



## Analysis of the three STR loci (D16S539, D7S820, D13S317) in a population sample of Marmara region of Turkey

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### Abstract

In this study, allele and genotype frequencies for the short tandem repeat (STR) loci D16S539, D7S820, and D13S317 were investigated in 138 unrelated individuals (124 for D13S317, 125 for D7S820, 138 for D16S539) living in Marmara region by PCR technique using an allelic ladder consisting of these loci as a standard size marker. It was evaluated that these STR loci could be valuable for forensic and paternity identification and the results were compared with other population studies. These loci were simultaneously amplified according to *Gene Print™ Silver STR™* triplex kit (Promega Corp.). Amplified products were separated on 6% denaturing polyacrylamide gel, visualised by silver staining. Seven alleles (8-14) were identified for D16S539, eight alleles (7-14) for D7S820, and seven alleles (8-14) for D13S317. No new alleles were found. The population investigated showed no deviation from Hardy-Weinberg equilibrium in the loci D16S539, D7S820 and D13S317. Population data showed a combined discrimination power of 0.9993 and a combined power of exclusion of 0.9523.

**Key words:** D16S539, D7S820, D13S317, STR, DNA typing

### Türkiye, Marmara bölgesinden bir popülasyon örneğinde üç STR lokusunun (D16S539, D7S820, D13S317) analizi

#### Özet

Bu çalışmada, Marmara bölgesinde yaşayan toplam 138 (D13S317 için 124, D7S820 için 125 ve D16S539 için 138 birey) akraba olmayan bireyden oluşan bir örnekte D16S539, D7S820 ve D13S317 STR lokuslarının allel ve genotip frekansları PCR tekniğiyle allelik cetvel kullanılarak belirlenmiştir. Belirtilen STR lokuslarının bu bölgede kriminal amaçlı kimliklendirme ve akrabalık ilişkilerinin tespitinde kullanılabilirliği istatistiksel olarak değerlendirilmiş ve elde edilen sonuçlar farklı popülasyon çalışmaları ile karşılaştırılmıştır. Bu lokuslar, Gene Print™ Silver STR (Promega Corp.) kitine göre PCR, denatüre poliakrilamid jel elektroforezi ve gümüş boyama yöntemi ile analiz yapılmıştır. Çalışmada D16S539 lokusu için 7 allel (8-14), D7S820 lokusu için 8 allel (7-14) ve D13S317 lokusu için 7 allel (8-14) tanımlanmıştır. Yeni allel bulunmamış olup, popülasyonun bu lokuslarda Hardy-Weinberg Eşitliğine göre dengede olduğu görülmüştür. 3 STR lokusunun birlikte ayırma gücü 0.9993, dışlama gücü ise 0.9523 olarak hesaplanmıştır.

**Anahtar Sözcükler:** D16S539, D7S820, D13S317, STR, DNA tiplemesi

## Introduction

Short tandem repeat (STR) loci comprise a class of highly polymorphic loci in human genome (Edwards et al., 1991; Edwards et al., 1992). Modern methods of DNA polymorphism typing are now widely used in criminology for identifying people whose biological traces are material testimony. The use of polymerase chain reaction (PCR) for amplifying polymorphic DNA regions (especially microsatellite STR sequences) helps determining phenotypes of DNA loci series even in very small and old biological evidences (Schumm, 1996). It is possible to simultaneously amplify a few STR loci in one PCR reaction (Schumm, 1993). This is the so called PCR multiplex reaction mixture and simultaneous multi-amplification of a few DNA loci. Examinations are performed at a smaller cost of time and labour. There is also a decreased risk of contamination with foreign DNA as well as the cost of expertise.

There is no need for expensive equipment for detection as manual detection using silver stain can be used. The multiplex STR products were typed by electrophoretic separation in denaturing polyacrylamide gels and detection by silver staining (Lins et al., 1997; Sprecher et al., 1997). The *Gene Print*<sup>TM</sup> SilverSTR<sup>TM</sup> III multiplex system is comprised from three tetranucleotide repeat loci, D16S539, D7S820, and D13S317 (Lins et al., 1998; Bär et al., 1997). The repeat sequences for each locus are listed in Table 1.

Prior to the introduction of a new DNA profiling method a study of the allele frequencies and genotype distribution for the population needs to be undertaken. The aim of this work was to examine allele frequencies for STR loci D16S539, D7S820, and D13S317 (Green et al., 1991; Hudson et al., 1995) in a population sample living in Marmara region.

## Materials and methods

The subjects studied were 138 unrelated individuals (124 for D13S317, 125 for D7S820, 138 for D16S539), randomly selected from criminal cases, and DNA isolation from bloodstains, and single hairs was performed essentially as previously described (Walsh et al., 1991; Comey et al., 1994; Dissing et al., 1996). Quantitation of DNA samples was performed using the slot-blot hybridization of method of Waye et al. (1989) using the QuantiBlot, human DNA quantitation kit (Roche Molecular Systems, Alameda).

The SilverSTR III<sup>TM</sup> Multiplex kit (Promega Corp., Madison, WI) was used to amplify the loci D16S539, D7S820 and D13S317. The PCR contained 1 to 2 ng of DNA, 2.5 µL of STR 10X buffer, 2.5 µL Multiplex 10X primer pair mix, and 0.75 units of Taq polymerase. Sterile water was used to adjust to a final volume of 25 µL. Amplification was performed in a Perkin-Elmer Gene Amp PCR System 9700 for 30 cycles according to the manufacturer's recommendations (Technical manual. Gene Print<sup>TM</sup> SilverSTR<sup>TM</sup> systems-Silver Stain Detection. Part#TMD004. Promega Corp.). Electrophoretic separation of the amplified alleles was performed in vertical, denaturing, polyacrylamide gels (6%) using a Sequi Gen GT gel apparatus (BioRad). The gels were pre-run at 60 W for 30 to 45 min. in order to reach an approximate temperature of 50°C. Following electrophoresis, the DNA was detected by silver staining (Bassam et al., 1991). Alleles were determined by comparison with the allelic ladders included in the kit and were designated according to the number of repeat units. This allelic ladders, included in the kit, contain the alleles 5, 8 to 15 for locus D16S539; 6 to 14 for locus D7S820; and 7 to 15 for locus D13S317 (Promega Corp., Madison, WI).

In order to confirm the hypothesis that the genotype frequency distributions of the loci

**Table 1:** STR locus characteristics.

Locus	Repeat sequence		Chromosome location	Known alleles	Allele size range (bases)
	Edwards*	ISFH†			
D16S539	AGAT	GATA	16q24-qter	5,8-15	264-304
D7S820	AGAT	GATA	7q11.21-22	6-14	215-247
D13S317	AGAT	TATC	13q22-q31	7-15	165-197

\*Edwards et al. 1991; †the DNA Commission of the International Society for Forensic Haemogenetics (Bär et al., 1997).

D16S539, D7S820, D13S317 conform to for Hardy-Weinberg equilibrium, the observed and expected genotype frequencies were calculated. Then exact test was performed by using the computer program GDA for the HWE (Weir, 1992; Guo and Thompson, 1992; Lewis and Zaykin, 2001). Power of Discrimination (Kloosterman et al., 1993), Polymorphism Information Content (Botstein et al., 1980), Paternity Index (Brenner and Morris, 1990), Power of Exclusion (Wiegand et al., 1993) and Matching Probability (Puers et al., 1993) were also calculated by using the Powerstats Software (Tereba, 1999). Moreover, the observed genotype frequencies were compared with the published genotype frequencies of different populations using G-test. Comparison of allele distribution for different populations was carried out using 2-way RxC contingency table.

## Results

A total of 138 (124 for D13S317, 125 for D7S820, 138 for D16S539) unrelated individuals in Marmara region were used for population genetic analysis regarding the allele and genotype frequencies of the loci D16S539, D7S820, and D13S317 were determined. The allelic frequencies observed for each of the three STR loci in Marmara population sample are shown in Table 2. Seven alleles (allele 8-14) were identified for D16S539, eight alleles (allele 7-14) for D7S820, and seven alleles (allele 8-14) for D13S317. Microvariant alleles were not observed. The most frequent allele types for each locus were D16S539 (allele 12), D7S820 (allele 10), and D13S317 (allele 12). The most common frequency ranged from 0.256 (D7S820) to 0.322 (D13S317). The most frequent genotypes for each locus were D16S539 (genotype 11-12), D7S820 (genotype 10-11), and D13S317 (genotype 11-12). The genotype frequencies are presented in Table 3.

The results of statistical calculations and additional forensic data for the loci D16S539, D7S820 and D13S317 are shown in Table 4. The power of exclusion probability ranged from 0.554 (D16S539) to 0.707 (D7S820), the power of discrimination from 0.899 (D16S539) to 0.917 (D13S317), the matching probability from 0.101 (D16S539) to 0.083 (D13S317), and the observed

**Table 2:** Observed allele frequency values for the STR loci D16S539, D7S820, and D13S317 in Marmara region of Turkey.

Alleles	Allele frequencies					
	D16S539		D7S820		D13S317	
7	-	(4)*	0.012	(3)	-	(3)
8	0.014	(4)*	0.200	(50)	0.121	(30)
9	0.105	(29)	0.104	(26)	0.077	(19)
10	0.083	(23)	0.256	(64)	0.109	(27)
11	0.301	(83)	0.240	(60)	0.262	(65)
12	0.312	(86)	0.164	(41)	0.322	(80)
13	0.167	(46)	0.020	(5)	0.089	(22)
14	0.018	(5)	0.004	(1)	0.020	(5)
All	1.000	(276)	1.000	(250)	1.000	(248)

\*Observed number of alleles (in parenthesis).

heterozygosity from 0.775 (D16S539) to 0.856 (D7S820).

## Discussion

The loci D16S539, D7S820, and D13S317 do not deviate from Hardy-Weinberg Equilibrium based on exact test. The PIC values for the three STR loci were highly informative (PIC>0.5). The most informative locus is D7S820 (PIC=0.77). Evaluation for forensic purposes was carried out by calculating the discrimination power (DP) for identity testing, the power of exclusion (PE) for paternity testing. The data demonstrate that a significant degree of discrimination can be obtained (PD=0.9993) when all three loci are used to characterize forensic biological evidence; the power of exclusion (PE) reaches 0.9523 for the 3 loci together.

The observed genotype frequencies in the Marmara population sample were also separately compared with those from some other populations. The results of these analyses are presented in Table 5. The distribution of alleles of three loci in Marmara region was either different ( $p<0.05$ ) from or similar ( $p>0.05$ ) to those of the Uruguayan (Pagano et al., 2001), the Southern Italian (Baldassarra et al., 2001), and the Caucasian-Mestizos Colombian (Yunis et al., 2001) populations when analyzed by G-test. But,

**Table 3:** Observed genotype frequency values for the STR loci D16S539, D7S820, and D13S317 in Marmara region of Turkey.

Genotypes	Genotype frequencies					
	D16S539		D7S820		D13S317	
7-7	-		0.008	(1)	-	
7-10	-		0.008	(1)	-	
8-8	-		0.016	(2)	0.016	(2)
8-9	-		0.056	(7)	0.032	(4)
8-10	0.007	(1)*	0.096	(12)	0.008	(1)
8-11	0.022	(3)	0.104	(13)	0.056	(7)
8-12	-		0.080	(10)	0.096	(12)
8-13	-		0.032	(4)	0.016	(2)
9-9	-		0.008	(1)	-	
9-10	0.014	(2)	0.056	(7)	0.008	(1)
9-11	0.063	(9)	0.040	(5)	0.016	(2)
9-12	0.084	(12)	0.032	(4)	0.064	(8)
9-13	0.035	(5)	0.008	(1)	0.032	(4)
9-14	0.007	(1)	-		-	
10-10	0.036	(5)	0.032	(4)	-	
10-11	0.022	(3)	0.152	(19)	0.048	(6)
10-12	0.029	(4)	0.128	(16)	0.096	(12)
10-13	0.022	(3)	-		0.048	(6)
10-14	-		0.008	(1)	0.008	(1)
11-11	0.072	(10)	0.064	(8)	0.088	(11)
11-12	0.196	(27)	0.056	(7)	0.177	(22)
11-13	0.138	(19)	-		0.048	(6)
11-14	0.014	(2)	-		-	
12-12	0.101	(14)	0.016	(2)	0.080	(10)
12-13	0.101	(14)	-		0.016	(2)
12-14	0.007	(1)	-		0.032	(4)
13-13	0.014	(2)	-		0.008	(1)
13-14	0.007	(1)	-		-	
Homozygotes	31		18		24	
Heterozygotes	107		107		100	
Total samples	138		125		124	
P**(Exact test)	0.056		0.055		0.076	

\*Observed number of genotypes (in parenthesis); \*\*Exact test based on 3200 shufflings.

highly significant differences were observed with the Southern Indian (Panneerchelvam et al., 2001), and the Vietnamese (Budowle and Moretti, 1998) populations. As seen in Table 5, the population in this region is similar to General Turkish population

(Çakır et al., 2001).

The obtained forensic efficiency values demonstrated that the three STR loci (D16S539, D7S820, D13S317) can be used for personal identification and paternity cases in Marmara region.

**Table 4:** Statistical parameters of forensic interest for the STR loci D16S539, D7S820, and D13S317 in Marmara region of Turkey.

Statistical parameters	D16S539	D7S820	D13S317
Ho (Heterozygosity observed)	0.775	0.856	0.806
He (Heterozygosity expected)	0.769	0.801	0.789
PD (Power of Discrimination)	0.899	0.915	0.917
MP (Matching Probability)	0.101	0.085	0.083
PE (Power of Exclusion)	0.554	0.707	0.611
PIC (Polymorphism Information Content)	0.73	0.77	0.76
TPI (Typical Paternity Index)	2.23	3.47	2.58
Combined PD		0.9993	
Combined PE		0.9523	

**Table 5:** Comparison of different populations for the STR loci D16S539, D7S820, and D13S317.

Locus	Population compared	<i>n</i>	G-statistic	<i>df</i>	P-value
D16S539	Uruguayan	188	5.031	6	0.540
	Southern Italian	60	7.053	6	0.316
	Caucasian-Mestizos Colombian	199	10.562	7	0.159
	Southern Indian	65	41.632*	6	0.001
	Vietnamese	210	35.278*	7	0.001
	General Turkish	479	13.177*	6	0.040
D7S820	Uruguayan	188	6.871	7	0.442
	Southern Italian	60	4.668	7	0.700
	Caucasian-Mestizos Colombian	206	10.334	8	0.242
	Southern Indian	65	79.118*	7	0.001
	Vietnamese	212	28.456*	7	0.001
	General Turkish	479	6.884	7	0.441
D13S317	Uruguayan	188	15.316*	7	0.032
	Southern Italian	60	5.467	7	0.603
	Caucasian-Mestizos Colombian	205	30.256*	7	0.001
	Southern Indian	65	67.823*	7	0.001
	Vietnamese	211	77.238*	7	0.001
	General Turkish	479	3.055	8	0.931

*n*-number of individuals examined; *df*-degrees of freedom; \**p* <0.05.

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