

A PRELIMINARY STUDY OF THE PALEOLITHIC AND NEOLITHIC CONTRIBUTION THE EUROPEAN MTDNA FLOW IN SHAPING THE GENETIC STRUCTURE OF RECENT BOSNIAN POPULATION

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Abstract

The mitochondrial DNA (mtDNA) polymorphisms in Bosnian human population was analyzed by means of hypervariable segment I and II (HVS I and HVS II) sequencing and restriction fragment-length polymorphism analysis of the mtDNA coding region. The results suggest that shaping the genetic structure of recent Bosnian population likely to be affected by the expansion from the European glacial refuges area at the end of the Last Glacial Maximum (LGM), postglacial expansions from southwestern refuges of Europe, the Italian Peninsula and the dispersion in periods of more recent historical events, from the East European Plain. Especially interesting feature of the Neolithic expansion in this area is the ancient African/South Asian haplogroup N1a with the HVS I variant 16147G, which is almost absent in Europe. The haplotypes HVS I with variant 16147G suggest the colonization of the Northeast Bosnia region by Neolithic communities in the Early Neolithic period of expansion through Europe, as evidenced by the archaeological remains of the Starcevo culture.

Keywords: Mitochondrial DNA, polymorphisms, Bosnia population

Introduction

Most previous studies on human populations of Europe based on the analysis of mitochondrial DNA (mtDNA) variations were focused on the effects of Late Glacial and post-glacial expansions. A founder analysis of mtDNA lineages in populations of Near East and Europe has demonstrated

that the majority of extant mtDNA lineages entered Europe in several episodes during the Upper Palaeolithic and there was a founder effect or bottleneck associated with the Last Glacial Maximum (LGM), from which derives the largest fraction of surviving lineages (Richards et al., 2000). The LGM took place about 21,000 years before the present (YBP) (Pala et al., 2009), and during this interval European human population was most probably concentrated in refugial areas in southwestern Europe, along Mediterranean, in the Balkans and on the east European plain (Gamble, Davies, Pettitt, Hazelwood & Richards, 2005). The oldest mtDNA haplogroup spreading with the first European settlers in the Early Upper Paleolithic is haplogroup U5 (Richards et al., 2000). Study Malyarchuk et al., (2010) suggest that the coalescence time estimate for the U5 is 25–30,000YBP. Analysis of mtDNA sequences in modern European populations revealed the presence of a bulk of U5 subclusters that demonstrated coalescence ages around 11–13,000 YBP (Tambets et al., 2003). Major components of Middle Upper Paleolithic (26,000YBP) were haplogroups U1, HV*, especially U4 and U2 (Richards et al., 2000). The main contribution to the subsequent population expansion in the Upper Paleolithic (14,000 YBP) have the major of haplogroups H, K, T*, W and X (Richards et al., 2000). Studies suggest that the basic signals of postglacial recolonization of Europe (after 15,000 YBP) from refuge Franco-Cantabria (southwestern Europe) are haplogroups H1, H3, V and U5b1b (Torroni et al., 2001; Achilli et al., 2004; 2005), from the Italian Peninsula haplogroup U5b3 (Pala et al., 2009; Fraumene et al., 2006), and from the East European Plain U4 and U5a (Pala et al., 2012; Malyrchuk et al., 2010). Significant clusters of the Neolithic expansion in Europe from the Near East (after 9,000 YBP) were represented by haplogroups J, T1, U3 and several subclusters by H and W (Richards et al., 2000). The most important genetic signature of the Early Neolithic farming population is haplogroup N1a (Palanichamy et al., 2010). Existing archaeological data in northern Bosnia (aged about 50,000 years) point to the fact that Bosnia and Herzegovina was inhabited back in the Paleolithic age (Imamovic et al., 1998). The youngest Paleolithic sites in Bosnia date back about 14,000 years and the known prehistoric period of civilization paths dates from the Neolithic (Imamovic et al., 1998). The oldest settlement from the early Neolithic period (6,700-7,700) up to now have been recorded in Northeastern Bosnia (Gornja Tuzla), and the main indicator of the Early and Middle Neolithic period in this area are archaeological remains of Starcevo culture (Tasic, 2000). Our study suggests that the results obtained by molecular mtDNA variations are correlated with the archaeological data found in the region north and northeastern Bosnia.

Materials and Methods

A total of 261 buccal samples have been collected from unrelated individuals in the area of Northeastern Bosnia. Prior to sampling all participants provided written informed consent for the collection of samples and subsequent analysis. Genomic DNA was extracted from dried swab samples of buccal mucosa, using salting out method (Miller, Dykes & Polesky, 1998). The method was slightly modified in order to optimize the extraction of DNA from buccal swab (Ahmic, 2009). Haplogroup affiliation was determined by RFLP (*Restriction Fragment Length Polymorphism*) method of analyzing haplogroup-specific markers of mtDNA coding region using the same primer pairs and amplification conditions for the PCR reaction (*Polymerase Chain Reaction*) as described by Torroni et al., (1996). The hypervariable segments I and II (HVS I and HVS II) in the mtDNA control region (CR) were amplified and sequenced for determination subtype haplogroup U and non-determined haplogroups. The set of primers F15971/R16410 was used for PCR reaction of the HVS I region sequence, while for HVS II region we used the F15/R484 primer set. PCR products were sequenced within facilities of the Macrogen Inc. Korea as their regular capillary DNA sequence services. Sequence analysis was performed with BioEdit software (Hall, 1999) and compared with the revised Cambridge Reference Sequence (rCRS; Andrews et al., 1999). Sequence classification into mtDNA subclusters was based on HVS I and HVS II motifs classification (Kayser, 2009; Richards et al., 2000; Macaulay et al., 1999; Malyarchuk, 2004). On the basis of observed differences analyzed with mtDNA Manager software (Bandelt, Lahermo, Richards & Macaulay, 2001), the sequences were classified into appropriate mtDNA subhaplogroups.

Results

The data obtained on the basis of haplogroup-specific markers of mtDNA coding region suggest the presence of haplogroups to the subsequent population expansion the Upper Paleolithic (H, K, T, W and X) and significant clusters of the Neolithic expansion (J and T). The subcluster diversification of oldest the haplogroup U, identified on the basis of HVS I and HVS II motifs, suggests that the genetic structure of the recent Bosnian population contains certain haplotypes which are related to different periods of the Late Glacial and post-glacial re-colonization of Europe. Our further study indicates and the presence of mtDNA signal of the first Neolithic migration (haplogroup N1a) on the European continent. Combinations of haplotypes identified on the basis of HVS I and HVS II motifs are given in Table 1. Frequencies of identified haplogroups are given in Table 2. In our study was observed the founder haplotype for phylogenetic cluster U5a1 (HVS I haplotype 16192-16256-16270) (Table 1). This ancestral cluster

(Richards et al., 2000) has been found in central Europe in Czechs (Malyarchuk et al., 2010). Of the total determinate phylogenetic clusters U5b1, U5b2 and U5b3 (Kayser, 2009) in our study were observed: U5b1 and U5b3. Significant feature of the genetic structure of analyzed population is haplogroup U5b3. This haplogroup is present in very low frequency in Europe (the exception of the Sardinian population 3,8 %) (Pala et al., 2009). The observed frequency in our study is close to 1%, which is close to the frequency in some Mediterranean populations (Pala et al., 2009). The most significant haplogroup of the Middle Upper Paleolithic found in our study are U4, U2, U1, HVO and HV2. We identified clusters U4a2a with a back mutation at nucleotide pair (np) 16356, subcluster U4a2c and U4c1 (Table 1). Phylogenetic cluster U4a2 is marked with the marker of ethnic history of Slavs and is considered to be of the central-eastern European origin (the coalescence age for U4a2 is $7,068 \pm 2,134$ YBP, respectively) (Malyarchuk et al., 2008). The cluster U4c1 was first identified in phylogenetic classification of the haplogroup U in the eastern and western Slavs (Malyrchuk et al., 2008). All determined haplotypes of the subhaplogroup U2e in our study (Table 1) include characteristic European HVSI motif 16129C (Macaulay et al., 1999). The haplotyps subhaplogroup U1a contain characteristic nucleotide motif 16189-16249 accompanied by 285T variant in HVSI. Determined haplotype of haplogroup HV2 in our study, which is characterized by HVS I motif 16217 in combination with 73G variant in HVS II is also very rare in European populations (Malyarchuk et al., 2003). Besides the standard determinants of Neolithic expansion (J, T), we noticed the presence of two ancestral African/Near East heliotypes with the HVSI variant 16147G (Table 1). Variants of haplotypes with 16147G are rarely present in Europe (Abu-Amero, González, Larruga, Bosley & Cabrera 2007; Abu-Amero, Larruga, Cabrera & González, 2008). HVSI heliotypes in our study include 147G-172-223-248-355, which was found in Ethiopia (Abu-Amero, González, Larruga, Bosley & Cabrera, 20007), the Arabian peninsula, (Abu-Amero, Larruga, Cabrera & González, 2008), Greece, Russia (Palanichamy et al., 2010) and Delnice (mountainous region Croatia) (Jeran et al., 2009) and 147G-172-223-248-295-355, which was found in study Hakk et al., in South-India (as cited in Palanichamy et al., 2010) and in the island of Pag (Croatia) (Jeran et al., 2009). According to the available literature data, except for the aforementioned Croatian populations, variants of HVSI haplotypes with 147G was not registered in the population of Southeastern Europe.

Table 1. mtDNA haplotypes of the observed subhaplogroups

HVSI (-16000)	HVSII	HG	N
192-256-270	73-263-309.1-315.1	U5a1	2
192-256-270		U5a1	1
256-270	73-263-309.1-315.1	U5a1a	1
75-256-270	73-263-309.1-315.1	U5a1a	1
189-193.1-195-234-256-270-311-330-	73-151-152-263-309.1-315.1	U5a	1
189-192-270	73-150-263-315.1	U5b1b1e	1
189-192-270-292	73-150-263-309.2-315.1	U5b1b1	1
93-189-194-195-270 -	73-150-263-315.1	U5b1b1c	1
192-270-304	73-150-228-263-315.1	U5b3	1
192-270-304	73-88.1-150-228-263-315.1	U5b3	1
356	73-195-263-310-	U4a2a	2
189-194-242-288-356-362	73-195-263-310-	U4a2c	1
179-356	73-195-235-263-315.1	U4c1	1
343	73-150-179-195-263-315.1	U3	1
261-343	73-150-263-315.1	U3	1
343-390	73-150-263-309.1-315.1	U3a	1
051-129-182-183-189-194-195-197-361	73-152-217-263-310-315.1	U2e	1
051-061-070-074-104-108-109-129-183-189-194-362	73-152-217-263-309.2-315.1-321-341-362-	U2e	1

044-050-051-082-091-129-179- 183-189-194	73-152-217-263-309.2-315.1	U2e	1
051-129-182-183-189-197	73-152-217-263-310-311-315.2- 324-	U2e	1
183-189-194-197-249	73-87-98-212-122-128-132-136- 141-150-160-163-171-178-285- 310	U1a	1
129-179-182-183-189-197-249- 263-304	73-263-285-309.2-315.1-362-366-	U1a	1
309 –318	73–152–263-309.1-315.1	U7	1
147G-172-223-248-355	73-199-204-263-315.1	N1a	1
147G-172-223-248-295-355	73-152-199-204-263-309.1-315.1	N1a	1
217-243-261	72-73-152-195-263-309.1-315.1	HV2	1
298-399	72-263-309.1-315.1	HVO	1
298-216	195-263-310-315.3	preV	1
126-148-309-318	73-146-151-152-195-263-309.1- 315.1	M37	1
	73-151-152-195-263-309.1-315.1	M37	1

* Mutations are shown indicating positions relative to the rCRS (Andrews, *et al.* 1999). The nucleotide positions in HVSI and II sequences correspond to transitions and transversions. The presence of insertions or deletions is indicated by .1, .2,.3 respectively, following the nucleotide position.

Table 2. Distribution (%) of the determined mtDNA haplogroups and subhaplogroups in the analyzed population

Haplogroup	N	Frequency
H	138	52,87
I	4	1,53
J	23	8,81
T	13	4,98
W	8	3,06
K	12	4,59
V	16	6,13
X	15	5,74
U7	1	0,38
U5a	6	2,29
U5b3	2	0,76
U5b1b	3	1,15
U4a2	2	0,76
U4a2c1	1	0,38
U4c1	1	0,38
U3	2	0,76
U3a	1	0,38
U2e	4	1,53
U1a	2	0,76
HVO	1	0,38
preV	1	0,38
HV2	1	0,38
M37	2	0,76

N1a	2	0,76
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Discussion

In subcluster diversification of the oldest haplogroup U5, we did not detect mtDNA haplotypes which clearly identify early clues of pre-LGM expansions and colonization of the area of Bosnia in the first migratory wave. However, there was found ancestral type of sequence 16192-16256-16270 (U5a1), with reverse mutation at 16192 np, whose evolutionary time dates 15,600-24,300YBP (according to Richards et al., 2000). The study Malarchuk et al., (2010) suggest that the coalescence time for subclusters U5a1 is about 16,000YBP, and for subclusters U5a1a about 14,800YBP. The observed subclusters U5a1 indicates the possibility that they were involved in the areas of the Northeastern Bosnia long time ago, probably at the end of LGM. The phylogeny U5b1 of the recent Bosnian population demonstrates the presence of younger subclusters of Mediterranean distribution (such as U5b1b1e—around 7,200YBP) and central European cluster (U5b1b1c around 7,200YBP) (Malyrchuk et al., 2010). Study Tambes et al., (2004) is suggested that the subcluster U5b1b spread to northern Europe from an Iberian source, via central/eastern Europe, in the post-LGM times, sometime after 15,000YBP. In addition to post-glacial re-expansion from the southwestern European refuges a significant impact on the medina pool of Bosnian population had the postglacial re-expansion during the Holocene from the refuge localized in Italian peninsula. Observed subclad U5b3 (coalescence time U5b3 are 10,000 YBP) is considered a potential signal of re-colonization from glacial refuge in Italian peninsula, whose expansion is linked to the Mediterranean coasts (Pala et al., 2009). A significant contribution to the genetic structure of the analyzed Bosnian population had dispersions of populations from the period of more recent historical events. All haplotypes U4a2 defined in our study include transition at np 310 in HVSI, which mostly prevailed in Slavic populations (in Russians and Poles) (Malyrchuk et al., 2008). The mtDNA lineages U4a2 and U4c1 are likely involved in the genetic structure of the Bosnian population by postglacial re-expansion of the Slavs from Eastern European refuge area. Besides detected mtDNA signals Late Glacial and postglacial expansion from the European refuge, area of the Northeast Bosnia has received traces of gene flow from East Asia (detected by the presence of M37), from the Anatolia (U7) and from Africa (N1a with 16147G). The haplogroup M37 represents a very important determinant of Bosnian population, because it is not a characteristic line of the European mtDNA flow. The origin of subhaplogroup M37 is India and the ages were estimated to be $28,300 \pm 11,900$ yr, respectively (Thangaraj et al., 2006). The presence U7 in the

Balkans suggests genetic flow into Europe via Anatolia (Tambes et al., 2003). A marking component of the genetic structure of recent Bosnian population is the most ancient eastern Africa/Southern Asia N1a lineage with the HV1 region variant 16147G. Haplogroup N1a has three phylogeographic subclusters: eastern Africa/Southern Asia which is characterized by control region motif 16147G and European/Central Asian characterized by control region motif 16147A (Palanichamy et al., 2010). The haplogroup N1a appeared on the European continent along with Linearbandkeramik (LBK) culture, which binds to the emergence of agricultural communes in Europe before 7,500 years (Burger et al 2006). Phylogenetic data indicate that the first European farmers were carriers of haplogroup N1, which is likely to spread from the Great Lakes region (East Africa) (Palanichamy et al., 2010). According to Palanichamy et al., (2010) all phylogeographic distribution of N1a subclusters and their coalescence time of 19,600-23,500 years suggest that the initial diversification of N1a appeared in the Near East followed by a westward dispersal of ancestral individual subhaplogroups in southern Europe and north-westward across the steppe zones of Central Asia towards Central Europe. The emergence of ancient eastern Africa/Southern Asia lineages N1a variant 16147G in the genetic structure of Bosnia population is the most significant feature of the Neolithic expansion in this area, which is also supported by the archeological data. Remains of the Starcevo culture found in this region (Tuzla region-Gornja Tuzla) is associated with sources of salt as places where ended one of expansion waves of the early Neolithic complex (Tasic, 2000). The fact that most of the Starcevo settlements in the Balkans were founded around the same time, the start of the sixth millennium (Tasic, 2000), might suggest in favor of the thesis that the early-Neolithic colonists needed a safe source of salts so as to settle in the Balkan area. The topology of the region of Northeast Bosnia and the resources of salt sites (Tuzla region), obviously was an extraordinary combination for the development of early Neolithic communities in this area. Based on the relatively archaeological relations, but also on the belief that for the first settlers in the area of Southeast Europe the discovery of salt sources was very important (Tasic, 2000), the observed ancestral haplotypes (with 16147G variant) suggest the early settlement of this region of Bosnia by Neolithic populations.

Conclusion

It is possible that the creation of such a mix of determinants of genetic structure the Bosnian population resulted from the original population heterogeneity of this region; such heterogeneity may have arisen during the beginnings of colonization, but also during the dispersal of newly arrived population. A previous studies which under different scopes have

covered the Balkan region, this study and future studies based on the analysis of the complete mitochondrial genome, represent a possible basis for a more accurate reconstruction of historical events and the potential scenario of settlement not only in the territory of Bosnia and Herzegovina, but also the Balkans.

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