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MtDNA variation in three main ethnic groups in Tuzla Canton of Bosnia and Herzegovina

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Abstract

This study was based on the analysis of mtDNA polymorphisms in three ethnic groups of Tuzla Canton of Bosnia and Herzegovina (Bosniaks, Croats and Serbs). The main aim of this study was to analyze the influences of the maternal gene flow on the genetic profile of ethnic groups. The analysis of mtDNA variation based on relevant restriction fragment length polymorphisms (RFLP) in combination with HVSI variations of the control region enabled the identification of the Western-Eurasian haplogroups (H, I, J, T, W, U, HV, HVO, K, V, X), African/Near East lineages N1a and Asian haplogroup M. Our data indicate a close gene similarity among maternal gene pools of the ethnic groups of Tuzla Canton as well as similar influence of the maternal gene flow on genetic structure of those populations. The presence of important maternal determinants of the Late Glacial expansion (U5a), postglacial re-colonisation of Europe from refugia of southwestern Europe (H, V, U5b1), central-eastern European Plain (U4), Italian Peninsula (U5b3) and Neolithic expansion (U3, N1a, J, T) was noted in the genetic structure of the ethnic groups in Tuzla Canton. Conclusions in our study are consistent with the results of previous studies based on the distribution of mtDNA haplogroups and Y-chromosome haplogroups in three main ethnic groups of modern Bosnia and Herzegovina, suggesting similar effects of the paternal and maternal gene flows on genetic structure of the three main ethnic groups of modern Bosnia and Herzegovina.

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Introduction

Mitochondrial DNA (mtDNA) is very informative genetic tool of the population studies in estimation of the genetic differentiation of populations, as well as genetic origin and diversification of the maternal lineages (Chiara et al., 2017; Behar et al., 2012;

Malyarchuk et al., 2002, 2003, 2006, 2008, 2010; Palanichamy et al., 2010; Malyarchuk & Derenko 2001; Richards et al., 1998, 2000, 2002; Macaulay et al., 1999; Pala et al., 2009, 2012; Torroni et al., 1996, 2001; Cvjetan et al., 2004; Kivisild et al., 1999; Grzybowski et al., 2007). Classification of the mtDNA variations indicates temporal and spatial

haplogroup/haplotype affiliation of different maternal lineages. Therefore, the European populations predominantly contain haplogroups of the Western-Eurasian mtDNA phylogeny (H, I, J, K, T, U, V, W and X) (Macaulay et al., 1999; Richards et al., 1998; Torroni et al., 1996). Most of mtDNA lineages in Europe are marked as paleolithic (U5a, U5b, U5b1b, U5b3, U4, U2, U1, HV, H, H1, H3, H5, V) whose expansion in Europe is correlated with the Upper Palaeolithic and postglacial recolonization of Europe after the Last Glacial Maximum (LGM) (Behar et al., 2012; Pala, et al., 2009, 2012; Malyarchuk et al., 2008, 2010; Richards et al., 2000). Other significant haplogroups in Europe are of Neolithic origin from the Near East (N1, J, T1, U3, subhaplogroups of the H and the W) (Derenko et al., 2013; Pala et al., 2012; Palanichamy et al., 2010; Richards et al., 2000).

The region of Tuzla Canton belongs to northeastern part of Bosnia. This region is very specific due to the presence of different archeological sites from ancient and recent past, rich deposits of mineral ores as well as different influence of the migrations of human populations. Many archaeological investigations demonstrate the presence of the prehistoric period of the civilization from the Neolithic in this region (Perić, 2002). During history, the Tuzla Canton, the rest of Bosnia and Herzegovina as well as Balkan region were settled by different Illyrian and Thracians tribes, then Avars, Slavs (Wilkes, 1992; Živković, 2002), new tribes such as the Croats and the Serbs (Marjanović et al., 2005). The expansion of the Ottoman Empire in the fifteenth century greatly influenced the structure of the populations of Bosnia (Malcolm, 1994). Due to its wealth in minerals, the Tuzla region was attractive to immigrants from all over the world. Namely, it has a very important geographic position which is connected to wider region of the Southeast and Central Europe. Therefore, Tuzla region has always been marked by demographic changes, which probably had an important impact on genetic structure of the human populations. Nowadays, this region is multinational and multireligious with three main ethnic groups (Bosniaks, Bosnian Croats and Bosnian Serbs). According to the demographic data of the register of the Federal Office for Statistics of Bosnia and

Herzegovina for 2013, the Tuzla Canton has the total population of 445,028 individuals, of which 392,356 Bosniaks, 23,592 Croats, 7,058 Serbs and a small percentage of other ethnic groups (<http://fzs.ba/>).

The previous population genetic studies of ethnic groups in Bosnia and Herzegovina were conducted by Kovačević et al. (2014) and Marjanović et al. (2005). The study by Kovačević et al. (2014) was based on the analysis of autosomal and haploid lineages of the populations from the Western Balkans, including three ethnic groups from Sarajevo and Zavidovići (Bosnian Bosniaks), Central Bosnia (Bosnian Croats), southern Bosnia and Herzegovina (Bosnian Croats) and Central Bosnia and Banja Luka (Bosnian Serbs). Study by Marjanović et al. (2005) based on the analysis of the Y-chromosome haplogroups in the three ethnic groups in Bosnia and Herzegovina suggests that paternal gene pool of these three groups is predominantly marked with a contribution of the post-glacial expansions from different refuges (the Balkans, Ukraine and Franco-Cantabrian) with lesser impact of the neolithic and postneolithic expansions from the Middle East. Our study is based on the preliminary analysis of mtDNA data in the three main ethnic groups of Tuzla Canton, in order to test the hypothesis on a similar influence of the maternal gene flow on genetic profile of these groups in Tuzla Canton and similar contribution of the paternal and maternal gene flow on gene pool of the three main ethnic groups in Bosnia and Herzegovina.

Materials and methods

Data of 241 mtDNA samples from northeastern Bosnia (Ahmić et al., 2014) were used in the analysis of the haplogroup affiliation of individuals from different ethnic groups of Tuzla Canton (Bosnian-Bosniaks N=201; Bosnian-Croats N=22; Bosnian-Serbs N=18).

The ratio of the number of samples for the sampled groups reflects ethnic structure of Tuzla Canton population according to the data of the Federal Office of the Statistics of the Federation Bosnia and Herzegovina ([web:www.fzs.ba](http://www.fzs.ba)). The haplogroup identification of the mtDNA in the three ethnic groups of Tuzla Canton was based on the analysis of

relevant restriction fragment length polymorphisms (RFLP) in combination with variations in HVSI of the control region of the mtDNA (Table 1). HVSI sequences were aligned using BioEdit software through ClustalIW multiple alignment (Hall, 1999) and compared with the revised Cambridge reference sequence (Anderson et al., 1981; rCRS, Andrews et al., 1999). For the haplogroup classification, mitomaster software (www.mitomap.com) and the PhyloTree build 17 were applied (van Oven and Kayser, 2009). The analyses of the mtDNA variations in the three ethnic groups of Tuzla Canton

and its comparison with neighboring European populations from narrower geographic region were done using the methods implemented in the Arlequin ver.3.11 software (Excoffier et al., 2005). The haplogroup diversity was calculated according to Nei (1987). Estimation of the genetic differentiation between the comparative groups was based on pairwise F_{ST} analysis (Weir & Cockerham, 1984). Multi-dimensional scaling plot was performed on pairwise F_{ST} values using SPSS Statistics 17.0 (Chicago, IL,USA).

Table 1. Haplogroups, method of detection of the coding region polymorphisms and HVSI haplotypes, and frequency (%) of haplogroups in three ethnic groups of Tuzla Canton

Haplogroup	Method detection of the haplogroups	Coding region polymorphisms and restriction enzymes	HVSI (+16000)	Bosnian-Bosnincs (N=201)	Bosnian-Croats (N=22)	Bosnia-Serbs (N=18)
HV	RFLP,sequencing	+7025 <i>AluI</i>	217-243-261	0.50		
HVO	RFLP, sequencing	+7025 <i>AluI</i>	298		4.55	
HVO	RFLP,sequencing	+ 7025 <i>AluI</i>	216-298	0.50		
H	RFLP	-7025 <i>AluI</i>		54.73	45.46	44.45
V	RFLP	-4577 <i>NlaIII</i>		6.46		5.56
I	RFLP	-1715 <i>DdeI</i> , +8249 <i>AvaII</i> , +10028 <i>AluI</i>		1.49	4.55	
J	RFLP	-13704 <i>BstOI</i>		8.96	4.55	11.11
T	RFLP	+13366 <i>BamHI</i> , +15606 <i>AluI</i>		4.48	4.55	11.11
W	RFLP	+8249 <i>AvaII</i> , -8994 <i>HaeIII</i>		3.48	4.55	
X	RFLP	- 1715 <i>DdeI</i>		4.48	4.55	5.56
K	RFLP	+12308 <i>HinfI</i> , - 9052 <i>HaeII</i>		3.48	4.55	5.56
U7	RFLP, sequencing	+12308 <i>HinfI</i>	309 – 318	0.50		
U5a1	RFLP, sequencing	+12308 <i>HinfI</i>	256-270		4.55	
U5a1	RFLP, sequencing	+12308 <i>HinfI</i>	75-256-270	0.50		
U5a1	RFLP, sequencing	+12308 <i>HinfI</i>	192-256-270	1.49		
U5a1	RFLP, sequencing	+12308 <i>HinfI</i>	189-192.1-195-234-256-270-311-330-362-384	0.50		
U5b1b1	RFLP, sequencing	+12308 <i>HinfI</i>	16189-192-270	1.00		
U5b1	RFLP, sequencing	+12308 <i>HinfI</i>	93–189–270			5.56
U5b3	RFLP, sequencing	+12308 <i>HinfI</i>	192–270–304	1.00		
U4a2	RFLP, sequencing	+12308 <i>HinfI</i>	356	1.00		5.56
U4a2	RFLP, sequencing	+12308 <i>HinfI</i>	189–242–288–356-362	0.50		
U4c1	RFLP, sequencing	+12308 <i>HinfI</i>	179–356	0.50		
U3	RFLP, sequencing	+12308 <i>HinfI</i>	343	0.50		
U3	RFLP, sequencing	+12308 <i>HinfI</i>	261-343		4.55	
U3a	RFLP, sequencing	+12308 <i>HinfI</i>	343–390	0.50		
U2e	RFLP, sequencing	+12308 <i>HinfI</i>	51-129-182-183-189-362	1.00		
U2e	RFLP, sequencing	+12308 <i>HinfI</i>	51-129-183-189-362			5.56
U2e	RFLP, sequencing	+12308 <i>HinfI</i>	129-179-182-183-189-362		4.55	
U1a	RFLP, sequencing	+12308 <i>HinfI</i>	183-189-249	0.50	4.55	
N1a	sequencing		147G-172-223-248-355	0.50		
N1a	sequencing		147G-172-223-248-295-355	0.50		
M	RFLP, sequencing	-10871 <i>MnI</i> , +10397 <i>AluI</i>	126-148-309-318	1.00		

Results and Discussion

MtDNA variation in the analyzed groups of Tuzla Canton

The analysis of mtDNA variation in the three main ethnic groups from Tuzla Canton enabled the identification of the typical Western-Eurasian haplogroups (H, I, J, T, W, U, HV, HVO, K, V, and X), African/Near Eastern lineages N1a and Asian haplogroup M (Table 1). The identified haplogroups in the groups of the Bosnian Bosniaks, the Croats and the Serbs of Tuzla Canton represent nearly entire mtDNA variation found in other European populations.

In subcluster diversification of the haplogroups, the mitochondrial haplogroups H, U5, U4, U2, U1, W, X, K and I whose origin is related to different periods of the Late Glacial and postglacial re-colonization of Europe (Pala, et al., 2009; Malyrchuk et al., 2008, 2010; Richards et al., 2000) as well as the Neolithic haplogroups U3, N1a, J and T (Palanichamy et al., 2010; Pala et al., 2009, 2012; Richards, et.al. 2000) were detected. All three ethnic groups of Tuzla Canton have a relatively high frequency of the macro-haplogroup H (haplogroup H): Bosniaks (54.73%), Croats (45.46%) and Serbs (44.45%). The observed frequencies of haplogroup H in the analyzed ethnic groups in Tuzla Canton are in accordance with the frequency of this haplogroup (including all the subhaplogroups) in the analyzed ethnic groups from the other regions of Bosnia and Herzegovina (Central Bosnia, the region of Sarajevo, Banja Luka) (Kovačević et al., 2014).

Namely, haplogroup H is a characteristic haplogroup of almost all European populations, and about 30-50% of the European mtDNA lineages are classified as the haplogroup H (Roostalu et al., 2007; Richards et al., 2002, 2000;). The haplogroup H entered Europe from the Near East ~20,000–25,000 years ago and expanded during the postglacial re-colonisation of Europe (~15,000 years ago) from southwestern European refuges (Hernandez et al., 2017; Pereira et al., 2005; Achilli et al., 2004; Richards et al., 2000; Torroni et al., 1998). The observed data of slightly increased frequency of the mitochondrial palaeolithic haplogroup H in all three ethnic groups in our study and the presence of the

R-M269 Y chromosome mutation in all three ethnic groups from Bosnia-Herzegovina (Kovačević et al., 2014; Marjanović et al., 2005), which according to the classical theory Semino et al. (2004) spread from the Franco-Cantabrian refuge, can be useful additional information in the consideration of the post-glacial expansion of populations from southwestern European refuges to the Balkan region. According to the data that the paternal haplogroup R and maternal haplogroups H1 and H3 share similar patterns of distribution and evolutionary history (Achilli et al., 2005), additional studies on diversification within haplogroup H in our study are necessary. The study by Kovačević et al. (2014) indicates the presence of sublineages H1 and H3 in all the three analyzed ethnic groups. The second most frequent haplogroup in the total population is the haplogroup U (represented with all known subhaplogroup). Subcluster diversification of the haplogroup U indicates that all three ethnic groups contain the oldest European haplogroup U5, (coalescence time of about 37,000 years) (Soares et al., 2010; Richards, et al., 1998), whose expansion started in central and southern Europe (Malyrchuk et al., 2010). Beside the haplogroup U5, U-haplotypes of the ethnic groups belong to U4a, U4c, U2e and U1 lineages, which spread with Paleolithic migrations in different periods of the Late Glacial and post-glacial re-colonization of Europe, as well as haplogroup U3 whose arrival in Europe is correlated with Neolithic expansion. In the Bosniak group, both clusters U5a and U5b are represented. The ancestral founder haplotypes U5a1 (with HVSI motif 16192-16256-16270) (Richards et al., 2000), U5b1 (which reached northern Europe from Franco Cantabria in the post-LGM times) (Pala et al., 2012) as well as U5b3 (which expanded along the Mediterranean coast in the Holocene) (Pala et al., 2009) were observed. Also, the Bosniak group contains almost all sublineages of the haplogroup U (U1, U2e, U3, U3a, U4a, U4c, U7). Interestingly, gene pool of the analyzed group of Bosniaks from the region of Sarajevo and Zavidovići (Kovačević et al., 2014) does not contain any of the sublineages of the subhaplogroup U. Bosnian Croat U-haplotypes, beside haplogroup U51a, belong to the U3, U2e and U1 lineages, which have been represented in the

population of the Croats in an earlier study by Cvjetan et al., (2004) while the study by Kovačević et al. (2014) indicates the presence of only U2e haplogroup in both groups of Bosnian Croats. Bosnian Serbian U-haplotypes, beside haplogroup U51b, belong to the components of Middle Upper Palaeolithic U4 and U2 (Richards et al., 2000). In our study, the frequency of the haplogroup U4a2 (5.56%) in Bosnian-Serbs is in accordance with the frequency of this haplogroup in the previous studies of the Serbian populations, 8.63% (Davidović et al., 2015) - 6.80 % (Cvjetan et al., 2004). Also, the ethnic group of Bosnian Serbs from central Bosnia and Banja Luka (Kovačević et al., 2014) indicates an increased frequency of this haplogroup (10.53%). This haplogroup is recognized as an indicator of postglacial re-colonization of Europe from the central-eastern European (after 15,000 YBP ; Pala 2012). The Y chromosome haplogroup R-M17, a possible paternal signal of the expansion of populations from the Ukrainian glacial refuge, (Semino et al., 2000), was observed in all three ethnic populations in modern Bosnia and Herzegovina (Kovačević et al., 2014; Marjanović et al., 2005). Other significant indicators of the population expansions and postglacial re-colonization of Europe from glacial refuge, such as the haplogroup V, which reach this region from Franco Cantabria refuge (Torroni et al., 2001; Richards et al., 2000) have been identified in analyzed ethnic groups from Tuzla Canton (excluding Bosnian-Croats). In our study, the observed mtDNA signals of the postglacial expansions from different glacial refuges are in accordance with the presence of specific paternal signals of the post-LGM event from refuges: Balkan, (I-P37), Ukraine (R-M17) and Franco-Cantabrian (RM269) in three ethnic groups of Bosnia and Herzegovina (Kovačević et al., 2014; Marjanović et al, 2005). The previous study by Dogan et al. (2016) based on the analysis of Y-chromosomal haplogroup distribution in Tuzla Canton indicates that the general population of Tuzla Canton contains about 50% of the I-P37, whose paleolithic origin is related to the expansion along the Balkan Peninsula (Marjanovic et al., 2005) and about 23% of the R1a which characterizes the populations of East Europe (Semino et al., 2000). All three ethnic populations

from northeastern Bosnia contain significant mitochondrial determinants of expansion of populations from the Near East to Europe during the Neolithic, such as haplogroups J, T and U3 (excluding U3 in the case of Bosnian Serbs). In our study, the haplogroup J is the third prevalent mitochondrial determinant in total population, with greater frequency in the population of Serbs (11.11%), than Bosniaks (8.96%) and Croats (4.55 %). Also, haplogroup J (subhaplogroups J1b and J1c) was observed in all the three analyzed ethnic groups from central Bosnia and the region of Sarajevo and Banja Luka (Kovačević et al., 2014). The studies by Kovačević et al. (2014) and Marjanović et al. (2005) reported the presence of neolithic Y-chromosome haplogroups E and J whose spread from Near East and Africa occurred during the Neolithic and post-Neolithic period (Cruciani et al., 2004). Interestingly, higher frequency of neolithic Y-chromosome haplogroup E-M78 was noted in the group of Bosnian-Serbs (19.8%) (Marjanović et al., 2005). In our study, gene pool of the Bosniak group contains African/Near East lineages N1a with mutation 16147G (Palanichamy et al., 2010), which occurred in Europe 7500 years ago (Haak et al., 2005). This haplotype is observed in the neighboring population from Serbia, in individuals who originated from Bosnia (Davidović et al., 2015). Other lineages of the European/Middle Eastern origin (T, K, X and W) with frequency < 5.00% in the ethnic populations were observed. Of interest is the presence of non-European haplogroup M in the sample of Bosniaks, which is also represented in gene pool of populations from Macedonia, Croatia and Serbia (Cvjetan et al., 2004; Malyrchuk et al., 2003) with low frequency.

MtDNA variation in the analyzed groups of Tuzla Canton

Table 2 shows the values of haplogroup diversity of the observed ethnic groups of Tuzla Canton. The value of the haplogroup diversity in the Bosnian-Bosniaks (0.6829) was lower in relation to the Bosnian-Croats and Bosnian-Serbs groups. The analysis of genetic differentiation between pairs of the observed groups (pFST) based on frequencies of the haplogroups is given in table 3.

Table 2. Diversity of the haplogroups in the analyzed ethnic groups

Populations	Genetic diversity
Bosnian-Bosniaks	0.6829 +/- 0.0353
Bosnian-Croats	0.8009 +/- 0.08790
Bosnian-Serbs	0.8039 +/- 0.09070

Table 3. Matrix of genetic differentiation (pFst) between analyzed ethnic groups

Populations	Bosnian-Bosniaks	Bosnian-Croats	Bosnian-Serbs
Bosnian-Bosniaks			
Bosnian-Croats	-0.00391		
Bosnian-Serbs	-0.01274	-0.03163	

No significant pairwise F_{ST} differences ($p > 0.05$) have been observed among three main ethnic populations from northeastern Bosnia. The F_{ST} values indicate close genetic similarity, which may suggest a common mtDNA pool of the ethnic groups. The results obtained in our study confirm the results of genetic differentiation ($< 0.1\%$) based on the analysis of 15 morphological and physiological parameters (Pojskić et al., 2003). Increased differentiation (5.8%) between ethnic groups of the Bosnian-Croats and Bosnian-Bosniaks, as well as Bosnian-Croats and Bosnian-Serbs observed in the study by Marjanović et al. (2005), based on analysis of the Y-chromosome haplogroups, is probably a consequence of internal immigration of the Croats from the region of Herzegovina. For clearer positioning of the analyzed ethnic groups of Tuzla Canton in the context of populations of former Yugoslavia, we compared the obtained data at the level of frequencies of haplogroups with those obtained in previous studies of the populations: Croatia, Herzegovina (Cvjetan et al., 2004), Serbia

Table 4. Haplogroup diversity in the analyzed populations and the compared ethnic groups

Populations	Diversity
Bosniaks (this study)	0.6814 +/- 0.0351
Bosnia (Malyrchuk et al., 2003)	0.7483 +/- 0.0358
Bosnia (Cvjetan et al., 2004)	0.7566 +/- 0.0269
Bosnian Croats (this study)	0.8052 +/- 0.0892
Croatia (Cvjetan et al. 2004)	0.7589 +/- 0.0230
Bosnian Serbs (this study)	0.8039 +/- 0.0907
Serbia (Cvjetan et al., 2004)	0.8078 +/- 0.0330
Serbia (Davidović et al., 2015)	0.7515 +/- 0.0359
Herzegovina (Cvjetan et al., 2004)	0.7931 +/- 0.0319
Slovenia (Malyrchuk et al., 2003)	0.7534 +/- 0.0406
Slovenia (Šarac et al., 2014)	0.7966 +/- 0.0289
Macedonia (Cvjetan et al., 2004)	0.8009 +/- 0.0290

(Davidović et al., 2015; Cvjetan et al., 2004), Slovenia (Šarac et al 2014 Cvjetan et al., 2004) and the previously studies populations from Bosnia and Herzegovina (Cvjetan et al., 2004; Malyrchuk et al., 2003). Lower values of the haplogroup diversity were observed between the Bosnian Bosniaks and the comparative European populations (table 4). Lower values of this parameter may be due to slightly higher frequencies of a specific haplogroup such as H in Bosniak group with regard to the compared populations. Table 5 shows the analysis of genetic differentiation between pairs of the observed populations (pFST). Significant pairwise F_{ST} differences ($p < 0.05$) between the Bosnian-Bosniaks and populations from Croatia, Serbia, Macedonia and Herzegovina were observed. Multi-dimensional scaling plot was created based on pairwise F_{ST} values (Figure 1). Genetic division between groups of Bosnian-Croats and Bosnian-Serbs and their neighboring populations is probably a consequence of a small sample size for these populations (Figure 1).

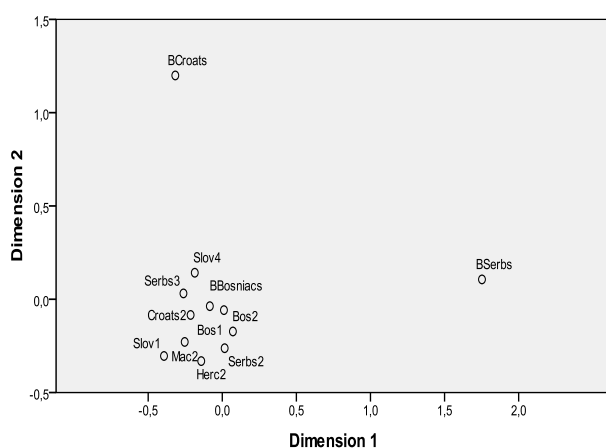
**Figure 1.** Multi-dimensional scaling plot of populations according to pairwise F_{ST} , constructed from the following data: BBosniaks - Bosnian Bosniaks; BCroats - Bosnian Croats; BSerbs - Bosnian Serbs (this study); Bos1 - Bosnian population; Slo1- Slovenian population (Malyrchuk et al., 2003); (Bos2 - Bosnian population; Croats2 - Croatian population; Serbs2-Serbian population; Her2 - Herzegovinian population; Mac2- Macedonian population (Cvjetan et al., 2004); Serbs3 - Serbian population (Davidović et al., 2015); Slov4 - Slovenian population (Šarac et al., 2014)

Table 5. Matrix of genetic differentiation (pFst) between the compared populations

	B-Bos	Bos ¹	Bos ²	B-Cro	Cro ²	B-Ser	Ser ²	Ser ³	Her ²	Slo ¹	Slo ⁴	Mac ²
B-Bos												
Bos ¹	0.0025											
Bos ²	0.0050*	0.0011										
B-Cro	-0.0115	0.01412	-0.0172									
Cro ²	0.0084*	0.0029	0.0037	-0.0108								
B-Ser	-0.0149	-0.0194	-0.0161	-0.0378	-0.0235							
Ser ²	0.0128*	0.0005	0.0002	-0.0152	0.0012	-0.0213						
Ser ³	0.0059	-0.0007	0.0003	-0.0145	0.0047	-0.0214	-0.0013					
Her ²	0.0103*	0.0004	0.0009	-0.0170	0.0044	-0.0236	-0.0014	-0.0018				
Slo ¹	0.0001	-0.0053	-0.0010	-0.0168	-0.002	-0.0248	-0.0033	-0.0026	-0.0015			
Slo ⁴	0.0291*	0.0164*	0.0174*	-0.0010	0.0010	-0.0227	0.0076	0.0165*	0.0090	0.0088		
Mac ²	0.0129*	0.0045	0.0044	-0.0166	-0.0004	-0.0260	-0.0007	0.0032	0.0016	0.0004	0.0008	

* $p < 0.05$; B-Bos - Bosnian Bosniaks; B-Cro - Bosnian Croats; B-Ser - Bosnian Serbs (this study); Bos1-Bosnian population (Malyarchuk et al., 2003); Bos2-Bosnian population; Cro2-Croatian population; Ser2-Serbian population; Her2-Herzegovinian population; Mac2-Macedonian population (Cvijetan et al., 2004); Ser3-Serbian population (Davidović et al., 2015); Slo1-Slovenian population (Malyarchuk et al., 2003); Slo4- Slovenian population (Šarac et al., 2014).

Conclusions

The results of our study suggest that mitochondrial DNA pool of the three main ethnic groups of Tuzla region was shaped by early and late migration events which marked the process of settlement of the Balkans. The presence of important maternal determinants of the Late Glacial expansion (U5a), postglacial re-colonisation of Europe from glacial refuges of southwestern Europe (H, V, U5b1), central-eastern European Plain (U4), Italian Peninsula (U5b3) as well as of neolithic expansion (U3, N1a, J and T) was noted in the genetic structure of the ethnic groups. Conclusions in our study are consistent with the results of previous study based on the distribution of Y-chromosome haplogroups in three main ethnic groups of modern Bosnia and Herzegovina (Kovačević et al., 2014; Marjanović et al., 2005), suggesting that the post-glacial expansion of populations from different refuges had a great influence on gene pool of the analyzed populations, while Neolithic and post-Neolithic gene flow had a less important role. There are no significant pairwise Fst differences observed between the three main ethnic groups from Tuzla Canton. These data can indicate common maternal pool of the ethnic groups, as well as similar contribution of the paternal and maternal gene flow on the genetic structure of the

three main ethnic groups of modern Bosnia and Herzegovina.

Conflict of interest

The authors report no conflicts of interest.

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References

- Achilli A, Rengo C, Battaglia V, Pala M, Olivieri A, Fornarino S, Magri C, Scozzari R, Babudri N, Santachiara-Benerecetti AS, Bandelt HJ, Semino O, Torroni A (2005) Saami and Berbers - an unexpected mitochondrial DNA link. *Am J Hum Genet*, 76:883–886.
- Ahmić A, Silajdžić E, Lasić L, Kalamujić B, Hadživadić V, Kapur-Pojškić, L, Bajrović K, Hadžiselimović R, Pojskić N (2014) Frequency of

- Main Western-Euroasian mtDNA Haplogroups and Paleolithic and Neolithic Lineages in the Genetic Structure of Population of Northeastern Bosnia. *Coll Antropol*, 38 (3): 819-827.
- Andrews RM, Kubicka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet*, 23:147.
- Anderson S, Bankier AT, Barrell BG, DE Bruijn, Coulson AR, Drouin J, Eperon IC, Nierlich ROE BA, Sanger F, Schreier PH, Smith AJB, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. *Nature*, 290: 457-465.
- Behar DM, Harmant Ch, Manry J, van Oven M, Haak W, Martinez-Cruz B, Salaberria J, Oyharçabal B, Bauduer F, Comas D, Quintana-Murci L (2012) The Basque paradigm: genetic evidence of a maternal continuity in the Franco-Cantabrian region since pre-Neolithic times. *Am J Hum Genet*, 90:486–493.
- Chiara B, Pille H, Vågane A, Zadik D, Eriksen H, Pamjav H, Antti S, Wetton J, Jobling M (2017) Population resequencing of European mitochondrial genomes highlights sex-bias in Bronze Age demographic expansions. *Sci Rep*, 7:12086.
- Cruciani F, La Fratta R, Santolamazza P, Sellitto D, Pascone R, Moral P, Watson E, Guida V, Colomb E B, Zaharova B, Lavinha J, Vona G, Aman R, Cali F, Akar N, Richards M, Torroni A, Novelletto A, Scozzari R (2004) Phylogeographic analysis of haplogroup E3b (E-M215) Y chromosomes reveals multiple migratory events within and out of Africa. *Am J Hum Genet*, 74:1014–1022.
- Cvjetan S, Tolik HV, Janičijević B, Martinović-Klakić I, Metspalu E, Peričić M, Juri P, Popović D, Terzić R, Villems R, Rudan P (2004) Frequencies of mtDNA haplogroups in Southeastern Europe. *Coll Antropol*, 28 (1):193-198.
- Davidović S, Malyarchuk B, M Aleksić J, Derenko M, Topalović V, Litvinov A, Stevanović M, Kovačević-Grujić N (2015) Mitochondrial DNA Perspective of Serbian Genetic Diversity. *Am J Phys Anthropol*, 156:449–465.
- Dogan S, Babić N, Gurkan C, Gösku A, Marjanović D, Hadziavdić V (2016) Y-chromosomal haplogroup distribution in the Tuzla Canton of Bosnia and Herzegovina: A concordance study using four different in silico assignment algorithms based on Y-STR data. *Homo*, 67 (6):471-483
- Excoffier L, Laval G, Schneider S 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol Bioinform Online*, 1:47–50.
- Grzybowski T, Malyarchuk BA, Derenko MV, Perkova MA, Bednarek J, Wozniak M. (2007) Complex interactions of the Eastern and Western Slavic populations with other European groups as revealed by mitochondrial DNA analysis. *Forensic Sci Int Genet*, 1:141–147.
- Hall TA 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp*, 41:95-98.
- Haak W, Forster P., Bramanti B, Matsumura S, Brandt G, Tänzer M, Villems R, Renfrew C, Gronenbor n D, Alt KW, Burger J (2005) Ancient DNA from the first European farmers in 7500-year-old Neolithic sites. *Science*, 310, 1016–1018.
- Hernández CL, Dugoujon JM, Novelletto A, Rodríguez JN, Cuesta P, Calderó R (2017) The distribution of mitochondrial DNA haplogroup H in southern Iberia indicates ancient human genetic exchanges along the western edge of the Mediterranean. *BMC Genet*, 18: 46.
- Kivisild T, Bamshad MJ, Kaldma K, Metspalu M, Metspalu E, Reidla M, Laos S, Parik J, Watkins WS, Dixon ME, Papiha SS, Mastana SS, Mir MR, Ferak V, Villems R (1999) Deep common ancestry of Indian and western-Eurasian mitochondrial DNA lineages. *Curr Biol*, 9:1331–1334.
- Kovačević L, Tambes K, Ilumäe AM, Kushniarevich A, Yunusbayev B, Solnik A, Bego T, Primorac D, Skaro V, Leskovac A, Jakovski Z, Drobnić K, Tolik HV, Kovačević S, Rudan P, Metspalu E, Marjanović D (2014) Standing at the Gateway to Europe - The Genetic Structure of Western Balkan Populations Based on Autosomal and Haploid Markers. *PLoS ONE*, 9(8):e105090.doi10.1371/journal.pone.0105090
- Macaulay V, Richards M, Hickey E, Vega E, Cruciani F, Scozzari R, Bonne Tamir B, Sykes B, Torroni A (1999) The emerging tree of west Eurasian mtDNAs: a synthesis of control-region sequences and RFLPs. *Am J Hum Genet*, 64:232–249.
- Malcolm N (1994) Bosnia. A Short History. Macmillan London Limited Copyright, London.
- Malyarchuk B, Derenko M, Grzybowski T, Perkova M, Rogalla U, Vanecek T, Tsybovsky I (2010) The peopling of Europe from the mitochondrial haplogroup U5 perspective. *PLoS ONE* 5:e10285.
- Malyarchuk B, Grzybowski T, Derenko M, Perkova M, Vanecek T, Lazur J, Gornolcack P, Tsybovsky I (2008) Mitochondrial DNA phylogeny in Eastern and Western Slavs. *Mol Biol Evol*, 25:1651–1658.

- Malyarchuk BA, Vanecek T, Perkova MA, Derenko MV, Sip M. 2006. Mitochondrial DNA variability in the Czech population, with application to the ethnic history of Slavs. *Hum Biol*, 78: 681–696.
- Malyarchuk BA, Grzybowski T, Derenko MV, Czarny J, Drobnič, K, Mišćicka-Sliwka D 2003 Mitochondrial DNA Variability in Bosnians and Slovenians. *Ann Hum Genet*, 67:412–425
- Malyarchuk BA, Grzybowski T, Derenko MV, Czarny J, Woźniak M, Mišćicka-Sliwka D (2002) Mitochondrial DNA Variability in Poles and Russians. *Ann Hum Genet*, 66:261–283.
- Malyarchuk BA, Derenko MV (2001) Mitochondrial DNA variability in Russians and Ukrainians: implication to the origin of the Eastern Slavs. *Ann Hum Genet*, 65:63–78
- Malcolm N (1994) *Bosnia. A Short History*. Macmillan London Limited Copyright, London.
- Marjanović D, Fornario S, Montagna S, Primorac D, Hadžiselimović R, Vidović S, Pojskić N, Achilli A, Drobnič K, Andjelinović S, Torroni A, Santachiara-Benerecetti S, Semino O (2005) The peopling of modern Bosnia-Herzegovina: Y chromosome haplogroups in the three main ethnic groups. *Ann Hum Genet*, 69(6):757-763
- Miller SA, Dykes DD, Polesky HF 1998 *Nucleic Acids*, 16:1215
- Nei M 1987 *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Palanichamy MG, Cai-Ling Zh, Bikash M, Malyarchuk B, Derenko M, Chaudhur K, Zhang Y-P (2010) Mitochondrial haplogroup N1a phylogeography, with implication to the origin of European farmers. *BMC Evol Biol*, 10:04.
- Pala M, Olivieri A, Achilli A, Accetturo M, Metspalu E, Reidla M, Tamm E, Karmin M, Kashani B, Perego UA, Carossa V, Gandini F, Pereira J, Soares P, Angerhofer N, Rychkov S, Al-Zahery N, Carelli V, Houshmand M, Hatina J, Macaulay V, Pereira L, Woodward W, Gamble C, Baird D, Semino O, Villems R, Torroni A, Richards BM (2012) Mitochondrial DNA Signals of Late Glacial Recolonization of Europe from Near Eastern Refugia. *A J Hum Genet*, 90:915–924.
- Pala M, Achilli A, Olivieri A, Hooshiar B, Perego A, Sanna D, Metspalu E, Tambets K, Tamm E, Carossa V, Lncioni H, Panara F, Huber G, Al-Zahery N, Brisighelli F, Francalacci P, Salas A, Behar D, Villems R, Semino O, Torroni A (2009) Mitochondrial haplogroup U5b3:A Distant Echo of the Epipaleolithic in Italy and the Legacy of the Early Sardinians. *Am J Hum Genet*, 84:814-821.
- Pereira L, Richards M, Goios A, Alonso A, Albarrán C, Garcia O, Behar DM, Gölge M, Hatina J, Al-Gazali L, Bradley DG, Macaulay V, Amorim A (2005) High-resolution mtDNA evidence for the late-glacial resettlement of Europe from an Iberian refugium. *Genome Res*, 15(1):19-24.
- Perić S (2002) Der kulturelle Charakter und die Chronologie der Starčevo-Elemente im Neolithikum der Westlichen Balkanregionen. *Starinar LI/2001*, Beograd.
- Pojskić N (2003) Kompleksna analiza različitih modela proučavanja genetičke distance i njenih mogućih faktora u stanovništvu BiH. Magistarska teza, Prirodno-matematički fakultet, Univerzitet u Sarajevu, COBISS.BH-ID:16505350
- Raymond M, Rousse F (1995) An Exact Test for Population Differentiation. *Evolution*, 49: 1280-1283.
- Richards M, Macaulay V, Torroni A, Bandelt HJ (2002) In search of geographical patterns in European mitochondrial DNA *Am J Hum Genet* 71:1168–1174.
- Richards M, Macaulay V, Hickey E, Vega E, Sykes B, Guida V, Rengo C, Sellitto D, Cruciani F, Kivisild T, Villems R, Thomas M, Rychkov S, Hill E, Bradley D, Romano V, Cali F, Demaine A, Papiha S, Stefanescu G, Hatina J, Belledi M, Novelletto A, Norby S, Al-zaheri N, Scozzari R, Torroni A, Bandelt HJ (2000) Tracing European founder lineages in the Near Eastern mtDNA pool. *Am J Hum Genet*, 67:1251-76.
- Richards MB, Macaulay VA, Bandelt HJ, Sykes BC (1998) Phylogeography of mitochondrial DNA in western Europe. *Ann Hum Genet*, 62:241–260.
- Roostalu U, Kutuev I, Loogväli EL, Metspalu E, Tambets K, Reidla M, Khusnutdinova EK, Usanga E, Kivisild T, Villems R (2007) Origin and expansion of haplogroup H, the dominant human mitochondrial DNA lineage in West Eurasia: the Near Eastern and Caucasian perspective. *Mol Biol Evol*, 24(2):436-
- Santos C, Lima M, Montiel R, Francalacci P (2004) Determination of Human European Mitochondrial DNA Haplogroups by Means of a Hierarchical Approach *Hum Biol*, 76 (3):432-453.
- Semino O, Passarino G, Oefner P J, Lin AA, Arbuzova S, Beckman, L E, De Benedictis G, Francalacci P, Kouvatsi A, Limborska S, Marcikiae M, Mika A, Mika B, Primorac D, Santachiara-Benerecetti A S, Cavalli-Sforza L L, Underhill P A (2000) The genetic legacy of Paleolithic Homo sapiens in extant Europeans: a Y chromosome perspective. *Science*, 290:1155–1159.

- Soares P, Achilli A, Semino O, Davies W, Macaulay V, Bandelt HJ, Torroni A, Richards MB (2010) The archaeogenetics of Europe. *Curr Biol*, 20:174–183.
- Tambets K, Rootsi S, Kivisild T, Help H, Serk P, Loogvali E, Tolk HV, Reidla M, Metspalu E, Pliss L, Balanovsky O, Pshenichnov A, Balanovska E, Gubina M, Zhadanov S, Osipova L, Voevoda M, Kutuev I, Bermisheva M, Khusnutdinova E, Gusar V, Grechanina E, Parik J, Pennarun E, Richard Ch, Chaventre A, Moisan JP, Barac L, Pericic M, Rudan P, Terzic R, Mikerezi I, Krumina A, Baumanis V, Koziel S, Rickards O, Gian F, Anagnou N, Pappa K (2004) The Western and Eastern Roots of the Saami—the Story of Genetic "Outliers" Told by Mitochondrial DNA and Y Chromosomes. *Am J Hum Genet*, 74:661–68.
- Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, Savontaus ML, Wallace DC (1996) Classification of European mtDNAs from an analysis of three European populations. *Genet*, 144:1835–1850.
- Torroni A, Bandelt HJ, Macaulay V, Richards M, Cruciani F, Rengo C, Martinez-Cabrera V, Villems R, Kivisild T, Metspalu E, Parik J, Tolk HV, Tambets K, Forster P, Karger B, Francalacci P, Rudan P, Janicijevic B, Rickards O, Savontaus ML, Huoponen K, Laitinen V, Koivumäki S, Sykes B, Hickey E, Novelletto A, Moral P, Sellitto D, Coppa A, Al-Zaheri N, Santachiara-Benerecetti AS, Semino O, Scozzari R (2001) A signal, from human mtDNA, of postglacial recolonization in Europe. *Am J Hum Genet*, 69: 844–852.
- Živković T (2002) South Slavs under the Byzantine rule (600–1025). Belgrade: Institute of History.
- Šarac J, Šarić T, Auguštin DH, Jeran N, Kovačević L, Cvjetan S, Perinić Lewis A, Metspalu A, Reidla M, Novokmet N, Vidovič M, Nevajda B, Glasnović A, Marjanović D, Missoni S, Villems R, Rudan P (2014) Maternal Genetic Heritage of Southeastern Europe Reveals a New Croatian Isolate and a Novel, Local Sub-Branching in the X2 Haplogroup. *Ann Hum Genetic*, 78:178–194.
- Wilkes J (1992) *The Illyrians*. Oxford (UK): Blackwell Publisher
- Van Oven M, Kayser M (2009) Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum Mutat*, 30 (2):386–394.
- Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population Structure. *Evolution*, 38:1358–137.