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Population Data for 15 Short Tandem Repeat Loci from Wallachia Region, South Romania

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Aim To determine allele frequencies' distribution for 15 short tandem repeat (STR) loci in a population sample of 1910 unrelated individuals from the region of Wallachia, South Romania.

Methods DNA was isolated using Chelex 100 method and an adapted version of AGOWA mag DNA Isolation Kit Sputum. Polymerase chain reaction amplification was done using AmpF*l*STR Identifiler kit (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA). For DNA typing, ABI PRISM 3100 Genetic Analyzer was used. Genetic parameters of forensic interest were calculated and comparisons with geographically close populations were performed.

Results With the exception of vWA locus ($P = 0.001$), no other significant deviations from Hardy-Weinberg expectations were found. Single locus comparisons with data on geographically close populations showed significant differences between the population of Wallachia and the population of Bucharest area, Greece, Turkey, Italy, Hungary, Belarus, and Poland, but no differences were found from the population from Croatia and Serbia.

Conclusion According to 15 analyzed STR loci, the population of Wallachia region was found to be genetically more similar to Slavic populations of Croatia and Serbia than to other surrounding populations.

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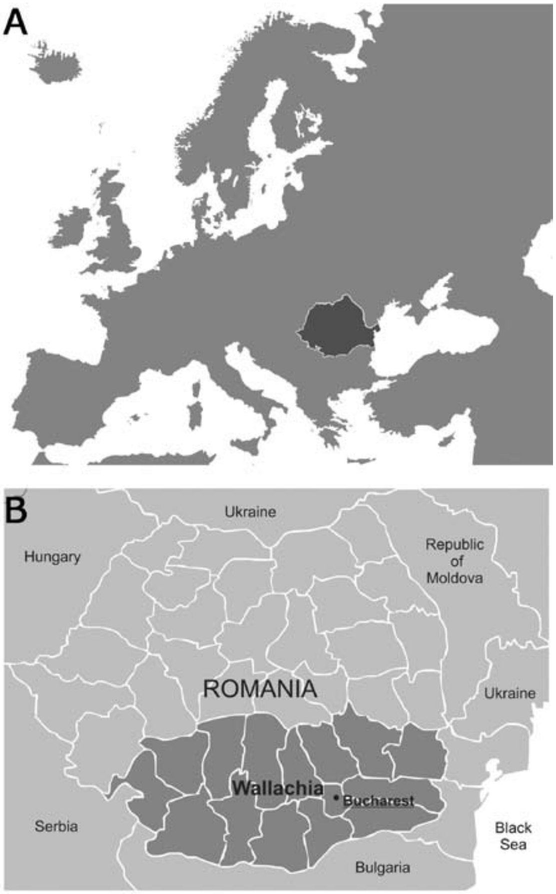
Short tandem repeats (STR) form approximately 3% of the total human genome and occur on average once in every 10 000 nucleotides (1). Due to the small dimensions and low mutation rate, these markers are intensely used in forensic and medical studies. With the growing number of laboratories which use STR marker technology, more and more population STR data are reported in Europe and all over the world, permitting regional population comparison.

Wallachia is a historical region situated on the northern side of Danube River and one of the 4 major geographical regions of Romania (Figure 1). It has approximately 6 879 709 inhabitants, 97% of whom are Romanians and 3% belong to other populations (Hungarians, Romany, Ukrainians, Germans, Russians, Turks, Tatars, Serbs, Croats, Slovenians, and others) (2). The present structure of Wallachian population is a result of many historical events such as Roman Empire conquests, the Slav migration from the north, and the Turkish southeast influence. To understand the historical influences that have contributed to the current population structure, we made allele frequency differentiations tests between the Wallachian and the surrounding populations. The obtained data are part of a larger study performed on the level of the entire country to determine the Romanians' genetic kinship relations in the European historical context.

Figure 1.



Romanian map. (**A**) Romania's location in Europe; (**B**) Wallachia's geographical location in Romania.

Beside the forensic and medical benefits of STR markers analysis, we find this kind of study very useful for further regional and worldwide human population meta-analyses.

MATERIAL AND METHODS

Population

We analyzed 1910 unrelated individuals from 14 of 15 counties in Wallachia region as follows: 259 samples from Argeş, 148 from Brăila, 10 from Buzău, 11 from Călăraşi, 215 from Dâmboviţa, 288 from Dolj, 114 from Giurgiu, 154 from Gorj, 117 from Ialomiţa, 113 from Ilfov, 122 from Mehedinţi, 20 from Olt, 331 from Prahova, and 8 from Teleorman. The region of the Romanian capital city, Bucharest, was excluded from the study. The samples for this study were collected from convicted offenders required to provide a DNA sample by Romanian Statute 76/2008, regarding the National DNA Database.

DNA analysis

DNA from 18 blood samples, 3 hair samples, and 1889 buccal swabs was extracted in 127 samples by Chelex 100 method (3) and in 1783 samples by an adapted version of AGOWA mag DNA Isolation Kit Sputum (AGOWA GmbH, Berlin, Germany) for Freedom Evo 150 Liquid Handling Platform (Tecan, Schweiz, AG, Switzerland) (4).

Approximately 1 ng target DNA was amplified using the commercial typing AmpF**l**STR Identifiler$^{\text{TM}}$ PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions, using a 9600 Thermal Cycler (Applied Biosystems)

Electrophoresis, detection of polymerase chain reaction products, and genotyping were carried out on the ABI PRISM 3100 Genetic Analyzer using Genescan and Genotyper Version 3.7 analysis software (Applied Biosystems).

TABLE 1. Allele frequencies and statistical parameters of 15 short tandem repeat loci in the Wallachia population (n = 1910)*

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
5	–	–	–	–	–	0.0003	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	0.2639	–	–	–	–	–	0.0003	–	–	–
7	–	–	0.0144	0.0003	–	0.1534	–	–	–	–	–	0.0021	–	0.0018	–
7.3	–	–	0.0003	–	–	–	–	–	–	–	–	–	–	–	–
8	0.0134	–	0.1482	0.0037	–	0.1204	0.1723	0.0115	–	–	–	0.4890	–	0.0008	–
9	0.0144	–	0.1584	0.0267	–	0.1955	0.0908	0.1194	–	0.0003	–	0.1128	–	0.0361	–
9.1	–	–	0.0008	–	–	–	–	–	–	–	–	–	–	–	–
9.3	–	–	–	–	–	0.2558	–	–	–	–	–	–	–	–	–
10	0.0657	–	0.2474	0.2636	–	0.0094	0.0565	0.1073	–	–	–	0.0589	0.0079	0.0772	–
10.2	–	–	–	0.0005	–	–	–	–	–	–	–	–	–	–	–
11	0.0835	–	0.2636	0.3280	–	0.0013	0.2704	0.3126	–	0.0037	–	0.3047	0.0113	0.2741	–
12	0.1029	–	0.1398	0.3157	0.0003	–	0.2974	0.2670	–	0.0976	0.0005	0.0317	0.1092	0.3997	–
12.2	–	–	–	–	–	–	–	–	–	0.0021	–	–	–	–	–
13	0.3094	–	0.0257	0.0518	0.0031	–	0.0893	0.1545	–	0.2874	0.0016	0.0005	0.1254	0.1990	–
13.2	–	–	–	–	–	–	–	–	–	0.0162	–	–	–	–	–
14	0.2414	–	0.0016	0.0086	0.0715	–	0.0225	0.0264	–	0.3024	0.1487	–	0.1715	0.0105	–
14.2	–	–	–	–	–	–	–	–	–	0.0335	–	–	–	–	–
15	0.1380	–	–	0.0008	0.2712	–	0.0008	0.0013	0.0005	0.1361	0.0932	–	0.1450	0.0008	–
15.2	–	–	–	–	0.0003	–	–	–	–	0.0401	–	–	–	–	–
16	0.0291	–	–	0.0003	0.2911	–	–	–	0.0374	0.0505	0.1929	–	0.1552	–	0.0008
16.2	–	–	–	–	–	–	–	–	–	0.0236	–	–	–	–	–
17	0.0024	–	–	–	0.1723	–	–	–	0.2042	0.0024	0.2940	–	0.1503	–	0.0005
17.2	–	–	–	–	–	–	–	–	–	0.0031	–	–	–	–	–
18	–	–	–	–	0.1764	–	–	–	0.1086	–	0.1908	–	0.0529	–	0.0123
18.2	–	–	–	–	–	–	–	–	–	0.0010	–	–	–	–	–
19	–	–	–	–	0.0134	–	–	–	0.0804	–	0.0668	–	0.0403	–	0.0921
19.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0003
20	–	–	–	–	0.0005	–	–	–	0.1539	–	0.0097	–	0.0154	–	0.1178
20.2	–	–	–	–	–	–	–	–	–	–	–	–	0.0003	–	0.0003
21	–	–	–	–	–	–	–	–	0.0262	–	0.0018	–	0.0110	–	0.1552
21.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0042
22	–	–	–	–	–	–	–	–	0.0238	–	–	–	0.0026	–	0.1665
22.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0147
23	–	–	–	–	–	–	–	–	0.1230	–	–	–	0.0010	–	0.1380
23.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0107
24	–	–	–	–	–	–	–	–	0.1165	–	–	–	0.0003	–	0.1631
24.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0024
25	–	0.0003	–	–	–	–	–	–	0.1105	–	–	–	0.0003	–	0.0822
25.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0016
26	–	0.0029	–	–	–	–	–	–	0.0131	–	–	–	0.0003	–	0.0291
26.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0010
27	–	0.0157	–	–	–	–	–	–	0.0013	–	–	–	–	–	0.0063
28	–	0.1285	–	–	–	–	–	–	0.0005	–	–	–	–	–	0.0010
29	–	0.2445	–	–	–	–	–	–	–	–	–	–	–	–	–
29.2	–	0.0024	–	–	–	–	–	–	–	–	–	–	–	–	–
30	–	0.1924	–	–	–	–	–	–	–	–	–	–	–	–	–
30.2	–	0.0505	–	–	–	–	–	–	–	–	–	–	–	–	–
31	–	0.0529	–	–	–	–	–	–	–	–	–	–	–	–	–
31.2	–	0.1018	–	–	–	–	–	–	–	–	–	–	–	–	–
32	–	0.0071	–	–	–	–	–	–	–	–	–	–	–	–	–
32.2	–	0.1505	–	–	–	–	–	–	–	–	–	–	–	–	–
33.2	–	0.0437	–	–	–	–	–	–	–	–	–	–	–	–	–
34.2	–	0.0063	–	–	–	–	–	–	–	–	–	–	–	–	–
35	–	0.0003	–	–	–	–	–	–	–	–	–	–	–	–	–
35.2	–	0.0003	–	–	–	–	–	–	–	–	–	–	–	–	–
Ho	0.7968	0.8434	0.7947	0.7047	0.7675	0.7780	0.7759	0.7722	0.8644	0.7801	0.7816	0.6240	0.8680	0.7062	0.8717
He	0.8040	0.8462	0.8020	0.7199	0.7758	0.7888	0.7890	0.7807	0.8728	0.7919	0.8048	0.6509	0.8705	0.7183	0.8722
P	0.236	0.188	0.499	0.062	0.138	0.967	0.217	0.845	0.468	0.769	0.001	0.011	0.940	0.060	0.095
MP	0.063	0.042	0.068	0.128	0.087	0.077	0.073	0.081	0.029	0.069	0.066	0.174	0.031	0.124	0.030
PD	0.937	0.958	0.932	0.872	0.913	0.923	0.927	0.919	0.971	0.931	0.934	0.826	0.969	0.876	0.970
PIC	0.78	0.83	0.77	0.67	0.74	0.76	0.76	0.75	0.86	0.76	0.78	0.59	0.86	0.67	0.86
PE	0.593	0.682	0.589	0.436	0.540	0.559	0.555	0.549	0.723	0.563	0.565	0.321	0.731	0.438	0.738
TPI	2.46	3.19	2.44	1.69	2.15	2.25	2.23	2.20	3.69	2.27	2.29	1.33	3.79	1.70	3.90

*Abbreviations: Ho – observed heterozygosity; He – expected heterozygosity; *P* – Hardy–Weinberg equilibrium exact test; MP – matching probability; PD – power of discrimination; PIC – polymorphism information content; PE – power of exclusion; TPI – typical paternity index.

TABLE 2. Population differentiation exact test probability values resulting from the comparison of Wallachia population with geographically close populations

	Wallachia vs (P value ± SE*)								
Locus	Bucharest (7)	Turkey (8)	Greece (9)	Italy (10)	Croatia (11)	Serbia (12)	Hungary (13)	Poland (14)	Belarus (15)
D8S1179	0.096 ± 0.011	0.001 ± 0.001†	0.194 ± 0.010	0.083 ± 0.011	0.348 ± 0.035	0.896 ± 0.006	0.001 ± 0.001†	0.016 ± 0.004†	–
D21S11	0.300 ± 0.023	0.416 ± 0.023	0.287 ± 0.017	0.018 ± 0.006†	0.069 ± 0.012	0.192 ± 0.020	0.001 ± 0.001†	0.001 ± 0.001†	–
D7S820	–	0.002 ± 0.001†	0.404 ± 0.023	0.120 ± 0.014	0.235 ± 0.017	0.042 ± 0.008	0.398 ± 0.021	0.164 ± 0.030	0.026 ± 0.008†
CSF1PO	–	–	0.956 ± 0.008	0.828 ± 0.011	0.758 ± 0.018	0.826 ± 0.012	–	0.223 ± 0.036	0.001 ± 0.001†
D3S1358	0.108 ± 0.017	0.462 ± 0.027	0.527 ± 0.025	0.231 ± 0.015	0.104 ± 0.015	0.858 ± 0.006	0.038 ± 0.010†	0.001 ± 0.001†	0.001 ± 0.001†
TH01	0.217 ± 0.022	–	0.431 ± 0.022	0.564 ± 0.022	0.842 ± 0.017	0.627 ± 0.011	–	0.002 ± 0.001†	0.001 ± 0.001†
D13S317	–	0.384 ± 0.015	0.276 ± 0.024	0.201 ± 0.013	0.528 ± 0.011	0.272 ± 0.009	0.084 ± 0.015	0.001 ± 0.001†	0.001 ± 0.001†
D16S539	0.154 ± 0.019	–	0.285 ± 0.023	0.036 ± 0.006†	0.114 ± 0.005	0.178 ± 0.014	–	0.001 ± 0.001†	0.001 ± 0.001†
D2S1338	0.142 ± 0.015	–	–	–	0.558 ± 0.022	0.170 ± 0.011	–	0.085 ± 0.022	–
D19S433	0.006 ± 0.001†	–	–	–	0.064 ± 0.011	0.778 ± 0.016	–	0.012 ± 0.002†	–
vWA	0.645 ± 0.024	0.100 ± 0.011	0.366 ± 0.022	0.017 ± 0.003†	0.081 ± 0.009	0.710 ± 0.015	–	0.007 ± 0.003†	0.001 ± 0.001†
TPOX	–	–	0.029 ± 0.009†	0.206 ± 0.015	0.295 ± 0.012	0.136 ± 0.008	–	0.034 ± 0.007†	0.001 ± 0.001†
D18S51	0.107 ± 0.022	0.001 ± 0.001†	0.088 ± 0.016	0.077 ± 0.010	0.115 ± 0.013	0.072 ± 0.011	0.001 ± 0.001†	0.043 ± 0.006†	0.001 ± 0.001†
D5S818	–	0.087 ± 0.011	0.164 ± 0.011	0.498 ± 0.020	0.443 ± 0.025	0.383 ± 0.012	0.165 ± 0.024	0.112 ± 0.016	0.001 ± 0.001†
FGA	0.534 ± 0.022	0.138 ± 0.013	0.542 ± 0.018	0.190 ± 0.015	0.838 ± 0.016	0.819 ± 0.015	0.014 ± 0.003†	0.007 ± 0.005†	–

*Standard error – an estimation of the error on the P value according to Arlequin software (5).
†Significant values ($P < 0.05$).

Statistical analysis

The Hardy-Weinberg equilibrium, expected heterozygosity, observed heterozygosity, and population differentiation tests were carried out with the Arlequin Software Version 3.1.1 (5). Matching probability, power of discrimination, polymorphism information content, probability of exclusion, and typical paternity index were calculated with a modified version of Powerstats Version 1.2 (6).

RESULTS AND DISCUSSION

The allele frequency distributions for the 15 STR loci studied in the Wallachia population and statistical parameters (Hardy-Weinberg equilibrium, expected heterozygosity, observed heterozygosity, matching probability, power of discrimination, polymorphism information content, probability of exclusion, and typical paternity index) are summarized in Table 1. No deviations from Hardy-Weinberg equilibrium were observed, with the exception of TPOX and vWA loci. After using the Bonferroni correction for the number of loci analyzed, only the departure observed at vWA locus was significant ($P < 0.05/15 = 0.003$). The expected heterozygosity and the power of discrimination calculated from the allele frequencies obtained for the Wallachia population revealed that in combination, the 15 STR loci have a high forensic efficiency.

Single locus comparisons with the data on geographically close populations (Table 2) showed significant differences between the population from Wallachia and the population from Bucharest area (7) at one locus – D19S433; between populations from Wallachia and Greece (9) at one locus – TPOX; between populations from Wallachia and Turkey (8) at 3 loci – D8S1179, D7S820, and D18S51; between populations from Wallachia and Italy (10) at 3 loci – D21S11, D16S539, and vWA; between population from Wallachia and Hungary (Budapest area) (13) at 5 loci – D8S1179, D21S11, D3S1358, D18S51, and FGA; between population from Wallachia and Belarus (15) at 10 loci – D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, vWA, TPOX, D18S51, and D5S818; and between population from Wallachia and Poland (14) at 11 loci – D8S1179, D21S11, D3S1358, TH01, D13S317, D16S539, D19S433, vWA, TPOX, D18S51, and FGA.

There were no significant differences between the population from Wallachia and Croatia (11) or Serbia (12), which means that Wallachia region is genetically more similar to Croatia and Serbia than to Greece, Turkey, Italy, Hungary, Belarus, and Poland population according to 15 analyzed STR loci. Difference between the Walachian region and Bucharest area can be a consequence of the high population heterogeneity, which in generally characterizes the metropolitan areas.

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