



Short population report

HLA profile of the Macedonian, Albanian and Macedonian Muslim donors in the Macedonian Bone Marrow Donor Registry

Meri Kirijas*, Olivija Efinska Mladenovska, Olgica Sibinovska, Boban Dobrevski, Teodora Brnjarchevska, Aleksandar Petlichkovski

Institute of Immunobiology and Human Genetics, Medical Faculty, University “Ss Cyril and Methodius”, Skopje, Republic of North Macedonia

A B S T R A C T

The aim of this study was to determine the HLA allele and haplotype frequencies of the volunteer donors from the Macedonian Bone Marrow Donor Registry (MBMDR). We analyzed 1541 donors, from different nationalities and presented the HLA allele and haplotype frequencies for Macedonian, Albanian and Macedonian Muslims, most numerous nationalities in MBMDR. Difference between the three groups was observed for allele frequencies in HLA-C and HLA-DRB1 loci. The most common haplotype in Macedonian was HLA-A*01-B*08-C*07-DRB1*03, while in Albanian and Macedonian Muslims HLA-A*02-B*18-C*07-DRB1*11. This study confirmed the close relationship between the populations that live in the Balkan Peninsula.

Republic of North Macedonia is located in south-east Europe, in the middle of the Balkan Peninsula with the neighboring Serbia and Kosovo in the north, Bulgaria in the east, Greece in the south and Albania in the west. It has around 2 million inhabitants, majority of which are with Macedonian nationality (64.18%), followed by Albanians (25.17%), Turks (3.85%), Roma (2.66%) and others [1].

The Macedonian origin goes back to the ancient Macedonians, people who lived between northern Greece (Thessaly) and Trace in the Balkans [2]. They were considered “non-Greek barbarians” and were never admitted to the Greek community, they did not speak Greek language rather another presently unknown language [3]. The Macedonian empire had its biggest expansion during the reign of Alexander the Great, from the Balkan Peninsula to the Himalayas and to North Africa [4]. In the next period, Macedonia was conquered by the Romans, Byzantium, Slavs and Bulgarian. Ottoman Turks controlled Macedonia for almost five centuries (1380–1912 CE) in which period the Christian population mostly fled to the mountains. After the World War II, Macedonia became part of the Socialist Federal Republic of Yugoslavia, and after the referendum in 1991 Macedonia gained its independence. Macedonians speak Macedonian language, member of the group of South Slavic languages and most of them are Orthodox Christians.

Albanians are the largest minority in the Republic of North Macedonia [1] and they mostly live in the western part of the country. It is believed that the ancestors of the actual Albanians are the South Illyrians, although mixtures with other ancient populations, such as Thracians, might have occurred [5]. After World War I and II, the territory inhabited by Albanians was divided among Albania, Macedonia

and Kosovo (Yugoslavia). They speak Albanian language, separate branch of the Indo-European language tree and are mostly Muslims.

Macedonian Muslims are a minority religious group within the community of ethnic Macedonians who are Muslims. They have been culturally distinct from the majority of Orthodox Christian Macedonian for centuries and are ethnically and linguistically distinct from the larger Muslim ethnic groups: Albanians, Turks and Roma [6]. They mostly live in the villages in western North Macedonia, speak Macedonian language, but mostly mate with other Muslim groups.

In this study we analyzed 1541 healthy individuals, enrolled in the Macedonian Bone Marrow Donor Registry (MBMDR). These individuals are volunteer donors that signed informed consent and became members of the MBMDR. This sample included 757 male (49.13%) and 784 female (50.87%) donors, age range between 18 and 60. The nationality was self-stated in the informed consent and 1283 were declared Macedonian (Orthodox), 128 Albanians, 76 Macedonian Muslims, 21 Turks, 6 Roma, 4 Bosnians, 1 Serbian and 22 didn't declare any nationality. In this study we presented the results for three groups: Macedonian, Albanian and Macedonian Muslims in MBMDR.

DNA was isolated from EDTA blood samples using *DuplicaPrep*, EuroClone S.p.A and was stored at the Institute of Immunobiology and Human Genetics, Skopje, Republic of Macedonia. HLA typing for HLA-A, -B, -C and -DRB1 was performed using the SSO method (ONE LAMBDA, INC, USA) according to the manufacturer's instructions.

Hardy-Weinberg equilibrium for all loci and allele frequencies was calculated using the Arlequin 3.5 software [7]. Haplotype frequencies were calculated using the Expectation-Maximization (EM) algorithm for unknown gametic phase as implemented in the Arlequin software. We

* Corresponding author at: Institute of Immunobiology and Human Genetics, Medical Faculty, 1109 Skopje, PO Box 60, Republic of North Macedonia.
E-mail address: meri.kirijas@medf.ukim.edu.mk (M. Kirijas).

calculated the Nei's genetic distance for HLA-A, -B and -DRB1 for the Macedonian, Albanian and Macedonian Muslims in MBMDR and 20 European population and unrooted tree was constructed using the Neighbor-Joining method implemented in the Phylip software. D. Middleton's website, www.allelefrequencies.net, was used for most of the data for the other population, accessed on October 2017 [8]. The populations in MBMDR were compared to these populations: Serbian (1992), Albanian (160), Bulgarian (55), Bosnian (134), Croatian (4000), Greece_pop7 (11250), Turkey_pop2 (228), Slovenia_pop3 (130), Austria (200), Albanians_Kosovo (120), Italy (159311), Hungarian (1644), Romania (348), Germany (11407), France Marseille (1000), Portugal Lisbon (17420), Russia Moscow (2650), Sweden_pop4 (966), Norway_pop2 (576) and Czech Republic Gypsy_pop2 (46).

All HLA data, allele and haplotype frequencies and genotype data for all three population in MBMDR are available in the Allele Frequencies Net Database under the population name Macedonia MBMDR – Macedonian, reference number 3613, Macedonia MBMDR – Albanian, reference number 3612 and Macedonia MBMDR – Macedonian Muslims, reference number 3611 [8].

The investigated population was in Hardy-Weinberg equilibrium ($p > 0.05$) for all four analyzed loci for the Macedonian, Albanian and Macedonian Muslims (Supplementary Table S1). The allele frequencies for HLA-A, -B, -C and -DRB1 for Macedonian, Albanian and Macedonian Muslims are shown in Supplementary Table S2. In HLA-A and HLA-B there was no difference in the most frequent allele groups in the 3 analyzed population. In HLA-A locus, HLA-A*02, A*01 and A*24 were the most frequent and HLA-B*35, B*18 and B*51 in HLA-B locus in all three groups. HLA-C*07, HLA-C*04 and HLA-C*12 were the most frequent in Macedonians and Albanians, while in Macedonian Muslims we observed different profile with HLA-C*07, HLA-C*04, HLA-C*02 and HLA-C*15 being the most frequent. In Macedonians and Albanians HLA-DRB1*11, DRB1*16 and DRB1*13 were the most common, whereas in Macedonian Muslims HLA-DRB1*11 and HLA-DRB1*16 were followed by HLA-DRB1*14.

We identified 907 different HLA-A-B-C-DRB1 four-loci haplotypes in Macedonian, 140 in Albanian and 74 in Macedonian Muslims. The most frequent in Macedonian was HLA-A*01-B*08-C*07-DRB1*03 (4.0%)

while HLA-A*02-B*18-C*07-DRB1*11 was the most frequent in Albanian and Macedonian Muslims (4.6% and 6.8%, respectively). All haplotype frequencies are shown in Supplementary Table S3. The comparison between the population in MBMDR and other European population is shown in the dendrogram on Supplementary Fig. 1. The results show the close relationship between the populations in the Balkan Peninsula.

There were previously described studies for HLA haplotypes in the Macedonian population. The first one included 172 individuals [9] and the second 286 [10] based on a family study. We observed that the allele and haplotypes frequencies are very similar in all studies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.humimm.2019.07.277>.

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