

# Historical Overview of the Human Population-Genetic Studies in Bosnia and Herzegovina: Small Country, Great Diversity

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## ABSTRACT

*Modern Bosnia and Herzegovina is a multinational and multi-religious country, situated in the western part of the Balkan Peninsula in South-eastern Europe. According to recent archaeological findings, Bosnia and Herzegovina has been occupied by modern humans since the Palaeolithic period. The structure of Bosnia-Herzegovina's human populations is very complex and specific, due to which it is interesting for various population-genetic surveys. The population of Bosnia and Herzegovina has been the focus of bio-anthropological and population genetics studies since the 19<sup>th</sup> century. The first known bio-anthropological analyses of Bosnia-Herzegovina population were primarily based on the observation of some phenotypic traits. Later examinations included cytogenetic and DNA based molecular markers. The results of all studies which have been done up to date showed no accented genetic difference among the populations (based on geographical regions) with quite high diversity within them. Human population of Bosnia and Herzegovina is closely related to other populations in the Balkans. However, there are still many interesting features hidden within the existing diversity of local human populations that are still waiting to be discovered and described.*

**Key words:** human populations, population genetic studies, molecular markers, Bosnia and Herzegovina

## Introduction

Due to its position and complex historical events, modern Bosnia and Herzegovina is multi-ethnic and multi-religious country with lots of smaller, isolated populations. It has always been the place where different civilisations would collide, which resulted in numerous migrations and constant mixing between various populations. All of this makes it very interesting area for population-genetics studies.

Recent archaeological studies from northern Bosnia indicate that first populations had inhabited this area in Palaeolithic period, ~100 000 years ago and they belonged to *Homo neanderthalensis*<sup>1</sup>. The earliest credible evidence of *Homo sapiens* in Europe is dating ~45 000 years ago. They apparently represent a population movement into the Balkans during a warm climate interval<sup>2</sup>. For the next period, which lasted for tens of thousands of years, there was no long absence of life in this area<sup>1</sup>.

In the early Bronze Age around 4<sup>th</sup> century BC, this region was populated by the Illyrians, which established probably the first known civilization in this part of Europe<sup>3</sup>. The name »Illyrians« was given by the ancient

Greeks to their northern neighbours, but it is not clear if they had been linguistically and culturally homogeneous or ever collectively regarded themselves as »Illyrians«<sup>4</sup>. Even though the origin of Illyrians has been discussed through history, it is believed that Illyrian tribes had been settled continuously in this area between the Bronze and succeeding Iron Age<sup>5</sup>.

From the end of 3<sup>rd</sup> century BC Romans began to emerge in this area<sup>1</sup>. They haven't conquered Illyrians at once, but rather it was the beginning of one hundred year long war, thence on, this area was controlled by Romans for more than half a millenium<sup>6</sup>. Although the conquered natives belonged to one category and Roman settlers to other<sup>1</sup>, during that time Latin speaking settlers from all over the Empire settled among the Illyrians and Roman soldiers were encouraged to retire in the region<sup>7</sup>.

The ancient Bosnia and Herzegovina populations were introduced to Christian faith rather early. The earliest literature data on the presence of new faith in this area are dating back to the 4<sup>th</sup> century AD. It is believed that the Slavs received the Christianity from indigenous, rath-

er than foreign missionaries<sup>1</sup>. Following history, including two mayor events along with the number of minor historical episodes, laid the foundations for what is contemporary structure of B&H human population. The first one is the large migration of people during 6<sup>th</sup> and 7<sup>th</sup> centuries, which led to the final collapse of Roman Empire and moved different Gothic and Slavic clans into the area. The second one, which greatly influenced the region of today Bosnia and Herzegovina, is the expansion of Ottoman Empire into this part of Balkan in the fifteen century and so caused numerous migrations during the following half of millennium<sup>8</sup>.

Last century was not less interesting regarding historical events in this area. Two world wars and war in Bosnia and Herzegovina between 1992 and 1995 launched many migrations including those within today's country border<sup>9</sup>.

The historical events left a big mark on the structure of today B&H population and made it attractive for population-genetic surveys. This paper gives a short historical review of previously published studies with new data of the latest population-genetic surveys of different levels and approaches.

### ***Previous bio-anthropological studies in B&H human population***

The first known bio-anthropological analyses of the population conducted by the Austro-Hungarian army doctor can be considered an initial phase in determining genetic structure<sup>10</sup>. In 1887, Austro-Hungarian doctor Himmel examined the specific phenotypic characteristics (average height, weight, eye colour, hair colour, and other anthropological – body measures) in a population of Bosnian soldiers, members of the Austrian army<sup>11</sup>. Later, Weisbach (1895, 1905) conducted tests of phenotypic markers in the population of Bosnia and Herzegovina and was the first to make a comparison of the observed phenotypic systems within the three constituent nations of Bosnia and Herzegovina<sup>12,13</sup>. However, the first exact results of human population-genetic studies were obtained in 1930's using analysis of genotype frequencies of blood group systems<sup>14,15</sup>.

During the 20<sup>th</sup> century, in the period 1960–1980, intensive efforts were made to study the diversity of morphologically complex phenotypic systems of qualitative and quantitative traits within the local, regional and ethnically defined human populations in Bosnia and Herzegovina<sup>16–33</sup>.

Period between 1980 and 1990 was the time of intense research<sup>34–40</sup>. More sophisticated studying approach has been employed, such as the analysis of genealogy and genetic distance of 7100 included subjects. Data on overall 15 morphological, biochemical and physiological markers were obtained: ABO blood groups, ABH antigen secretion, PTC tasting, red colour vision, green colour vision, ear lobe type, fissured tongue, chin dimple, midphalangeal hairiness, nail form, position of distal phalange of the

little finger, digital index, tongue rolling, distal and proximal extensibility of the thumb<sup>36</sup>. In general, in the group of local populations studied, relatively low levels of genetic heterogeneity and differential genetic specificity were found. The effects of genetic drift and the high degree of propagational isolation were considered the most possible causes of such findings.

Research on phenotypic systems has continued into the 21<sup>st</sup> century. Genetic structure of both pre-war and post-war populations in Bosnia and Herzegovina were compared based on the analysis of ten qualitative traits. Large migration has been taken as a turning point and their effects on the structure of the small populations were observed<sup>41</sup>.

Simultaneous monitoring of genetic diversity of a large number of carefully selected phenotypic characteristics in population-genetic studies may result in significant information about the micro-changes in the genetic structure of observed populations<sup>42</sup>.

### ***Molecular genetic markers used for human population genetic studies in B&H***

After a pause which took place during 1992–2000, research process on local human populations in B&H within the context of its demographic structure, ethnic and cultural milieu has been continued. Revitalisation and modernisation of the capacities for population-genetic research enabled the application of highly sophisticated genetic markers for detection of human population variation. The main objectives of those studies were to determine the diversity of local human populations, especially isolated ones, as well as three main ethnic groups (Bosnian Bosniaks, Bosnian Serbs and Bosnian Croats).

The first molecular genetic analysis of Bosnian population included an analysis of autosomal STR loci as well as analysis of HV1 and HV2 region of mitochondrial DNA in isolated populations of rural areas<sup>43–47</sup>. The results of these studies indicated that genetic variation is not diminished in these isolated populations and analysis of genetic distance obtained from DNA typing showed consistency with initial hypothesis based on ecological, geographical, social and cultural factors.

In 2005, Marjanovic et al. published a paper on 28 Y-chromosome biallelic markers within 256 males (90 Croats, 81 Serbs and 85 Bosniaks) from Bosnia and Herzegovina, with the aim of providing new clues about their origin and the ancient events of gene flow which have influenced this area located in the heart of Europe<sup>48</sup>. High frequencies of hg I-P37 were observed in all three ethnic groups. The highest frequencies were observed in the population of Croats (~71%) and could be partially attributed to genetic drift and founding events on this ethnic group. High frequencies were also observed in the Bosniaks (~44%) and Serbs (~31%). This shows that different ethnic groups in Bosnia and Herzegovina share a large subset of their paternal lineages, affected by a major demographic event, the post-LGM expansion. A population

with a high frequency of I-P37 from one of the refuges, located possibly in the Balkans, played a great role in the peopling of Bosnia and Herzegovina and surrounding areas.

Y STR marker analysis was also conducted on 100 unrelated male individuals from different regions of Bosnia and Herzegovina. Distribution of allele frequencies for 12 Y-chromosomal STR was analyzed. Eighty-one different Y-STR haplotypes were detected: 69 of them were unique, 7 appeared twice, 4 appeared three and only 1 five times<sup>49</sup>.

Population genetic research of representative sample of Bosnians and Herzegovinians were continued in 2006<sup>50</sup>. Allelic frequencies for the 15 STR loci included in the PowerPlex 16 kit were obtained from a multi-ethnic sample of 100 unrelated individuals born in Bosnia and Herzegovina. The authors compared B&H data with data obtained from geographically closer European populations in order to put molecular genetic diversity of B&H into regional and European context.

Also, complex studies involving the application of various molecular genetic markers to study the genetic structure of human populations in Bosnia and Herzegovina have been performed<sup>51</sup>. In order to investigate concordance of different genetic markers, Pojskic et al. used 10 Alu loci, 15 autosomal STR loci and 6 blood groups (genotyped using PCR-RFLP and PCR-SSP methods). Also, polymorphism of 10 phenotypic traits have been estimated within 300 individuals in human populations placed in 10 regions across Bosnia and Herzegovina. The main goal was to compare validity of human population genetic analyses implementing different type of genetic markers. Modest correlation between results of population genetic analyses based on different markers has been detected, especially between biallelic markers. Strong and clear correlation between biallelic and multiallelic markers has not been detected<sup>52</sup>.

Extensive study based on molecular-genetic typing of various blood group systems had been done in 2010<sup>53</sup>. Six blood group systems (Rh, MN, Duffy, Kidd, Kell and Lutheran) were investigated among three ethnical groups as well as ten regional subpopulations of Bosnia and Herzegovina, with some of these blood group systems typed for the first time in the population of Bosnia and Herzegovina. This study showed that there is no accentuated differentiation between geographically defined populations in Bosnia and Herzegovina, and there are no significant genetic differences between the three main ethnic groups. These results are in agreement with results of previous studies, which indicates that three ethnical subpopulations of Bosnia and Herzegovina belong to the same gene pool and have the same origin.

The mitochondrial DNA (mtDNA) polymorphisms, determined in terms of mtDNA haplogroups, were analysed in many studies of B&H populations. One of these studies included a total sample of 245 unrelated individuals from the area of North-eastern Bosnia (also known as Tuzla region). The study revealed that 95.51% of the analyzed individuals belonged to the typical Western-Eurasian hap-

logroups: H, I, J, K, T, U, V, W or X. The most frequent haplogroup in the analysed population was the haplogroup H (52.65%) which, due to its increased frequency, represents a marking haplogroup of the population of North-eastern Bosnia. The results of intergroup genetic analysis showed that Bosnian-Herzegovinian population is genetically closer to previously studied populations of Herzegovinians (part of Bosnia and Herzegovina), Slovenians and Croats in relation to other neighbouring populations located in South-eastern Europe. This study also suggests that population genetic structure of Tuzla region is dominated by mutations that are classified as »Palaeolithic«. These mutations were probably brought to the area of North-eastern Bosnia through waves of prehistoric and historic migrations, but the impact of any pre-Neolithic, Neolithic or some »later« migrations, with a slightly lower contribution to the genetic structure of this population, also can-not be neglected<sup>54</sup>.

In 2014, population genetic study focusing on mtDNA haplogroups in Bosniaks was done<sup>55</sup>. This study included 227 samples from different geographical regions across Bosnia and Herzegovina (Eastern Bosnia, Middle Bosnia, Central Bosnia, Sarajevo region, Central Herzegovina, Eastern Herzegovina, and Krajina). This research has shown somewhat different frequency distributions of the observed haplogroups in the analysed regions. Statistically significant difference was not recorded, meaning that the geographical distribution has no differentiation character in the population of Bosniaks in Bosnia and Herzegovina. Since no genetic differences between the seven observed regions (populations) were observed, the conclusion is that the distribution of mtDNA haplogroups is not geographically conditioned. This research has confirmed the assumption based on the previous human population genetic studies in Bosnia and Herzegovina that the Bosnian population (hence the Bosniak population) is specific, relatively heterogeneous, without a significant differentiation from the characteristics of the European gene pool.

Primorac<sup>56</sup> analysed 180 samples from unrelated volunteers in Herzegovina. Populations were divided into three regions according to geographical characteristics: Central Herzegovina with Neum, Eastern Herzegovina and Western Herzegovina, as well as three ethnic groups: Bosniaks, Bosnian Croats and Bosnian Serbs. Total of nine Western-Eurasian haplogroups (i.e. H, I, J, K, T, U, V, W and X) were evenly distributed in three geographical regions. In relation to ethnic groups, Bosniaks displayed a significant increase in the frequency of haplogroup H while Bosnian Serbs showed significantly increased frequency of haplogroup U. The difference in the frequency can be explained as the result of genetic drift and increased emigration of the population in this area. Historically, this difference can also be explained by the mild climate which enabled survival of the tribes after end of the last Ice Age and by an interesting history of Herzegovina which was independent until the arrival of the Ottoman Empire. The frequencies of haplogroups in total

Herzegovinian population were in accordance with European average<sup>56</sup>.

In 2013, Pojskić et al. published a paper on Alu polymorphisms in 10 geographical regions across Bosnia and Herzegovina (Krajina, Posavina, North-Eastern Bosnia, Central Bosnia, Middle Bosnia, Western Herzegovina, Sarajevo Region, Eastern Bosnia, Central Herzegovina, Eastern Herzegovina). The sample comprised of 278 Bosniaks, 134 Bosnian Croats and 94 Bosnian Serbs. Conclusion of this study was that the results are in agreement with most previous human population studies in Bosnia and Herzegovina, indicating that the three populations of Bosnia and Herzegovina have the same genetic background. Also, there was no statistically significant differentiation among regional populations, pointing to the absence of geographic influence. Therefore, authors concluded that the whole population of Bosnia and Herzegovina is homogeneous considering Alu polymorphisms and there is no scientific basis for its genetic stratification<sup>57</sup>.

Another great study involving the investigation of polymorphisms of autosomal SNP markers in human populations of the Western Balkan Peninsula was published in 2014<sup>58</sup>. In this study, the genetic variation of 660000 autosomal SNPs, mitochondrial DNA markers and non-recombining part of Y chromosome was assessed in three ethnic groups from Bosnia and Herzegovina (Bosniaks, Bosnian Croats and Bosnian Serbs) and four other Slavic-speaking Western Balkan populations: Serbians, Croatians, Macedonians from the republic of Macedonia, Montenegrins, and Albanian-speaking Kosovars. Comparison of the variation within autosomal and haploid data sets of

studied Western Balkan populations revealed their genetic closeness regardless of a genetic system inspected, in particular among the Slavic speakers. In a more general perspective these results reveal clear genetic continuity between the Near Eastern and European populations, lending further credence to extensive, likely multiple and possibly bidirectional ancient gene flows between the Near East and Europe, cutting through the Balkans<sup>58</sup>.

In this respect, Bosnia and Herzegovina represents a unique region and source of genetic information discovered previously, but also, yet to be discovered in future studies.

## Conclusion

Due to the complex historical background and migration events in Balkan region, overall B&H population shows quiet high degree of diversity between its regional subpopulations, considering specific genetic characteristics of isolated populations. There is no major genetic difference between three main ethnic groups in Bosnia and Herzegovina (Bosniaks, Bosnian Serbs, and Bosnian Croats) indicating that they represent the same gene pool. Comparison of B&H population with neighbouring and other European populations shows no significant genetic difference between them. These findings clearly suggest that all three ethnic groups and total population of B&H belong to the European gene pool. This conclusion suggests that any differences between three main ethnic groups in Bosnia and Herzegovina do not have the genetic background but are primarily of social and cultural manner.

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## POVIJESNI PREGLED GENETSKIH ISTRAŽIVANJA POPULACIJE BOSNE I HERCEGOVINE: MALA ZEMLJA, VELIKA RAZNOLIKOST

### SAŽETAK

Moderna Bosna i Hercegovina je višenacionalna i multi-religiozna zemlja, nalazi se na zapadnom dijelu balkanskog poluotoka u jugoistočnoj Europi. Prema posljednjim arheološkim nalazima, Bosna i Hercegovina je okupirana od strane modernih ljudi od razdoblja paleolitika. Struktura ljudske populacije Bosne i Hercegovine je vrlo složena i specifična, zbog čega je zanimljiva za razna populacijsko-genetička istraživanja. Stanovništvo Bosne i Hercegovine je u fokusu bio-antropoloških i populacijskih (genetika) studija još od 19. stoljeća. Prva poznata bio-antropološka analiza bosansko-hercegovačkog stanovništva prvenstveno se temeljila na promatranju nekih fenotipskih osobina. Kasniji pregledi su uključili citogenetske i DNA molekularnih markera u istraživanja. Rezultati svih studija koje su do sada napravljene do sada nisu pokazale značajnu genetsku razliku među populacijama (na temelju geografskih regija) s vrlo visokom raznolikošću unutar njih. Ljudsko stanovništvo Bosne i Hercegovine je usko povezano s drugim populacijama na Balkanu. Međutim, još uvijek postoje mnoge zanimljive značajke skrivene unutar postojeće raznolikosti lokalnih populacija koje još uvijek čekaju da budu otkrivene i opisane.