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MtDNA VARIATION WITHIN LOCAL HUMAN POPULATIONS IN BOSNIA AND HERZEGOVINA

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Being a crossroad of many ancient and recent historical migrations, Bosnia and Herzegovina (B&H) represents unique spot of multicultural and social diversity. The main aim of this study was to assess genetic structure of three local populations of mountain area from central part of B&H using mtDNA HVS-1 as an informative marker for population genetics studies.

A 444 bp HVS-1 segment of control region of mtDNA extracted from buccal swabs was PCR amplified and sequenced. Haplotype and nucleotide diversity, average number of nucleotide differences, AMOVA and pairwise F_{ST} based on mtDNA haplotype and haplogroup frequencies were calculated. NJ tree was constructed based on pairwise F_{ST} results. Tajima's D was calculated to evaluate population demographic status.

Key words: human *mtDNA* diversity, *HVS1* motif, haplogroups, population structure

INTRODUCTION

Rural parts of B&H are characterized by the presence of numerous local semi-nomad communities characterized by accentuated geographical, ethnical, ecological, religious and cultural isolation (HADZISELIMOVIC *et al.,* 1981). There are a number of villages scattered in the B&H alpine area such as Bjelasnica-Treskavica located southwest from B&H capital Sarajevo inhabited with Bosniacs. Three populations observed in this study from Bobovica, Lukomir and Dejcici were agriculturally oriented with limited connection to the urban parts of country. Sharing ethnographic and geographic background, propagation isolation, migratory status

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(mostly emigration), similar population effective size, sex ratio and predominant patrilocality may have had an influence on their genetic structure. Interestingly, historical data also mention this region as a refuge of Bogumils (Bosnian heretics) during the period of intense Christianization from East (Orthodox) and West (Roman Catholic Church) and Islamization under the invasion of Ottomans. In that sense it is expected to find a haplogroup distribution that could be, at some extent, similar to population reality of medieval period. Furthermore, evident spatial isolation in recent history could also have had an important effect on the observed population genetic specificity (POJSKIC *et al.,* 2005). These three populations have been already observed and their level of isolation and genetic structure were investigated using sixteen *STR loci*. Comparison to 18 isolated populations from three Croatian Adriatic islands (Hvar, Brac and Korcula) revealed structural similarity of Croatian – island and Bosnian – mountain populations (MARJANOVIC *et al.,* 2004).

The main aim of this study was to dissect genetic structure and evaluate its nature and variation within local populations of Bobovica, Lukomir and Dejcici using mitochondrial DNA sequence as marker and make a comparison to available corresponding data for small and isolated local populations within the context of mitochondrial genetic variability of small local populations in the Southeast European region.

MATERIALS AND METHODS

Population. Study sample was consisted of 80 unrelated individuals from Bobovica, Lukomir and Dejcici - residing in the Bjelašnica-Treskavica mountain area, located around 40 kilometers southwest from Sarajevo (Figure 1).

Figure 1. Geographic map showing the location of study local human populations in Bosnia and Herzegovina

DNA isolation and analysis. Samples for genetic analysis were obtained from saliva specimens taken with sterile cotton swabs and transferred into labeled sterile microcentrifuge tubes where air-dried and kept until further analysis. Genomic *DNA* was isolated using Qiagen™ Dnaeasy Tissue Kit (Qiagen, Hilden, Germany). High quality *DNA* extract in quantity of 100 ng was used as a template in a 25µl PCR reaction volume containing 10mM Tris HCl pH=8.3, 50mM KCl, 1.5 mM $MgCl_2$, 0.2 µM each primer (forward*: 5′ TAACTCCACCATTAGCACC 3′* and reverse: *5′ CACGGAGGATGGTGGTCAAG 3′*) and 2.5 units of AmpliTaq DNA polymerase. Conditions for amplification of 444 bp *mtDNA* segment and sequencing (Big Dye terminator chemistry) were as described elsewhere (HOLLAND, 1993). Sequences containing *HVS-1* region between nucleotide positions 15971 and 16414 were consecutively aligned in BioEdit software implementing ClustalW alignment. 377 bp segment between nt 16024 and 16400 was checked against the CRS (Cambridge Reference Sequence) (ANDERSON *et al.,* 1981; ANDREWS *et al.,* 1999) for haplotype identification. Assignment of haplotypes to corresponding *mtDNA* haplogroups was done comparing the *HVS-1* sequences to motifs defined by MACAULAY *et al*., (1999) and reanalyzed matching to available haplogroup data sets (MALYARCHUK *et al.,* 2003; BABALINI *et al.,* 2005). For population structure comparisons data from BABALINI *et al.* (2005) was used.

Population genetics analysis. Haplotype and nucleotide diversity NEI (1987) and average number of nucleotide differences (TAJIMA, 1983) were calculated. Analysis of molecular variance (AMOVA) (EXCOFFIER *et al.,* 2005) was used to detect among-group (Bosnian and Croatian local populations, respectively), among-population within group and within-population proportion variation. Inter- and intra-population relations were estimated using average *pairwise* differences (NEI, 1987). Additionally, *mtDNA* haplogroup diversity of local Bosnian populations was estimated and compared to Croatian coastal and island local populations (BABALINI *et al.,* 2005). *Pairwise Fst* matrix was created from calculations with 1000 permutations using methods implemented in Arlequin 3.11. (EXCOFFIER *et al.,* 2005) and *Neighbor-Joining tree* (SAITOU and NEI, 1987) was drawn from this output.

Tajima's D (TAJIMA, 1989) is based on the probability of having a number of haplotypes greater or equal to the observed number in sample drawn from a stationary population. Since, this test can be considered as test of deviation of the observed data from neutral predictions expected in constant-size population, we used it to estimate the population deviation from equilibrium in sense of recent population expansion. For all above-mentioned calculations, Arlequin ver. 3.11 (EXCOFFIER and SCHNEIDER 2005) and MEGA3 (KUMAR *et al*., 2004) software was used.

RESULTS

The 377bp segment in the control region of *mtDNA* belonging to *HVS1* for 80 unrelated individuals was successfully obtained (Table 1). Total of 41 lineages was detected as defined by 59 substitution sites with 5 transversions. The most frequent haplotype (10%) found in all observed populations is motif 16343G-16390A belonging to haplogroup U3. It was also noted that 30% of all listed haplotypes occurred only once, 7.5% twice, 8.75% three times and 3.75% four times. The most frequent haplogroup – H (37.5%) was represented with nine different sequence motifs. Other detected haplogroups were in varying frequencies: U3 – 12.5%, V – 10%, W – 7.5%, K – 6.25%, J – 5% and U and I – 3.75%. Haplogroups J2, U4, X, U5b1, U5a1, B, M, T2 and C occurred once each (1.25%).

Table 1. MtDNA variability within Bosnian local human populations of three mountain villages from central part of the country

Population	No of reps	HVS-I Haplotype	Status at nt73	Haplogroup
Bobovica	1	16051 G 16312 G	A	H
	3	16069 T 16126 C 16209 C 16235 G	G	J
	3	16343 G 16390 A	G	U3
	3	16354 T	A	H
	3	16298 C	A	V
	1	16079 G 16162 G 16264 T	G	H
	1	16093 C 16224 C 16311 C 16362 C	G	K
	1	16111 T 16343 G	G	U3
	1	CRS	A	H
	1	16343 G	A	U3
	1	16129 A 16172 C 16223 T 16311 C 16319 A 16391 A	G	I
	1	16129 A 16145 A 16223 T 16391 A	G	I
	1	16153 A 16298 C	A	V
	1	16189 C 16356 C 16362 C	A	H
	1	16223 T 16292 T 16311 C	G	W
	1	16223 T 16292 T	G	W
	1	16269 G	G	U
	1	16304 C	A	H
Lukomir	3	16051 G 16312 G	A	H
	3	16343 G 16390 A	G	U3
	1	16129 A 16145 A 16223 T 16391 A	G	I
	1	16069 T 16126 C 16193 T	G	J2
	1	16092 C 16293 G 16311 C	A	H
	1	16179 T 16356 C	G	U4
	1	16189 C 16193.1 C 16223 T 16278 T	G	X
	1	16189 C 16193.1 C	A	H
	2	16217 C 16243 C 16261 T	G	U
	2	16223 T 16292 T 16311 C	G	W
	2	16223 T 16292 T 16295 T 16324 C	G	W
	1	16224 C 16304 C 16311 C	G	K

	1	16293 G 16311 C	A	H
	2	16304 C	A	H
	3	16311 C	A	H
	2	16362 C 16400 T	A	H
Dejcici	1	16051 G 16312 G	A	H
	2	16343 G 16390 A	G	U3
	2	16153 A 16298 C	A	V
	2	16311 C	A	H
	2	CRS	A	H
	2	16298 C	A	V
	1	16294 T 16362 C 16400 T	A	H
	1	16293 G 16311 C	A	H
	3	16224 C 16311 C	G	K
	1	16189 C 16270 T 16301 T	G	U5b
	1	16145 A 16189 C 16193.1 C 16193.2 C 16311 C	A	H
	1	16144 C 16189 C 16193.1 C 16270 T	G	U5b1
	2	16129 A 16182 C 16183 C 16189 C 16234 T	G	B
	1	16129 A 16223 16291 T 16298 C	G	M
	1	16126 C 16294 T 16296 T 16304 C	G	T2
	1	16063 C 16069 T 16126 C 16348 T	G	J
	1	16092 C 16261 T 16293 G 16311 C	G	H
	1	16093 C 16223 T 16234 T 16288 C 16298 C 16327 T	G	C
	1	16114 A 16192 T 16256 T 16270 T 16294 T	G	U5a1

Haplotype diversity ranges between 0.960 for Lukomir to 0.974 for Dejcici. Comparisons of these data with seven Croatian coastal and island local populations (BABALINI *et al.,* 2005) show similarity with Dejcici population having highest level of haplotype diversity (Table 2).

Average number of pairwise differences of three observed local human populations in B&H fit the range of Brac and Hvar (island) and Zivogosce (coast) Croatian local populations. The Bosnian populations show bell-shaped distribution of HVS-1 pairwise differences with negative and significant Tajima's D values. The nucleotide diversity indices within populations range from 0.011 for Bobovica to 0.013 for Dejcici (Table II). These values are higher than nucleotide diversity of costal southeast Croatian population, but in the range of islanders from Brac and Hvar (Table 2).

Table 2. mtDNA HVS-1 sequence and haplogroup variation in three observed local populations compared to small Croatian coastal and island populations

Population	n[a]	h[b]	h_s[c]	hg[d]	hg_d[e] ± SD	h_d[f] ±SD	π[g] ±SD	k[h]	Tajima's D[i]	P
Bosnians										
Bobovica	26	18	14	8	0.849±0.042	0.963±0.021	0.011±0.006	4.172±2.143	-1.742	0.031
Lukomir	27	16	8	9	0.749±0.079	0.960±0.018	0.012±0.007	4.348±2.218	-1.621	0.031
Dejcici	27	19	12	12	0.871±0.047	0.974±0.016	0.013±0.007	5.094±2.550	-1.941	0.013
Croatians/ coastal										
Krilo Jesenice	35	21	13	9*	0.684±0.079	0.960±0.017*	0.009±0.005*	3.422±1.792	-1.331	0.091
Mimice	24	17	11	10*	0.844±0.062	0.971±0.019*	0.010±0.006*	3.993±2.068	-1.591	0.049
Zaostrog	13	9	5	7*	0.897±0.054	0.949±0.042*	0.012±0.007*	4.615±2.421	-0.867	0.215
Zivogosce	24	14	9	8*	0.692±0.095	0.924±0.038*	0.008±0.005*	3.373±1.791	-1.573	0.051
Croatians/ island										
Brac	105	54	39	18*	0.843±0.023	0.968±0.008*	0.011±0.006*	4.265±2.131	-1.942	0.014
Hvar	108	51	31	16*	0.880±0.012	0.973±0.006*	0.014±0.008*	5.642±2.727	-1.429	0.069
Korcula	98	44	31	15*	0.626±0.055	0.944±0.013*	0.008±0.005*	3.335±1.727	-2.113	0.006

*[a]Sample size, [b]number of haplotypes, [c]number of single haplotypes, [d] number of observed haplogroups, [e]diversity of haplogroups, [f]haplotype (gene) diversity, [g]nucleotide diversity, [h]average number of nucleotide differences, [i]Tajima'D and corresponding P-value, *Data from Babalini et al.(2005)*

Average *pairwise* differences among local populations of Bosnians shows similar values for all observed populations with highest distance between Lukomir and Dejcici. *AMOVA* calculations clarified hierchical apportioning of mtDNA haplotypes variance in three observed Bosnian and Herzegovinian local populations (Bobovica, Lukomir and Dejcici) and Croatian local populations. This analysis shows significant variation within populations with 96.47% of total variance, while variation among populations is 3.05%. The same analyze among groups of local populations (Bosnian vs. Croatian local populations) showed 0.48% variation.

There is no significant *pairwise Fst* between observed local human populations in B&H (Bobovica vs. Lukomir, pFst=0.016 P=0.135; Bobovica vs. Dejcici, pFst=0.000 P=0.495; Lukomir vs. Dejcici, pFst= 0.02648 P= 0.090). When we compared these populations with Croatian coast and island populations (BABALINI *et al.,* 2005) we found significant *pairwise Fst* between Bobovica and Zivogosce (pFst=0.053, P=0.045), Hvar (pFst=0.030, P=0.000), Korcula (pFst=0.077, P=0.000), then Lukomir and Brac (pFst=0.024, P=0.045), Hvar (pFst=0.048, P=0.009) and finally between Dejcici and Korcula (pFst=0.056, P=0.000). Constructed *Neighbor-Joining tree* based on *pairwise Fst* shows similarity of Bobovica and Dejcici populations, since Lukomir population is closer to Croatian local populations with lower haplogroup diversity (Figure 2).

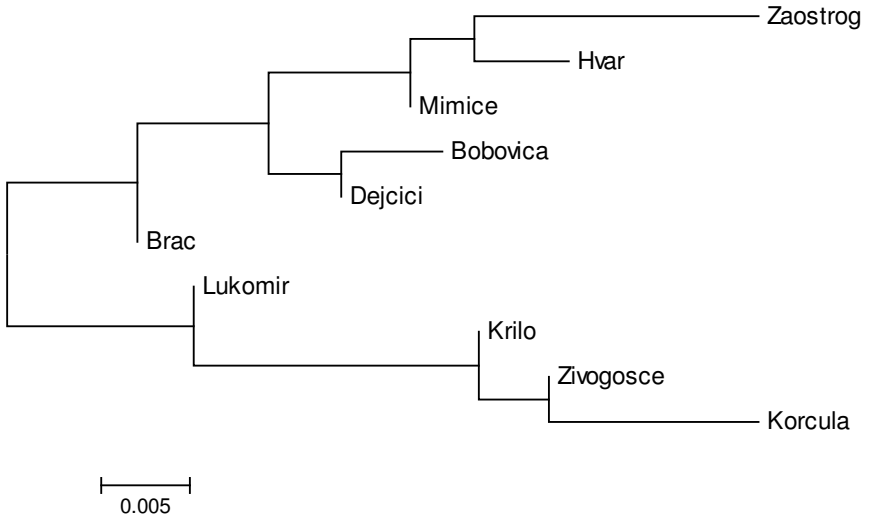

Figure 2. *Neighbor-Joining tree* based on matrix of *pairwise Fst* among Bosnian-Herzegovianian and Croatian coastal and island populations

DISCUSSION

Frequencies and distribution of defined haplotypes reveal typical pattern for European populations (RICHARDS *et al.,* 2000) and small local populations from coastal Croatia (BABALINI *et al.,* 2005). The most frequent haplogroup found in Bosnian sample, is of Near- East origin - haplogroup H. This outcome is in agreement with the results of mtDNA study involving northeast region of Bosnia and Herzegovina (AHMIC *et al.*, 2013). When compared to other reports for Balkan countries Slovenians and Bosnians (Croatian and Serbian origin) (MALYARCHUK *et al.,* 2003) and Croatian local populations (BABALINI *et al.,* 2005) the sample of local populations from B&H have some additional characteristics. Interestingly, occurrence of haplogroups B and C, that have East Asian origin, have not been observed in Bosnian population previously, but haplogroups M and T2 occured at lesser frequency, like was found by MALYARCHUK *et al.* (2003). Haplogroup M in both surveys was represented with same *HVS1* motif - 16129A-16223T-16291T-16298C, while L1, characteristic of African lineages that has been detected in previous studies of Bosnian sample (MALYARCHUK *et al.,* 2003) was not found within our study sample.

The number of single haplotypes and observed haplogroups within Bobovica, Lukomir and Dejcici populations is similar to coastal populations, since Tajima'D with significant P-value is more similar to island then coastal Croatian populations.

Diversity indices within Bobovica, Lukomir and Dejcici population are in the range both coastal as well as island Croatian populations (BABALINI *et al.,* 2005). This indicate that m*tDNA* diversity in three observed B&H local populations has characteristics of isolated populations which have been already detected across Adriatic coast and islands of Croatia. The same process has occurred in local populations in mountain area of B&H. The effect of emigration on genetics diversity within local human populations in Bosnia and Herzegovina have already studied using STRs (MARJANOVIC *et al.*, 2004), Y-chromosome haplogroups (MARJANOVIC *et al.*, 2005) and Alu insertions (POJSKIC *et al.*, 2013). Although, such indicators could also suggest that reduced population size does not necessarily implies on rapid reduction of gene diversity in local population. Nevertheless, the influence of small size of local populations on their *mtDNA* diversity must not be ignored and lower gene diversity could be result of the effect inbreeding rather than isolation, as it is previous described by BABALINI *et al.,* (2005).

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*Mt*DNK VARIJACIJA UNUTAR LOKALNIH LJUDSKIH POPULACIJA U BOSNI I HERCEGOVINI

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Izvod

Bosna i Hercegovina u prošlosti je bila raskrsnica migracijskih puteva ljudskih populacija. Takvi događaji ostavili su traga i u genetičkoj strukturi bh. stanovništva. *MtDNK* regoni su informativni markeri u populacijsko-genetičkim studijama. Koriste se ponajprije za određivanje genetičkog porijekla ljudskih skupina, kao i za procjenu filogenetičkih odnosa. Izolirane populacije su u tom smislu vrlo zanimljive, jer zbog svoje veličine, odnosa polova, inbridinga, te smanjenog stepena migracija mogu biti značajne u uočavanju različitih populacijsko-genetičkih fenomena. Takve su bosansko-hercegovačke planinske populacije Bobovice, Lukomira i Dejčića koje su analizirane sa namjerom određivanja i uočavanja potencijalno zanimljivih promijena i procesa koje se događaju na nivou *mtDNK* unutar i između populacija. Analizirane populacije su po pitanju haplotipskog diverziteta *HVS-1 mtDNK* regiona uspoređene sa izoliranim hrvatskim ostrvskim, kao i sa kopnenim priobalnim. Haplotipski diverzitet bh. populacija kreće se u rasponu od 0,960 (Lukomir) do 0,974 (Dejčići). Frekvencija i distribucija haplotipova analiziranih bh. populacija odgovara obrascu većine evropskih populacija. Najfrekventnija haplogrupa analiziranog uzorka je haplogrupa H (37,5%). Haplogrupe istočnoazijskog prefiksa potvrđene su u vrlo niskim frekvencijama, dok afrički signal u ovoj i prethodnim studijama nije potvrđen. Uočeni *mtDNK* diverzitet vrlo je sličan prethodno analiziranim izoliranim hrvatskim ostvrskim i priobalnim populacijama. Uočeni nukleotidni diverzitet analiziranih bh. populacija nešto je viši u poređenju s hrvatskim populacijama obalnog pojasa, a odgovara stanju populacija unutrašnjosti ostrva Brača i Hvara. Prosječni *pairwise Fst* među analiziranim lokalnim bh. populacijama pokazuju slične vrijednosti za sve analizirane populacije, sa najvećom diferencijacijom između Lukomira i Dejčića. Nisu zabilježene značajne vrijednosti *pairwise Fst-a* među analiziranim bh. lokalnim populacijama. Broj unikatnih haplotipova, kao i uočenih haplogrupa unutar tri bh. populacije odgovaraju priobalnim populacijama, dok *Tajima'D* sa statistički značajnom vrijednošću više sliči ostvrskim nego obalnim hrvatskim jadranskim populacijama. Iz svega navedenog se može zaključiti da pokazatelji diverziteta unutar populacija Bobovica, Lukomir i Dejčići su u opsegu ostvrskih i priobalnih kopnenih populacija. To ukazuje da planinske izolirane populacije u Bosni i Hercegovini imaju iste genetičke karakteristike izoliranih populacija detektovanih u prethodnim studijama u priobalnom i ostvrskom području Republike Hrvatske. No, isto tako, pokazatelji *HVS-I* mtDNK diverziteta ukazuju na činjenicu da reducirana veličina populacije ne dovodi nužno do smanjenog genetičkog diverziteta. Ipak se uticaj malog uzorka lokalne populacije na mtDNK diverzitet ne smije zanemariti, te manji genetički diverzitet može biti usljed povećanog inbridinga prije nego same izolacije

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