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Original scientific paper

Genetic Polymorphisms of 15 STR Loci within Turkish Student Population Living in Sarajevo, Bosnia and Herzegovina

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A B S T R A C T

Allele frequencies of 15 STRs included in the PowerPlex 16 System (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX and FGA) were calculated from the referent sample of 100 unrelated individuals of both sexes from Turkish student population living in Sarajevo, Bosnia and Herzegovina. Buccal swab, as a source of DNA, was collected from the volunteers from whom the informed consent form was obtained. DNA extraction was performed using QIAamp DNA Micro kit by Qiagen. DNA template ranging from 0.5 to 2 ng was used to amplify 15 STR loci by PCR multiplex amplification which was performed by using the PowerPlex 16 kit (Promega Corp., Madison, WI, USA) according to the manufacturer's protocol. The amplifications were carried out in a PE Gene Amp PCR System thermal cycler (Applied Biosystems) and capillary electrophoresis was carried out in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's recommendations. The frequency of each locus was calculated from the numbers of each observed genotype. Deviation from Hardy-Weinberg equilibrium and observed heterozygosity were calculated. Data were analyzed by using Microsoft Excel workbook template – Powerstats V12 and the power of discrimination (PD), power of exclusion (PE), as well as other population genetic indices for the 15 STR loci were calculated. Obtained results contribute to existing Turkish DNA database, as well as insight of differences and similarities in comparison to population of Bosnia and Herzegovina. In addition, 13 autosomal STR loci frequencies (D3S1358, TH01, D21S1 1, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSFIPO, Penta D, VWA, D8S1 179, TPOX, and FGA) were studied in 15 different worldwide populations (Turkish, Bosnian, Croatian, Serbian, Montenegrin, Macedonian, Albanian, Kosovan, Greek, Russian, Japanese, Korean, Lithuanian, Iraqi, Belarusian). For the proof of corresponding data, two different Turkish population STR data obtained from previously published articles were compared with our data and this showed that our data correspond to these 2 previously published data. Further, STR allele frequency data for 13 loci for each population were obtained from previous scientific articles and the allele frequencies and genetic diversity among the 15 sample populations were compared. In addition, even though the populations are from different nationalities, the STR data are similar among the geographically close populations. The phylogenetic tree established among worldwide populations and genetic distance values show a great affinity among the 15 populations. Our data is useful for anthropological and further comparative genetic studies of populations.

***Key words**: STR loci, allele frequencies, DNA typing, Turkish population data, polymorphisms, phylogenetic tree*

Introduction

STRs (Short tandem repeats) are the genetic markers which exist with different frequencies in different populations and population STR databases containing frequencies of STR loci are available to evaluate the DNA samples. They provide highly informative DNA data for the human identification purposes and comparative ge-

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Fig. 1. Geographical Locations of the 15 compared populations in the study.
1 – Turkish, 2 – Bosnian, 3 – Croatian, 4 – Serbian, 5 – Montenegrin, 6 – Macedonian, 7 – Albanian, 8 – Kosovan, 9 – Greek, 10 – Russian, 11 – Japanese, 12 – Korean, 13 – Lithuanian, 14 – Iraqi, 15 – Belarusian

netic studies among the populations. These STR markers have been in use for decent years in forensic genetics, parental testing, paternity testing, individual identification studies and comparative genetic analysis[1-3]. These markers are very useful in forensic and population genetics studies. They had proved their efficiency in terms of determination of relationships among related individuals and populations and they are especially valuable for the study of genetic relationships between closely related populations[4-5]. However, STRs can be used to investigate genetic similarities between the sampled population and other populations that are not from the same bio-geographical zone. Furthermore, a phylogenetic tree can be generated between different populations by using STR frequencies and they will provide an idea about historical human migrations associated to sample populations[6].

Bosnia and Herzegovina is located in Southeastern Europe region, on the Balkan Peninsula and according to the preliminary 2013 census data; its population totals about 3,8 million people. Its capital and largest city is Sarajevo with an estimated urban population of 440,000 people. For ages, population of Bosnia and Herzegovina has been influenced by different populations because of the historical solidarity. Presence of Ottoman Empire in this region is the one of the factors which influenced the structure of population in Bosnia and Herzegovina. Bosnian and Turkish populations share a history about 600 years and this historical fidelity influenced both populations for centuries. For this study, Turkish students who currently study at the International Burch University in Sarajevo, Bosnia and Herzegovina are randomly chosen as a sample of temporary Turkish population situated in Bosnia. This population study was carried out in order to compare this Turkish population primarily with the Bosnian and homeland Turkish populations. Additionally comparison was performed with other worldwide populations including Croatian, Serbian, Montenegrin, Macedonian, Albanian, Kosovan, Greek, Russian, Japanese, Korean, Lithuanian, Iraqi and Belarusian.

In this study, we used 15 STRs of PowerPlex 16 kit to provide the allelic distributions in the Turkish population and estimate their forensic parameters. In addition, a phylogenetic tree (Figures 2 and 3) was established among worldwide populations and genetic distance values were calculated among populations.

Materials and Methods

Population

Reference sample consisted of 100 unrelated healthy individuals of both sexes (38 females and 62 males) from Turkish population living in Bosnia and Herzegovina. These unrelated individuals mean not belonging to the

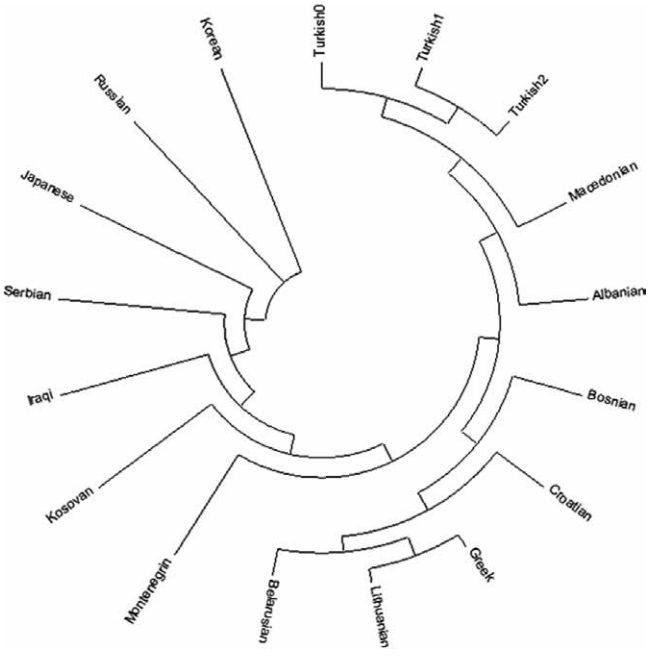

Fig. 2. NJ- Circular Phylogenetic Tree of 13 STRs for compared populations.

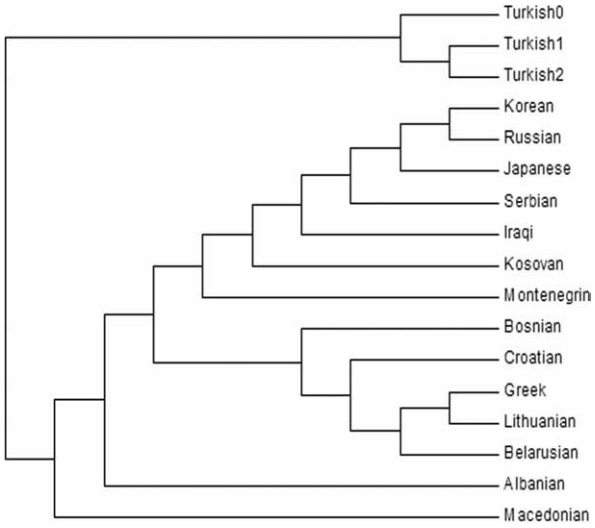

Fig. 3. NJ- Rectangular Phylogenetic Tree of 13 STRs for compared populations.

same nuclear family. Samples were collected from Turkish students from whom the informed consent form was obtained at the International Burch University in January 2013.

Sample preparation

Collected buccal swabs were used as a DNA source (N=100). All specimens were air-dried, placed in paper envelopes and stored at +4 °C until DNA extraction at the Molecular Biology Laboratory in International Burch University. After 3 weeks, samples were transported to the laboratory at the Institute for Genetic Engineering and Biotechnology for the further steps.

DNA extraction

Genomic DNA was extracted from buccal swabs by Qiagen extraction method[7] and DNA extraction was performed by using QIAamp DNA Micro kit (Qiagen, California, USA) and extracted DNA samples were stored at –20 °C until use.

PCR amplification (STR amplification)

Multiplex PCR amplification of 15 STR loci (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX and FGA) and sex determination locus Amelogenin was performed using the PowerPlex® 16 System (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. PCR was carried out in a 25 μL volume using; 2.5 μL template DNA (2.5 μL of 10X reaction Gold buffer, 1.0 μL of AmpliTaq Gold DNA polymerase, 2.5 μL of 10X primer pair mix and 16.5 μL nuclease-free water following the procedure recommended by the manufacturer with little modifications. PCR was performed using a GeneAmp® PCR System thermal cycler (Applied Biosystems) with the with the manufacturer's recommendations.

DNA typing (Capillary Electrophoresis)

PowerPlex 16 PCR amplicons were analyzed on ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Collected data were analyzed and allele designations and profiles were obtained by using GeneMapper® ID Software Version 3.2. Amplified alleles sizing was performed using the ABI 310 Genetic Analyzer according to the PowerPlex® 16 System PCR Amplification protocol. Internal Lane Standard (ILS) 600 that is provided in the kit was included with every sample to allow automatic sizing of alleles. All work flow for sample preparation and capillary electrophoresis was driven according to the recommendations of Promega and ABI.

Statistical analysis

Allele designations were determined by comparison of the sample fragments with those of the allelic ladders provided with the kit. At each locus the frequency of each allele was calculated from the number of each genotype in the Turkish sample set. Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosities were calculated. Data were analyzed by using Microsoft Excel workbook template-Powerstats V12[8] and the power of discrimination (PD), power of exclusion (PE), as well as other population genetic indices[9] for the 15 STR loci were calculated. Also, exact test of population differentiation was performed by Arlequin ver 3.5 (2010)[10].

Phylogenetic analysis

STR allele frequencies of 15 worldwide populations (Figure 1) were analyzed to calculate genetic distance values using neighbor-joining (NJ) program within Poptree2 software[11] (Table 1) and Phylogenetic tree

TABLE 1
COMPARED POPULATIONS IN THE STUDY

Populations	Sample Size	Reference
Turkish[0]	100	This Study
Turkish[1]	116	Cakir et al. 2003[13]
Turkish[2]	500	Yavuz and Sarikaya 2005[14]
Bosnian	100	Marjanovic et al. 2006[15]
lCroatian	195	Projic et. al. 2007[16]
Serbian	200	Veselinovic et. al. 2007[17]
Montenegrin	101	Jeran et. al. 2007[18]
Macedonian	100	Havas et. al. 2007[19]
Albanian	100	Robino et. al. 2001[20]
Kosovan	136	Kubat et al. 2004[21]
Greek	318	Kovatsi et al. 2006[22]
Russian	386	Stepanov et al. 2010[23]
Japanese	164	Hara et al. 2004[24]
Korean	452	Hong et al. 2013[25]
Lithuanian	300	Caplinskiene et al. 2011[26]
Iraqi	103	Barni et al. 2007[27]
Belarusian	176	Rebela et al. 2007[28]

was built using MEGA v5.1 software[12]. Also two different Turkish populations STR data obtained from previously published studies were compared with our Turkish data by using Poptree2 software and MEGA v5.1 software.

Results

All 100 individuals' DNA samples were successfully typed for 15 STR loci and amelogenin locus. Table 2 displays observed allele frequencies of 15 STR loci and ob-

TABLE 2
OBSERVED ALLELE FREQUENCIES IN TURKISH POPULATION SAMPLE LIVING IN BOSNIA AND HERZEGOVINA FOR 15 AUTOSOMAL LOCI (N=100)

Allele	D3S1358	THO1	D21S11	D18S51	PENT.E	D5S818	D13S317	D7S820	D16S539	CSF1P0	PENT.D	vWA	D8S1179	TPOX	FGA
5	–	–	–	–	0.060	–	–	–	–	–	–	–	–	–	–
6	–	0.270	–	–	0.005	–	–	–	–	–	–	–	–	–	–
7	–	0.135	–	–	0.145	0.005	–	0.040	–	–	0.005	–	–	0.005	–
8	–	0.155	–	–	0.005	0.010	0.120	0.160	0.025	0.005	0.010	–	0.025	0.490	–
9	–	0.250	–	–	0.010	0.065	0.100	0.080	0.135	0.035	0.185	–	0.020	0.095	–
9.3	–	0.165	–	–	–	–	–	–	–	–	–	–	–	–	–
10	–	0.025	–	0.005	0.075	0.115	0.075	0.255	0.080	0.220	0.150	–	0.095	0.120	–
11	–	–	–	0.005	0.110	0.255	0.315	0.255	0.280	0.305	0.210	–	0.070	0.240	–
12	–	–	–	0.160	0.160	0.375	0.295	0.185	0.285	0.375	0.155	–	0.115	0.050	–
13	–	–	–	0.145	0.125	0.170	0.080	0.020	0.175	0.055	0.175	0.005	0.290	–	–
14	0.075	–	–	0.155	0.050	0.005	0.010	0.005	0.020	0.005	0.085	0.075	0.200	–	–
15	0.235	–	–	0.165	0.050	–	0.005	–	–	–	0.020	0.110	0.150	–	–
15.2	–	–	–	–	–	–	–	–	–	–	–	0.005	–	–	–
16	0.270	–	–	0.100	0.055	–	–	–	–	–	0.005	0.220	0.025	–	–
17	0.215	–	–	0.120	0.065	–	–	–	–	–	–	0.285	0.005	–	–
18	0.185	–	–	0.065	0.045	–	–	–	–	–	–	0.195	0.005	–	0.005
19	0.015	–	–	0.035	0.025	–	–	–	–	–	–	0.085	–	–	0.055
19.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.010
20	0.005	–	–	0.025	–	–	–	–	–	–	–	0.020	–	–	0.100
20.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
21	–	–	–	0.005	0.005	–	–	–	–	–	–	–	–	–	0.230
21.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.015
22	–	–	–	0.015	0.005	–	–	–	–	–	–	–	–	–	0.110
22.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.010
23	–	–	–	–	0.005	–	–	–	–	–	–	–	–	–	0.130
23.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.005
24	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.130
25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.110
26	–	–	0.005	–	–	–	–	–	–	–	–	–	–	–	0.080
27	–	–	0.015	–	–	–	–	–	–	–	–	–	–	–	0.005
28	–	–	0.135	–	–	–	–	–	–	–	–	–	–	–	–
29	–	–	0.250	–	–	–	–	–	–	–	–	–	–	–	0.005
29.2	–	–	0.005	–	–	–	–	–	–	–	–	–	–	–	–
30	–	–	0.180	–	–	–	–	–	–	–	–	–	–	–	–
30.2	–	–	0.045	–	–	–	–	–	–	–	–	–	–	–	–
31	–	–	0.065	–	–	–	–	–	–	–	–	–	–	–	–
31.2	–	–	0.130	–	–	–	–	–	–	–	–	–	–	–	–
32	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
32.2	–	–	0.115	–	–	–	–	–	–	–	–	–	–	–	–
33.2	–	–	0.050	–	–	–	–	–	–	–	–	–	–	–	–
35	–	–	0.005	–	–	–	–	–	–	–	–	–	–	–	–

TABLE 3
HWE TEST FOR 15 STR LOCI IN TURKISH POPULATION SAMPLE LIVING IN BOSNIA AND HERZEGOVINA (N=100)

	D3S1358	THO1	D21S11	D18S51	PENT.E	D5S818	D13S317	D7S820	D16S539	CSF1P0	PENT.D	vWA	D8S1179	TPOX	FGA
He	0.78950	0.79849	0.85196	0.87573	0.90583	0.75161	0.78111	0.80573	0.78804	0.71724	0.84095	0.81101	0.82869	0.67975	0.87352
Ho	0.72000	0.80000	0.81000	0.88000	0.92000	0.85000	0.83000	0.78000	0.86000	0.66000	0.85000	0.87000	0.76000	0.68000	0.84000
PD	0.920	0.922	0.957	0.965	0.971	0.861	0.908	0.929	0.910	0.860	0.945	0.925	0.942	0.844	0.963
PE	0.460	0.599	0.618	0.755	0.836	0.695	0.656	0.562	0.715	0.369	0.695	0.735	0.527	0.398	0.675
MP	0.080	0.078	0.043	0.035	0.029	0.139	0.092	0.071	0.090	0.140	0.055	0.075	0.058	0.156	0.037
TPI	1.790	2.500	2.630	4.170	6.250	3.330	2.940	2.270	3.570	1.470	3.330	3.850	2.080	1.560	3.130
PIC	0.750	0.760	0.830	0.860	0.890	0.710	0.750	0.770	0.750	0.660	0.820	0.780	0.800	0.630	0.860
CPE 0.999474523															
CPD 0.9999999999999999957															

He – expected heterozygosity, Ho – observed heterozygosity, PD – power of discrimination, PE – power of exclusion, – MP – matching probability, TPI – typical paternity index, PIC – polymorphism information content, CPD – combined power of discrimination, CPE – combined power of exclusion

TABLE 4
NEI'S GENETIC DISTANCES AMONG COMPARED POPULATIONS

	Turkish[1]	Turkish[2]	Bosnian	Croatian	Serbian	Montenegrin	Macedonian	Albanian	Kosovan	Greek	Russian	Japanese	Korean	Lithuanian	Iraqi	Belarusian
Turkish[0]	0.001	0.009	0.022	0.016	0.032	0.020	0.014	0.018	0.021	0.015	0.056	0.048	0.300	0.021	0.022	0.019
Turkish[1]		0.000	0.014	0.009	0.026	0.010	0.007	0.005	0.013	0.008	0.056	0.036	0.290	0.016	0.013	0.008
Turkish[2]			0.015	0.011	0.025	0.014	0.009	0.011	0.016	0.010	0.060	0.038	0.295	0.015	0.015	0.012
Bosnian				0.013	0.028	0.020	0.017	0.018	0.017	0.012	0.054	0.059	0.311	0.013	0.029	0.015
Croatian					0.025	0.018	0.013	0.015	0.017	0.010	0.054	0.056	0.309	0.014	0.023	0.013
Serbian						0.031	0.029	0.030	0.029	0.023	0.066	0.068	0.321	0.028	0.039	0.027
Montenegrin							0.017	0.018	0.022	0.017	0.065	0.055	0.305	0.019	0.032	0.019
Macedonian								0.014	0.020	0.012	0.063	0.053	0.305	0.016	0.019	0.018
Albanian									0.022	0.015	0.058	0.051	0.304	0.019	0.024	0.018
Kosovan										0.013	0.055	0.056	0.311	0.016	0.028	0.016
Greek											0.049	0.052	0.306	0.009	0.023	0.011
Russian												0.097	0.351	0.048	0.077	0.053
Japanese													0.280	0.053	0.054	0.052
Korean														0.311	0.306	0.314
Lithuanian															0.031	0.012
Iraqi																0.030
Belarusian																

served heterozygosity, power of discrimination (PD), power of exclusion (PE), matching probability (MP), typical paternity index (TPI) and polymorphism information content (PIC) values are shown in Table 3. The most frequent allele among all allelic variants over all loci was the allele 8 at TPOX (0.490). The heterozygosity of the 15 STR loci studied in this study ranged from 0.66 to 0.92 (Table 3), indicating that high heterogeneity of alleles was detected. Thus, we can confirm that these markers have high value in forensic DNA analysis.

The comparison of the proportion of the observed heterozygosity across loci showed the highest value for PENTA E (0.920), and the lowest for CSF1P0 (0.660). No significant deviation from Hardy-Weinberg equilibrium was found for any of the observed loci. Table 3 shows that all markers have high PD values (>0.844). The highest values of PD and PIC were observed for PENTA E and the lowest ones were observed for TPOX. PENTA E was the most powerful marker for paternity testing with the highest values of PE and TPI and it was the most polymorphic marker with 18 alleles.

Discussion and Conclusion

As we have expected, comparison of our Turkish population STR data and previously published Turkish pop-

ulation STR data showed that our data correspond to these 2 previously published data[13,14]. As shown in phylogenetic tree and confirmed by genetic distances in Table 4 and Figures 2 and 3, Turkish population seems to be nearest to the Macedonian, Greek, Bosnian, Croatian and Albanian populations than the Russian, Korean and Japanese populations. Geographical proximity among the Turkish and Balkan countries and joint history could be essential explanations for this affinity. By comparison to the Turkish population and Russian, Korean and Japanese populations was relatively distant (Table 4 and Figures 2 and 3). Geographical constraints may explain this limitation among these populations. It could be concluded that results obtained in this study could contribute to existing Turkish DNA database, as well as insight of differences, similarities and genetic distances among the 15 worldwide populations. At the end we could easily conclude that Turkish population living in Bosnia and Herzegovina could be recognized as the genetically representative sample of homeland Turkish population.

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GENETIČKI POLIMORFIZMI 15 STR LOKUSA U POPULACIJI TURSKIH STUDENATA KOJI ŽIVE U SARAJEVU, BOSNA I HERCEGOVINA

S A Ž E T A K

Učestalost alela 15 STR-a uključenih u PowerPlex 16 System (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX i FGA) izračunate su iz reprezentativnog uzorka 100 nesrodnih pojedinaca oba spola iz populacije turskih studenata koji žive u Sarajevu, Bosna i Hercegovina. Bukalna sluznica, kao izvor DNK, uzeta je dobrovoljcima, nakon davanja informiranog pristanka. Izolacija DNK izvršena je pomoću QIAmp DNA Micro kit, proizvođača Quiagen. Korišten je DNK predložak, u rasponu od 0,5 do 2 ng, za amplificiranje 15 STR lokusa pomoću PCR multiplex metode i PowerPlex 16 kita (Promega Corp., Madison, WI, SAD), prema protokolu proizvođača. Amplifikacije su izvedene u PE Gene Amp PCR System termalnom cirkulatoru, a kapilarna elektroforeza na ABI PRISM 310 Genetic Analyzeru (Applied Biosystems), u skladu s preporukama proizvođača. Učestalost svakog lokusa izračunata je po broju svakog dobivenog genotipa. Izračunata su odstupanja od Hardy-Weinbergove ravnoteže i heterozigotnost. Podaci su analizirani koristeći Microsoft Exel Powerstats V12, a izračunata je diskriminacijska moć (PD), moć isključivanja (PE), kao i drugi populacijsko-genetički izračuni za 15 STR lokusa. Dobiveni rezultati pridonijeli su postojećoj bazi podataka DNK Turaka, kao i uvidu u razlike i sličnosti s populacijom Bosne i Hercegovine. Dodatno, istraženo je 13 autosomnih STR lokusa (D3S1358, TH01, D21S1 1, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSFIPO, Penta D, VWA, D8S1 179, TPOX i FGA) na 15 različitih svjetskih populacija (Turska, Bosna, Hrvatska, Srbija, Crna gora, Makedonija, Albanija, Kosovo, Grčka, Rusija, Japan, Koreja, Litva, Irak, Bjelorusija). Kao dokaz za podudarnost podataka, usporedili smo dvije različite već objavljene turske populacije STRa s našim podacima te je to pokazalo podudarnost naših rezultata i prethodno objavljenih rezultata. Nadalje, uspoređene su učestalost STR alela i genetička različitost unutar uzorka od 15 populacija. Dodatno, iako su populacije različitih nacionalnosti, STR analiza pokazala je sličnost između geografski bliskih populacija. Filogenetičko stablo postavljeno od svjetskih populacija i vrijednosti genetičke udaljenosti pokazuju veliki afinitet između 15 populacija. Naši podaci i rezultati korisni su za antropološke i daljnje komparativne genetičke studije populacija.