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Allele distribution data for 16 short tandem repeat loci in Bolu

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Aim: To examine the short tandem repeat (STR) data of Bolu population and compare the data with previously published population studies and with the data of a neighboring province, Düzce (a former district of Bolu), which became a province after the earthquake in 1999.

Materials and methods: Blood samples were taken from 175 unrelated individuals. DNA was isolated using a DNA Kit and the amplification was performed using an AmpFℓSTR Identifier kit. Genotyping was carried out by an ABI Prism 310 genetic analyzer by using a reference ladder. Several parameters, such as allele frequencies, Hardy-Weinberg equilibrium, power of exclusion, power of discrimination, pairwise comparison, were calculated and correction test was used to confirm significant differences found in the comparative analysis.

Results: According to their power of exclusion and power of discrimination values, the most discriminating loci were D18S51 and D2S1338 whereas TPOX appears to be the least. The most discriminating loci and paternity index were found to be different in Bolu and its former district, Düzce, which is an interesting result.

Conclusion: The results indicate the importance of local population studies, because in regions where migration occurs and marriages between members of different ethnic groups are not socially acceptable, genetic data are affected.

Key words: DNA analysis, short tandem repeats, population genetics, Bolu, Turkey

Bolu'da 16 otozomal STR lokusunun allel dağılımı

Amaç: Bu çalışmanın amacı, Bolu populasyonunda STR verilerini belirlemek, daha önce yapılmış populasyon çalışmaları ve önceden kendisine bağlı bir ilçe iken 1999 depreminden sonra il olan Düzce'nin verileri ile karşılaştırma yapmaktır.

Yöntem ve gereç: Birbirleriyle akraba olmayan 175 kişinin kan örnekleri alınmıştır. DNA Kit kullanılarak DNAlar izole edilmiş ve AmpFℓSTR Identifier kit ile çoğaltılmıştır. ABI Prism 310 genetik analizörü ile referans dizi kullanılarak tiplendirme yapılmıştır. Allel sıklığı, Hardy Weinberg eşitliği, dışlama ve ayırt etme değerleri gibi farklı parametreler hesaplanmış ve karşılaştırmalı analizlerde belirgin farklılıkların doğruluğunun kontrolü için testler uygulanmıştır.

Bulgular: Dışlama ve ayırım gücü değerlerine göre, D18S51 ve D2S1338 lokuslarının ayrım gücü en yüksek, TPOX lokusunun ise en düşük lokuslar olduğu belirlenmiştir. Bolu ile eski ilçesi Düzce'nin en ayırt edici lokus ve paternite indeksi açısından farklı özelliklere sahip olduğunun ortaya çıkarılması ilginç bir bulgu olmuştur.

Sonuç: Elde edilen sonuçlar,özellikle göç yaşanan ve farklı etnik gruplar arasında kız alıp vermenin sosyal açıdan uygun görülmediği bölgelerde genetik verilerin etkilendiğini ve yerel populasyon çalışmalarının önemini göstermiştir.

Anahtar sözcükler: DNA analizi, kısa ardışık tekrarlar, populasyon genetiği, Bolu, Türkiye

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Introduction

Population Data

Short tandem repeat (STR) loci show variability among individuals in population and that makes these sequences important in genetic mapping, linkage analysis, identity testing in forensic cases, paternity testing, missing persons investigations, and mass disaster victim identification. In order to determine the probability of a particular genotype, population data must be gathered with a proper sample size to make an estimate of the frequency of each possible allele and genotype. The literature on STR allele frequencies contains over 1000 papers from various countries and population groups (1). However, population studies are not sufficient to make reliable interpretation about polymorphism of STR loci in Turkey. For this reason, it is necessary to perform population studies on a province and region level and collect all data in order to form a forensic database.

Bolu is a province in the northwestern part of Turkey. The province is between İstanbul and the capital city, Ankara. The known history of Bolu began with Bithynians, in 2000 BC. Later, Roman, Byzantine, Seljuk, and Ottoman Empires ruled the city. The area was affected by consistent migration because of being a neighboring province of İstanbul. The most important migration movements were from the Caucasia region in the second half of the 19th century. Most of the immigrants were settled mostly at the Düzce area in 1860s. Following the establishment of the Turkish Republic in 1920 and because almost half of the Turkish industry was set up there, this region heavily urbanized after 1950s (2).

Düzce (a former district of Bolu) became a province after the earthquake in 1999 to provide a better financial support. What makes this province socially distinctive is that a part of its population is made up of diverse subpopulations preserving their traditional structure due to the fact that marriages between members of these subpopulations are considered unacceptable. Determination of the genetic data of the region that has long and complex population dynamics have become important particularly for the forensic point of view.

Materials and methods

To characterize the patterns of STR variation in Bolu, first a statistically significant number of individuals were calculated. After obtaining informed consents, blood samples were taken from 175 unrelated individuals. Samples were placed on FTA® paper (Whatman Bioscience) and sent to Ankara University. DNA analyses of the samples were conducted in the Forensic DNA Laboratory in the Forensic Medicine Department of Ankara University.

DNA was isolated using the Bio Basic Inc. (Canada) UNIQ-10 Spin Column DNA Kit. The amplification of 15 STR loci was performed using AmpFℓSTR Identifier Kit according to the manufacturer's recommendation (Applied Biosystems, Foster City, CA, USA). Genotyping was carried out by ABI Prism 310 genetic analyzer using a reference ladder.

Allele frequencies were calculated by Power Stats V12 program (3) and Hardy-Weinberg equilibrium was assessed by means of Chi-square ($\chi 2$) test using POPGENE (version 1.32). The level of significance (P) was 0.05 for all analyzed loci. Pairwise comparison with different regional studies of Turkish, Northern Iraqi, Jordanian, Greek, Iranian and Russian populations were calculated by Fst values, according to Weir and Cockerham (4). Bonferroni correction test was used to confirm significant differences found in the comparative analysis (5-6).

Results and discussion

The observed allele frequencies for the 15 STR loci and results of forensic efficiency parameters for Bolu are shown in Tables 1 and 2. At each loci, a different number of alleles were observed with frequencies ranging between 0.003 (D19S433- allele 11) and 0.596 (TPOX-allele 8). The highest heterozygosity is observed for D18S51 whereas the smallest heterozygosity value is obtained for TPOX. All loci but FGA (0.0270) and TPOX (0.0147) met Hardy-Weinberg expectations ($P > 0.05$). After employing modified Bonferroni correction proposed by Jaccard and Wan (1996) for the number of loci analyzed, the

Table 1. Allele frequencies at 15 STR loci in Bolu population.

Allele	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA
6	-	-	-	-	-	-	-	-	-	-	-	-	0,311	0,011	-
7	-	-	-	0.009	0.026	-	-	-	-	-	-	-	0,226	-	-
8	-	-	-	-	0.167	0.026	0.177	0.026	-	-	-	-	0,080	0,596	-
9	0.051	-	-	0.017	0.106	-	0.069	0.157	-	-	-	-	0,194	0,109	-
9,3	-	-	-	-	-	-	-	-	-	-	-	-	0,174	-	-
10	0.269	-	-	0.074	0.277	0.043	0.051	0.109	-	-	-	-	0,015	0,049	-
11	0.260	-	-	0.343	0.206	0.063	0.337	0.268	0.009	0.003	-	-	-	0,209	-
12	0.360	-	-	0.383	0.189	0.057	0.326	0.209	0.117	0.091	-	-	-	0,017	-
13	0.060	-	-	0.157	0.020	0.283	0.031	0.211	0.180	0.280	-	-	-	0,009	-
13,2	-	-	-	-	-	-	-	-	-	0.026	-	-	-	-	-
14	-	-	0.060	0.017	0.009	0.354	0.009	0.020	0.180	0.411	-	-	-	-	0,149
14,2	-	-	-	-	-	-	-	-	-	0.023	-	-	-	-	-
15	-	-	0.240	-	-	0.120	-	-	0.094	0.074	-	-	-	-	0,126
15,2	-	-	-	-	-	-	-	-	-	0.040	-	-	-	-	-
16	-	0.020	0,354	-	-	0.043	-	-	0.183	0.026	-	-	-	-	0,154
16,2	-	-	-	-	-	-	-	-	-	0.026	-	-	-	-	-
17	-	0.177	0.177	-	-	0.011	-	-	0.134	-	-	0,009	-	-	0,323
18	-	0.143	0.169	-	-	-	-	-	0.046	-	-	-	-	-	0,151
19	-	0.177	-	-	-	-	-	-	0.040	-	-	0,066	-	-	0,097
20	-	0.069	-	-	-	-	-	-	0.017	-	-	0,129	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	0,174	-	-	-
22	-	0.031	-	-	-	-	-	-	-	-	-	0,149	-	-	-
23	-	0.220	-	-	-	-	-	-	-	-	-	0,213	-	-	-
24	-	0.051	-	-	-	-	-	-	-	-	-	0,131	-	-	-
25	-	0.109	-	-	-	-	-	-	-	-	-	0,089	-	-	-
26	-	0.003	-	-	-	-	-	-	-	-	-	0,040	-	-	-
27	-	-	-	-	-	-	-	-	-	-	0,040	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	0,057	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	0,157	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	0,315	-	-	-	-
30,2	-	-	-	-	-	-	-	-	-	-	0,026	-	-	-	-
31	-	-	-	-	-	-	-	-	-	-	0,071	-	-	-	-
31,2	-	-	-	-	-	-	-	-	-	-	0,197	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	0,003	-	-	-	-
32,2	-	-	-	-	-	-	-	-	-	-	0,117	-	-	-	-
33,2	-	-	-	-	-	-	-	-	--	-	0,017	-	-	-	-
Hom	53(30%)	24 (14%)	52(30%)	45(26%)	37(21%)	37 (21%)	45 (26%)	39(22%)	18 (10%)	50 (29%)	38(21,7%)	21(12%)	42(24%)	75(43%)	28(16%)
Het	122(70%)	151(86%)	123(70%)	130(74%)	138(79%)	138(79%)	130(74%)	136(78%)	157(90%)	125(71%)	137(78,3%)	154(88%)	133(76%)	100(57%)	147(84%)
Total	175	175	175	175	175	175	175	175	175	175	175	175	175	175	175

Table 2. Tests performed to determine the suitibility of markers for forensic and paternity studies.

	Forensic Statistics						Paternity Statistics			
Locus	MP	PD	PIC	CPD	PE	PI	CPE	Ho	He	$\chi 2$	P
CSF1PO	0,135	0,865	0,68	0,99999999999999997	0,424	1,65	0,999997	0,700	0,697	17,989	0,0551
D2S1338	0,057	0,943	0,83		0,720	3,65		0,863	0,863	35,258	0,0851
D3S1358	0,099	0,901	0,71		0,433	1,68		0,702	0,703	16,793	0,0790
D5S818	0,138	0,862	0,65		0,498	1,94		0,745	0,743	26,885	0,0995
D7S820	0,069	0,931	0,78		0,578	2,36		0,788	0,789	39,184	0,0780
D8S1179	0,089	0,911	0,74		0,578	2,36		0,760	0,789	50,705	0,0530
D13S317	0,117	0,883	0,70		0,498	1,94		0,743	0,743	31,887	0,0600
D16S539	0,071	0,929	0,77		0,557	2,24		0,709	0,777	32,612	0,0507
D18S51	0,047	0,953	0,84		0,790	4,86		0,900	0,897	60,104	0,0654
D19S433	0,100	0,900	0,70		0,451	1,75		0,800	0,714	57,629	0,0980
D21S11	0,059	0,941	0,79		0,557	2,24		0,782	0,777	61,247	0,0537
FGA	0,063	0,937	0,84		0,755	4,17		0,886	0,880	54,081	0,0270
THO1	0,087	0,913	0,74		0,527	2,08		0,761	0,760	18,983	0,0864
TPOX	0,228	0,772	0,54		0,258	1,17		0,567	0,571	27,771	0,0147
vWA	0,073	0,927	0,78		0,675	3,13		0,843	0,840	19,706	0,0998

MP; Matching Probability, PD; Power of Discrimination, PIC; Polimorphism Information Content, PE; Power of Exclusion, PI; Paternity Index, Ho; Observed Heterozygosity, He; Expected Heterozygosity, P; Probability of Homozygosity

departure observed at these loci was not significant. All loci but CSF and TPOX were more variable with expected heterozygosity greater than 0.7. The most discriminating loci according to their power of exclusion (PE) and power of discrimination (PD) values were D18S51 and D2S1338 whereas TPOX appears to be the least. The low discrimination power of TPOX is accounted for by the high frequency (0.596) of a single allele. Nonetheless, the combined power of discrimination of all 15 loci attains a value of 0.99999999999999997, which should be sufficient for the identification of any individual even for an extremely large population size. All 15 loci provide a combined probability of exclusion in non-paternity of 99.9%. Furthermore, observed and expected heterozygosity values are in good agreement for 12 of the 15 loci. In the former district Düzce, FGA and D21S11 were found to be the most discriminating loci according to PD and PE values (7).

While these 15 loci have routinely been used for forensic purposes, there are still very limited numbers of Turkish population studies. In one of them, D2S1338 and FGA were determined using AmpFℓSTR SGM Plus kit as the loci with the highest PD and PE (8). According to PD and PE values, locus TPOX was found to be the least discriminating locus for both provinces (Bolu; PD: 0.77-PE:0.28 and Düzce PD: 0.82-PE: 0.43). In some previous studies on subpopulations located in Turkey, these values were 0.81–0.39 and 0.83–0.34 and in 0.68 –0.41 for Kosovo Turks ((9–11).

The allele frequencies of Bolu were compared with Düzce and also with the published data of the 3 different regional studies of Turkish, Northern Iraqi, Jordanian, Greek, Iranian and Russian populations (Table 3) (7,11-19). D21S11 was found to be the most differentiated locus among all compared populations.

Table 3. Comparison of the allele frequencies of different population studies.

Loci	Population (n)									
	İstanbul (311)	Düzce (193) (173)	Marmara Region (116)	Eastern Turkey (950)	Turkey (200)	Northern Iraq	Jordan (95)	Greece (205)	Iran (150)	Russia (402)
CSF1PO	0.0677	NS	-	0.0247	-	NS	0.0308	0.0196	NS	-
D2S1338	-	NS	NS	-	-	NS	-	-	NS	-
D3S1358	-	0.0336	0.0356	NS	NS	NS	0.0483	0.0336	0.04498	0.02666
D5S818	-	NS	-	NS	NS	NS	NS	NS	0.02706	0.01998
D7S820	0.0466	NS	-	0.0167	0.0360	NS	-	0.0101	NS	NS
D8S1179	-	NS	NS	NS	0.0191	NS	0.0156	NS	NS	NS
D13S317	0.0154	NS	-	NS	NS	NS	NS	NS	0.01612	0.01910
D16S539	0.0210	NS	0.0180	0.0257	NS	-	0.0219	0.0165	NS	-
D18S51	-	NS	NS	NS	NS	-	NS	NS	NS	NS
D19S433	-	0.0171	0.0173	-	-	-	-	-	0.07616	-
D21S11	-	0.0277	0.0399	0.0334	0.0417	0.0105	0.0443	0.0271	_	0.01940
FGA	-	NS	NS	NS	0.0186	NS	NS	NS	NS	NS
THO1	0.0146	NS	0.0119	0.0186	-	NS	0.0287	0.0146	0.02148	-
TPOX	0.0186	NS	-	0.0192	-	NS	0.0358	0.0126	NS	-
VWA	NS	NS	NS	NS	0.02450	NS	NS	NS	NS	NS

NS: Not significant
(-): Not typed loci

According to the data of 13 CODIS STR loci from 11 European population groups, Budowle and Chakraborty emphasize that, Fst value of 0.01 is conservative for forensic applications. They found THO1 and D3S1358 as the most differentiated loci (20). The comparison between Bolu and Düzce populations revealed significant differences for D3S1358, D19S433, D21S11 using the same fst value (Fst > 0.01). However, the comparison between İstanbul and Bolu populations revealed significantly different loci, CSF1PO, D7S820, D13S317, D16S539, THO1, and TPOX. However, in a study performed in the Eastern Anatolia Region of Turkey, no difference found for STR loci except THO1 and FGA (14).

It could be concluded that the AmpFℓSTR Identifiler Kit, except the TPOX locus, is suitable for forensic and paternity tests in this area. These results also indicate the importance of population studies with a statistically sufficient number of individuals especially, in regions where migration occurs. In addition, some economic, social, and political factors (particularly in societies where marriages between members of different social groups are not socially acceptable) affect the genetic data.

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