-3
8	9-2-5	9-1-3
	2-1-3	9-1-4
9	9-1-2	9-1-3
	10-1-3	9-1-4
10	4-1-4	10-2-5
	9-1-4	9-1-5
11	8-1-5	9-1-4
	9-1-5	9-1-5

Discussion

In a previous study, it was shown that 92 percent of Turkish SMA families had deletion type of mutations[13]. The main purpose of the present study is to define the haplotypes associated with SMN gene deletions in Turkish SMA patients using microsatellite markers.

The markers lie with the order of D5S679, D5S125/SMA/MAP1B5' from the centromeric to telomeric position. A1 of D5S125 and A5 of MAP1B5' are the most common alleles observed on both normal and mutant chromosomes. Allelic distributions of D5S125 and MAP1B5' markers on normal and mutant chromosomes are not significantly different from each other. The A9 allele of D5S679 is the predominant allele, with significant selection for the mutant chromosomes.

These results indicate that the markers used to analyze the allele segregations are polymorphic in the normal Turkish population and that a risk haplotype for the mutant alleles cannot be stated.

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