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HLA-A, -B, -C and -DRB1 allele and haplotype frequencies in the Macedonian population based on a family study

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ABSTRACT

Aim: The aim of this study was to determine HLA allele and 2-, 3- and 4-loci haplotype frequencies in a sample from Macedonian population with defined haplotypes based on family history.
Material and Methods: We analysed 286 unrelated individuals with Macedonian origin, parents of patients who needed stem cell transplantation, in the period of 01.01.2003 till 31.12.2016. Allele and haplotype frequencies, as well as Hardy-Weinberg equilibrium were calculated using the Arlequin3.5 software. Population comparison was calculated using the PHYLIP software.
Results: We identified 18 HLA-A, 26 HLA-B, 13 HLA-C and 13 HLA-DRB1 allele group families. The most frequent allele groups in our population were HLA-A*02 (29.0%), HLA-A*24 (13.8%), HLA-B*35 (16.1%), HLA-B*51 (14.7%), HLA-B*18 (14.7%), HLA-C*07 (27.9%), HLA-DRB1*11 (25.5%) and HLA-DRB1*16 (14.8%). The most frequent four loci haplotype was HLA-A*01-B*08-C*07-DRB1*03 (2.7%). Our comparison showed that the Macedonian population is closely related to the neighbouring countries in the Balkan Peninsula.
Conclusion: This study provides data about the HLA diversity in the Macedonian population, which can be very important in the process of unrelated donor search, and in addition yields control group for future disease association studies in our population.

1. Introduction

HLA genes are group of genes located on the short arm of the 6th chromosome (6p21.3), whose protein products mediate human responses to infective diseases and the outcome of tissue and organ transplantation [1,2]. According to their function, the genes in this system are divided in three regions, HLA class 1, class 2 and class 3. Due to the abundant polymorphism of these genes, and in order to avoid graft versus host disease, HLA typing is a necessity in the process of stem cell transplantation. The minimum number of loci required for successful stem cell transplantation is 4, HLA-A, HLA-B and HLA-C in class 1 and HLA-DRB1 in class 2 [3].

The frequency of the HLA alleles in different populations is a point of interest for many population studies, to determine the human history and the mechanisms of molecular evolution [4]. Equally important are the association studies with different diseases and the organization of network of national registries of bone marrow volunteers in order to facilitate the process of matched donor search [5].

The Republic of Macedonia has around two million inhabitants, most of which are declared Macedonians (6418%) [6]. The Republic of Macedonia is located in south-east Europe, in the middle of the Balkan Peninsula and its borders with Serbia and Kosovo on the north, with Bulgaria in the east, with Greece in the south and with Albania in the west (Fig. 1). Due to its location, it became a crossroad for many people and civilizations throughout history. There were always admixtures between the different populations present in this region, so it is very interesting to determine the HLA allele frequency of the Macedonian population and their association with the neighbouring and other European populations.

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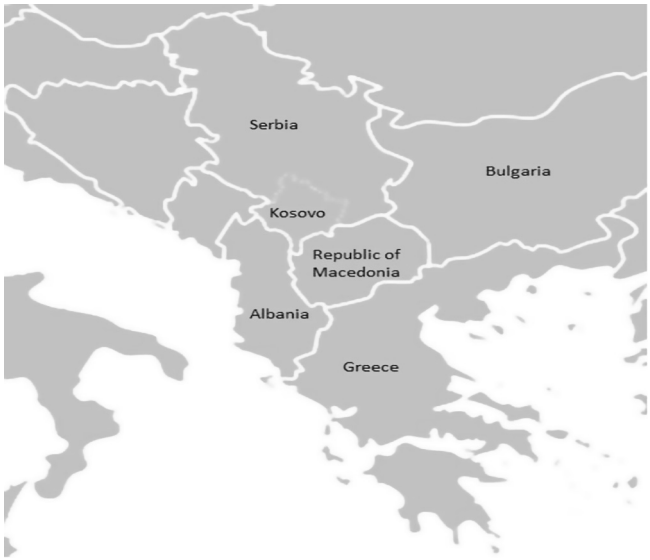

Fig. 1. Map of Republic of Macedonia and neighbouring countries in the Balkan Peninsula.

The aim of our study was to determine HLA allele frequencies, as well as 2-, 3- and 4-loci haplotype frequencies in the Macedonian population based on a family study.

2. Material and methods

2.1. Population

In this study we analysed 286 healthy, unrelated individuals with Macedonian origin. They are parents of the patients sent to the Institute of Immunobiology and Human Genetics, Skopje for HLA typing for potential transplantation of hematopoietic stem cells. Due to the known family history, the samples are with defined haplotypes. The samples are collected in the period from 01.01.2003 till 31.12.2016.

2.2. Methods

Signed consent was obtained from all individuals and DNA was isolated from periphery blood with standard phenol-chloroform method of isolation [7]. All DNA samples are stored at the Institute of Immunobiology and Human Genetics, Skopje [8]. HLA typing with low resolution was performed using the methods of Reverse Line Strip (RLS) and Sequence Specific Oligonucleotides (SSO) according to the manufacturers' recommendations. The method of Reverse Line Strip was performed using kits from INNOGENETICS, Belgium and the nitrocellulose strips were analysed using the LiRAS™ software. Part of the samples were analysed using the LABType SSO Typing Tests from ONE LAMBDA, INC, USA and the Fusion3.0 software.

2.3. Statistical analysis

Allele and haplotype frequencies were estimated using the Arlequn3.5 software package [9]. Hardy-Weinberg equilibrium was tested by applying modified hidden Markov chain with 10,00,000 steplength approach and 1,00,000 dememorization steps, as is implemented in the Arlequin software. Haplotype data was calculated for two-, tree- and four-HLA loci with known genotypic data. Furthermore, linkage disequilibrium (D) and relative linkage disequilibrium (D') were calculated as previously described [10]. The Macedonian population was compared with 31 populations from all over the world using Nei's genetic distance and unrooted tree was constructed using Neighbour-Joining method [11] implemented in the Phylip software. The data for the other populations were obtained from D. Middleton's website www.allelefrequencies.net accessed on June 2017 [12] and available published papers [13–23]. The Macedonian sample was compared with these populations: Serbians (1992), Albanians (160), Bulgarians (55), Bosnians (134), Croatian (4000), Greece_pop7 (11,250), Greece_North (500), Turkey_pop2 (228), Slovenia_pop3 (130), Austria (200), Albanians_Kosovo (120), Italy (1,59,311), Hungarians (1644), Romania (348), Germany (11,407), France Marceille (1000), France Lyon (4813), Spain Murcia (173), Spain Majorca (407), USA OPTN Caucasians (8525), United Kingdom Central (135), United Kingdom North (330), Morocco (647), Portugal Lisbon (17,420), Portugal Porto (7937), Russia Moscow (2650), Sweden pop4 (966), Norway_pop2 (576), Czech Republic Gypsy pop2 (46) and Brazil Parana Caucasian (2775).

3. Results

3.1. Allele frequencies

We analysed the Hardy-Weinberg equilibrium for all 4 HLA loci (HLA-A, HLA-B, HLA-C and HLA-DRB1) and the results are shown in Table 1. The population is in Hardy-Weinberg equilibrium ($p > .05$) for all investigated loci.

The frequencies of the allele groups in HLA-A, HLA-B, HLA-C and HLA-DRB1 loci are shown on Table 2. We identified 18 different allele groups in the Macedonian population for HLA-A. The most frequent HLA-A allele groups were HLA-A*02 (29.0%), HLA-A*24 (13.8%), HLA-A*01(12.6%) and HLA-A*03 (10.1%). These four alleles constitute around 65.5% of all HLA-A alleles in the sample. The other alleles are with frequencies less than 10%.

In the Macedonian population we identified 26 different allele groups for HLA-B, with highest frequency for HLA-B*35 (16.1%), HLA-B*51 (14.7%) and HLA-B*18 (14.7%). These are followed by HLA-B*44, HLA-B*07, HLA-B*08, HLA-B*40 and HLA-B*27.

13 different allele groups were observed for HLA-C in the analysed population. The most frequent HLA-C alleles were HLA-C*07 (27.9%), HLA-C*04 (18.3%) and HLA-C*12 (12.7%). They account for 58.9% of all HLA-C allele groups in the population. The other allele groups were with frequencies less than 10%.

Regarding the HLA class 2, we analysed the HLA-DRB1 locus in the Macedonian population. We discovered 13 different allele groups in the HLA-DRB1 locus. HLA-DRB1*11 (25.5%), HLA-DRB1*16 (14.7%),

Table 1
Hardy-Weinberg equilibrium for the four analysed HLA loci.

HLA locus	No of individuals	No of allele groups identified	Observed heterozygosity	Expected heterozygosity	p-value	S.D.
A	286	18	0.88811	0.85708	.68125	0.00026
B	286	26	0.90559	0.90999	.94459	0.00012
C	286	13	0.87413	0.85244	.12552	0.00026
DRB1	286	13	0.88811	0.87041	.80615	0.00021

S.D., standard deviation.