



## Short communication

## Genetic data from Y chromosome STR and SNP loci in Ukrainian population

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## ABSTRACT

We have tested a sample of 154 unrelated males from Lviv region (Ukraine) for 11 Y-chromosomal single nucleotide polymorphisms (SNPs) and 17 Y-chromosomal STR loci (DYS19, *DYS385a*, *DYS385b*, *DYS389I*, *DYS389II*, *DYS390*, *DYS391*, *DYS392*, *DYS393*, *DYS437*, *DYS438*, *DYS439*, *DYS448*, *DYS456*, *DYS458*, *DYS635*, *YGATA\_H4.1*). Haplotype and haplogroup diversity values were calculated for the population under study. Genetic distances ( $R_{ST}$ ) to 9 other Slavic populations were calculated based on 12 Y-STR loci. Haplotype frequencies and MDS plots were constructed based on genetic distances. Haplogroup frequency patterns revealed in Ukraine are similar to those characteristic of other European populations. However, it also allowed for identification a specific genetic component in Ukrainian sample which seems to originate from areas dwelled by Western Slavs, i.e. subhaplogroup R1a1a7, at frequency of 13.65%. Analysis of  $R_{ST}$  distances and AMOVA revealed high level of heterogeneity between Slavic populations inhabiting the south and north part of Europe, determined geographically rather than by linguistic factors. It has also been found a closer similarity (in the values of  $R_{ST}$ ) between Ukrainian and Slovak populations than between Ukrainians and other Slavic population samples.

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## 1. Population

Dried blood samples on paper were collected from 154 unrelated males from Lviv region (western part of Ukraine). The permission to perform this study was granted by the Bioethics Committee at the Collegium Medicum of the Nicolaus Copernicus University, Bydgoszcz, Poland (approval no. KB 466/2010).

## 2. DNA extraction

Total genomic DNA was extracted from blood samples by means of cell lysis in the presence of proteinase K and 2% SDS, followed by standard phenol/chlorophorm extraction.

## 3. PCR and typing

## 3.1. Y-STR analysis

Samples were amplified using AmpFISTR Yfiler™ kit including 17 loci (*DYS19*, *DYS385a*, *DYS385b*, *DYS389I*, *DYS389II*, *DYS390*,

*DYS391*, *DYS392*, *DYS393*, *DYS437*, *DYS438*, *DYS439*, *DYS448*, *DYS456*, *DYS458*, *DYS635*, *YGATA\_H4.1*) (Applied Biosystems) according to manufacturer's recommendations. Alleles were separated and detected using ABI 3130 capillary sequencer (Applied Biosystems). Haplotyping was performed using GeneMapper ID v.3.2 software (Applied Biosystems) according to manufacturer's recommendations. Alleles were named according to the published nomenclature and the updated ISFG guidelines for Y-STRs [1].

## 3.2. Y-SNP analysis

Haplogroup affinity was tested using selected SNP markers that are characteristic of major Eurasian haplogroups and subhaplogroups. The SNP markers used in the study are as follows: M9, M17, M35, M45, M89, M170, M172, M269, M458, P25, SRY10831.2. The SNP markers were assayed by SNaPshot analysis. Amplification of 11 Y-SNPs in two multiplex PCRs (MY-1, MY-R1) and subsequent single base extension were carried out according to Onofri et al. [2]. Additionally we extend the MY-R1 multiplex with a new SNP marker M458 (which is common for Slavic population) based on Underhill et al. study [3]. Detection of SNaPshot products and genotyping were carried out on the ABI 3130 capillary sequencer (Applied Biosystems) using the GeneMapper ID v3.2 software (Applied Biosystems). The Y-SNP haplogroup nomenclature used here complies with the recommendations of the Y Chromosome Consortium [4].

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**Table 1**  
Allele frequencies and gene diversity values at 17 AmpFISTR Yfiler loci in population sample from Lviv, Ukraine.

Allele	Loci															Haplotype	DYS385
	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	Y H4		
8									0.006							9–9	0.149
9					0.065				0.045	0.026						10–13	0.052
10					0.552				0.448	0.188				0.006		10–14	0.214
11					0.364	0.864			0.403	0.338				0.377		11–11	0.013
12		0.110			0.019	0.045	0.052		0.104	0.312				0.448		11–12	0.013
13	0.078	0.740				0.019	0.864			0.104		0.039		0.162		11–13	0.019
14	0.110	0.149				0.071	0.065	0.591		0.019		0.052	0.006	0.006		11–14	0.006
15		0.305					0.019	0.286		0.006		0.403	0.221			11–15	0.071
15/16	0.006															11–17	0.006
16	0.396								0.123			0.299	0.331			12–14	0.006
17	0.104											0.188	0.286			12–15	0.084
18											0.019	0.019	0.130			0–16	0.058
18.2											0.006					13–13	0.026
19											0.136		0.026			13–14	0.006
20											0.734			0.039		13–15	0.026
21				0.019							0.078			0.117		13–16	0.065
22				0.052							0.019			0.149		13–17	0.019
23				0.117							0.006			0.558		14–14	0.006
24				0.390										0.136		14–15	0.039
25			0.006	0.390												14–16	0.013
26				0.032												14–17	0.006
27																14–18	0.019
28			0.097													15–15	0.006
29			0.247													15–16	0.006
30			0.364													15–18	0.006
31			0.201													16–18	0.013
32			0.078													17–18	0.013
33			0.006													18–20	0.026
																18–18	0.006
GD	0.722	0.420	0.756	0.683	0.562	0.248	0.248	0.558	0.628	0.746	0.439	0.713	0.747	0.636	0.635		0.750

GD: gene diversity.

**4. Statistical analysis**

Arlequin software version 3.1 [5] was used to calculate allele and haplotype frequencies, gene diversities, haplotype diversity,  $R_{ST}$  distances and AMOVA. The statistical significance tests for  $R_{ST}$  were performed at 1000 permutations and for AMOVA at 20 000 permutations. Our population data were compared with the previously described neighboring European populations belonging to Slavic speaking group. These populations included Poland [6–8], Slovakia [8,9], Czech Republic [8,10], Croatia [11,12], Macedonia [13], Montenegro [14], Serbia [14,15], Belorussia [16] and Russia [17–19]. For those population samples data for twelve Y-STR loci were available (DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439). Genetic distances were obtained in the form of  $R_{ST}$  values and illustrated by creating a MDS plot using the software package STATISTICA v. 7.1 (StatSoft, Inc., Tulsa, USA).

Because some Ukrainian populations that are only available from the YHRD 3.0 [20] have been studied by means of 9 Y-STR loci (DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393), we have used an AMOVA tool of the YHRD to measure the genetic distance between populations ( $R_{ST}$  statistics).

**4.1. Quality control**

A quality control check was performed using the proficiency testing of the Y-STR Haplotyping Quality Assurance Exercise 2000 (YC000039). Haplotype data were submitted to YHRD and received the accession number YA003749.

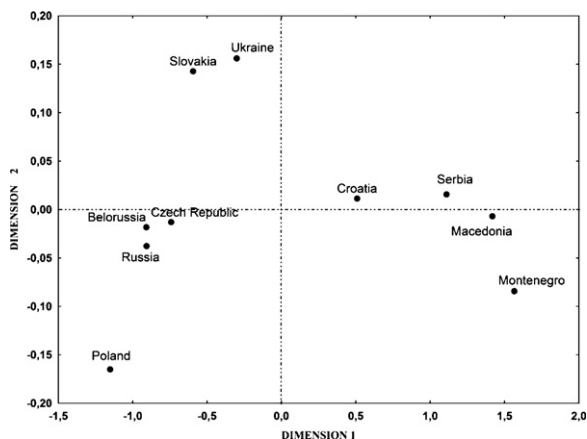
**5. Results**

Allele frequency distribution and gene diversity values calculated for each locus are listed in Table 1. Pairwise  $R_{ST}$  values

**Table 2**  
 $R_{ST}$  calculation for 12 Y-STR loci available for 10 Slavic populations.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1. Poland		+++	+++	+++	+++	+++	+++	+++	+++	++
2. Slovakia	0.018		+	+++	+++	+++	+++	+++	–	+
3. Czech Republic	0.019	0.005		+++	+++	+++	+++	+++	+++	+++
4.Croatia	0.139	0.067	0.096		+++	+++	+++	+++	+++	+++
5. Macedonia	0.242	0.140	0.170	0.048		–	–	+++	+++	+++
6. Montenegro	0.258	0.161	0.193	0.063	0.000		++	+++	+++	+++
7. Serbia	0.203	0.114	0.145	0.025	0.002	0.009		+++	+++	+++
8. Russia	0.011	0.010	0.015	0.109	0.204	0.218	0.167		+++	–
9. Ukraine	0.037	0.006	0.026	0.035	0.106	0.125	0.078	0.027		++
10. Belorussia	0.008	0.010	0.021	0.105	0.208	0.218	0.165	0.002	0.021	

Above the diagonal  $R_{ST}$  P values are indicated as “–”  $P > 0.05$ ; “+”  $0.05 > P > 0.005$ ; “++”  $0.005 > P > 0.001$ ; “+++”  $P < 0.001$ .



**Fig. 1.** MDS plot based on  $R_{ST}$  values for 12 Y-STR haplotypes showing relationships among 10 Slavic populations. Stress value for two dimensional MDS is 0.000603.

for the compared Slavic populations are presented in Table 2. Variations among the Ukrainian and other nine Slavic populations can be seen at the MDS plot (Fig. 1). Haplotype and haplogroup data of the Ukrainian population are given in Supplementary material, Table S1.

## 6. Other remarks

Ukrainians speak a language of Eastern Slavic subgroup of the Slavic language group. Ukrainian population is relatively poorly studied as far as Y chromosome STR-polymorphism is regarded. Previous studies have employed mainly low resolution approaches, based on the analysis of 5-loci Y-STR haplotypes only [21] or the minimal Y-STR haplotypes [20]. The main purpose of this study was to expand published Y chromosome genetic data from the Ukrainian population using the information from the new Y-SNP and Y-STR loci, as well to present some additional information about the Y-STR genetic relationships of Ukrainian populations with the populations from the neighboring Slavic peoples.

In the 154 Ukrainian samples analyzed in the study, a total of 147 different Y-STR haplotypes were observed. 140 haplotypes (95.24%) were unique, while the remaining 7 haplotypes appeared twice. In one sample we found a duplicated allele at DYS19 locus. We have also found intermediate alleles at loci DYS385a and DYS448 in two samples. Haplotype and haplogroup diversity values in our sample were 0.9995 and 0.7947, respectively. The highest gene diversity was observed for the single locus marker DYS389II ( $H = 0.756$ ) and for a two-locus system DYS385 ( $H = 0.750$ ).

Haplogroups that were shown to be present in Lviv population were (in the order of their relative frequency): R1a1/M17 (31.17%), I\*/+M170 (28.57%), R1a1a7/+M458 (13.65%), E1b1b1/+M35 (7.79%), R1b1/+P25 (5.19%), F\*/+M89 (4.54%), J2\*/+M172 (3.25%), R1b1a2/+M269 (2.59%) and K\*/+M9 (1.95%). The data indicated that Y chromosome haplogroup frequency patterns revealed in Ukraine are similar to those characteristic of other European populations [22]. However, it also allowed for identification a specific genetic component in Ukrainian sample which seem to originate from areas dwelled by Western Slavs, i.e. subhaplogroup R1a1a7 [3].

To analyze similarities and differences between our Ukrainian sample and other Slavic populations, the analysis of pairwise interpopulation genetic distances  $R_{ST}$  was performed (Table 2). Calculations as illustrated with the MDS plot (Fig. 1) has shown high level of heterogeneity between Slavic populations inhabiting the south and north part of Europe. The results of molecular variance analysis (AMOVA) based on variability of 12 Y-STR loci in Slavic populations grouped according to geographical or linguistic criteria (Table 3) has shown that the observed heterogeneity between southern and northern Slavic populations is determined geographically rather than by linguistic factors.

Comparing the results of this study to those described previously [6] it is interesting to note the position of Ukraine population on the MDS plot. In our study this position suggests closer similarity between Ukrainian and Slovak populations (non-significant  $R_{ST}$  value) than between Ukrainians and other Slavic population samples. Since Ukrainian sample studied here originated from western part of Ukraine, it is reasonable to analyze between-population relationships relying on population samples from other parts of the country. Database YHRD contains information on 9-loci Y-STR haplotypes in four Ukrainian populations representing western (Lviv, Uzhgorod) and eastern (Kiev, Lugansk) parts of Ukraine. Therefore, using the YHRD AMOVA tool we have performed analysis of variability of 9 STR loci in 5 Ukrainian populations and 5 neighboring populations from Belarus (Ivanava) [16], Poland (Lublin) [23], Slovakia (Bratislava) [6], Czech Republic (Central Bohemia) [10] and Russia (Orel) [24]. As seen (Table 4), all but one (Uzhgorod) Ukrainian population as well as Slovaks are clustered together, showing the lowest values of  $R_{ST}$  statistics.

These observations seem to challenge previous results obtained by Rębała et al. [6], where Ukrainian sample was indistinguishable in terms of  $F_{ST}$  distances from Russian, Belorussian, Polish and Slovak populations. However, it is worth noting that Ukrainian sample analyzed in the Rębała et al. study [6] was much smaller than our sample and comprised of haplotypes obtained only for 9 Y-STRs. This example emphasizes the need for further studies of Slavic populations, utilizing high-resolution approach, to elucidate subtle differences between those closely related groups.

**Table 3**

AMOVA calculation results with  $P$ -values (in parentheses) for 12 Y-STRs in 10 Slavic population samples.

Grouping criteria	Analysis of molecular variance (%)		
	Among groups	Among populations within groups	Within populations
A	–	<b>8.60</b> (0.00000 ± 0.00000)	<b>91.40</b> (0.00000 ± 0.00000)
B	<b>8.91</b> (0.00939 ± 0.00072)	<b>1.67</b> (0.00000 ± 0.00000)	<b>89.42</b> (0.00000 ± 0.00000)
C	<b>14.74</b> (0.00365 ± 0.00042)	<b>1.58</b> (0.00000 ± 0.00000)	<b>83.68</b> (0.00000 ± 0.00000)

The calculations were performed for: A – all Slavic populations grouped together; B – Slavic populations grouped on the basis of linguistic criteria: Eastern Slavs (Russia, Ukraine, Belorussia), Western Slavs (Poland, Czech Republic, Slovakia) and Southern Slavs (Croatia, Serbia, Macedonia, Montenegro); C – Slavic populations grouped on the basis of geographical criteria: Slavic nations living at the central and eastern part of Europe (Russia, Ukraine, Belorussia, Poland, Czech Republic, Slovakia) and southern part of Europe (Croatia, Serbia, Macedonia, Montenegro).

**Table 4**R<sub>ST</sub> calculation for 9 Y-STR loci available for 10 Slavic populations, including 5 Ukrainian samples.

	1	2	3	4	5	6	7	8	9	10
1. Bratislava (Slovakia)	–	+++	–	–	++	–	–	+	+	–
2. Central Bohemia (Czech Republic)	0.018	–	+	+++	+++	+++	++	++	++	+++
3. Ivanava (Belarus)	0.010	0.0449	–	–	–	–	–	–	++	+
4. Kiev (Ukraine)	–0.003	0.0334	0.0074	–	+++	–	–	+	++	+
5. Lublin (Poland)	0.024	0.0583	0.0075	0.0198	–	+	+++	+	+++	+++
6. Lugansk (Ukraine)	0.002	0.046	0.012	–0.003	0.017	–	–	+	++	+
7. Lviv-1 (Ukraine)	–0.004	0.018	0.024	0.002	0.042	0.007	–	++	–	–
8. Orel (Russia)	0.017	0.026	0.002	0.019	0.012	0.025	0.032	–	+++	+++
9. Uzhgorod (Ukraine)	0.023	0.032	0.073	0.039	0.104	0.044	0.007	0.082	–	–
10. Lviv-2 (Ukraine)	0.005	0.028	0.041	0.011	0.056	0.018	–0.003	0.052	0.002	–

Above the diagonal R<sub>ST</sub> P values are indicated as “–”  $P > 0.05$ ; “+”  $0.05 > P > 0.005$ ; “++”  $0.005 > P > 0.001$ ; “+++”  $P < 0.001$ . Lviv-1 – YHRD data (Population ID YP000230); Lviv-2 – present study.

All population data described in this paper has been uploaded to the YHRD [25]. This paper follows the guidelines for publication and population data requested by the journal [26].

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fsigen.2012.05.007>.

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