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HLA CLASS II ANTIGENS AND HAPLOTYPES IN THE VOJVODINA POPULATION: RELATIONSHIP TO OTHER POPULATIONS

ABSTRACT: The aim of the study was to assess the distribution of HLA DR and DQ antigens, haplotypes and their gametic associations, in the population of Vojvodina. HLA II class profile of the population of Vojvodina has been compared with those of other European populations in order to provide additional information regarding the history of their origin. The study included 142 unrelated healthy persons from all regions of Vojvodina. The antigens with the highest frequencies were DQ1 (68.2%), DQ3 (52%), DQ2 (38%) and DR11(5) (34.5%). The HLA haplotypic associations with the highest frequencies included HLADR11(5)DQ7(3) (6.69%), DR3DQ2 (5.28%) and DR2DQ1 (4.75%). The antigen DR11(5) showed the strongest association with DQ7(3) (Δ=0.0389, fr.=6.69%). Our results proved the closest relationship between the Croats, Greeks, Italians, Romanians and Slovenians, while the Scottish and Finnish, as examples of geographically distant populations, showed greater genetic distances from the population of Vojvodina. Present distribution of HLA class II antigens in the Vojvodina population may be the result of historical influence of neighbouring populations in the course of history.

KEY WORDS: Antigens – HLA – Genetical distances – Frequencies

INTRODUCTION

The province of Vojvodina, in the northern part of Serbia, extends on an area of 21.506 km^2. It is located at the confluence of three important rivers: Danube, Tisa and Sava that cut the province into three regions: Banat, Bačka and Srem (*Figure 1*). Throughout history, Vojvodina had been a part of Dacia, the Roman Empire, the Hunnic Empire, the Avarian Kaganate, the Gepidic Kingdom, the Byzantine Empire, Bulgaria, Hungary, the Ottoman Empire, Austria, Austro-Hungary, Yugoslavia, Serbia and Montenegro, and finally Serbia, as noted by Bihalji-Merin *et al.* (1968), Fotić *et al.* (1991) and Grubač (1961).

During the early medieval migrations, the Slavs (including the Serbs) settled on the territory of present-day Vojvodina in the 6th and 7th centuries. Serbs were recorded as a population which lived in northern Banat since AD 567, according to Grubač (1961) and Marković (1984). In the 9th century, the territory of present-day Vojvodina became a part of Bulgaria. At the end of the 14th century and at the beginning of the 15th century, Serbs started their large-scale migration from the Kosovo province and from the area around the Morava River, provoked by Turkish invasion to the Balkan Peninsula. Serbs passed the rivers Danube and Sava and settled down in the south parts of the Pannonian Plain where they were ordered to be a border guard of Austro-Hungarian Monarchy. In the 18th century, after a partial emigration of Serbs to Russia caused by the tendency of Catholic Church to force the Serbs to adopt the Catholic or Uniate religions or to pay tributes and enter serfdom relations, the territory of Vojvodina was occupied by various ethnics: Germans, Hungarians, Slovaks, Ruthenians, Romanians a. o. Marković (1984) and Šalinović (1999) argued that Vojvodina thus gradually obtained its ethnic diversity including more than 29 ethnic groups in the region, which makes it very interesting for investigation.

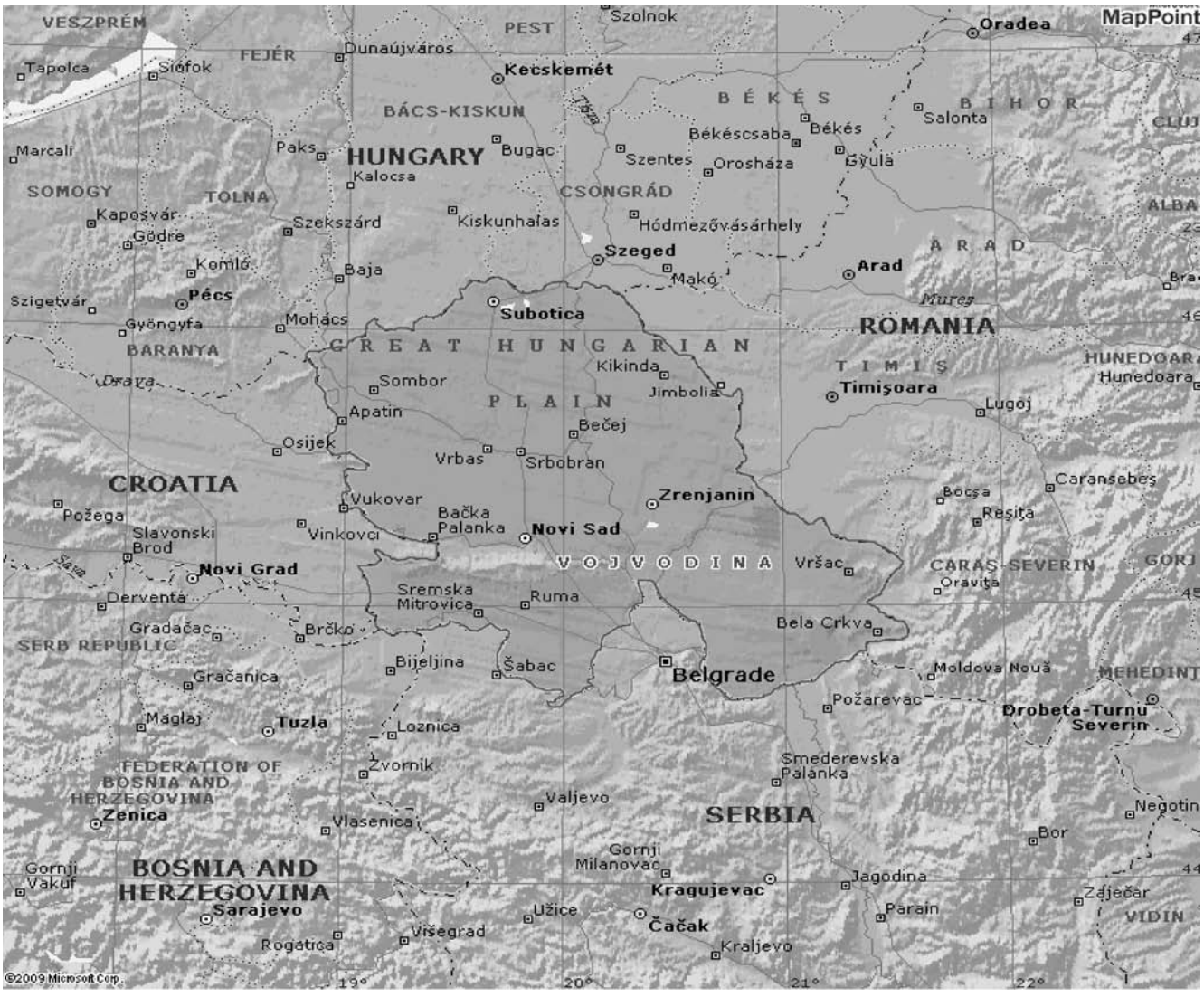

FIGURE 1. Map showing the location of Vojvodina.

In present work we studied HLA class II antigens in the Vojvodina population sample in order to document genetically the historical events recorded in modern Serbian history.

MATERIAL AND METHODS

Blood samples from 142 healthy unrelated individuals from all regions of Vojvodina were used for HLA typing and phylogenetical calculations. HLA DR and DQ antigens were determined using immunomagnetic technique as noted by Stolić *et al*. (1991) and Vartdal *et al.* (1986, 1987). The gene frequencies were estimated by following formula: gf=1-√1-af, where af is phenotype frequency of a given allele, as described by Vogel *et al.* (1986) and Kaštelan (1991). Association between HLA DR and DQ loci and linkage disequilibrium (LD) coefficient (Δ) were calculated according to following formula: Δ=F–2p1q1, where: F=actual (observed) frequency, p1q1=expected frequency of a given gene combination. Statistical significance of observed and expected two-locus haplotype frequencies were calculated using Chi-squared test as noted by Hartl (1983), Kraljević-Balalić *et al.* (1981), Hedrick *et al*. (1986), Thomson *et al.* (1987), Weir *et al*. (1989) and Pasekov (1982). Phylogenetic trees (dendrograms) were constructed applying the Neighbour-Joining (NJ) method established by Saitou *et al.* (1987, 1992), Nei *et al.* (1979), and Li (1981), with genetic distances between populations according to Cavalli-Sforza *et al.* (1971), and using MEGA 4.0 software as noted by Tamura *et al*. (2007, 2008).

RESULTS

The observed antigen frequencies of HLA-DR and HLA-DQ loci are presented in *Table 1*. Thirteen HLA-DR antigens were found in the population of Vojvodina. The common HLA-DR antigens were DR11(5) (34.5%), DR2 (33.7%) and DR3 (26.7%). Among eight HLA-DQ antigens found in our

TABLE 1. Phenotype and gene frequencies of HLA-DR and-DQ antigens in Vojvodina.

Allele	af		gf		Allele	af		gf	
DR1	0.126		0.065		DQ1	0.591		0.360	
DR2	0.190		0.100		DQ5(1)	0.021	0.682	0.010	0.405
DR15(2)	0.133	0.337	0.068	0.175	DQ6(1)	0.070		0.035	
DR16(2)	0.014		0.007		DQ2	0.380		0.212	
DR3	0.239		0.127		DQ3	0.225		0.119	
DR17(3)	0.021	0.267	0.010	0.140	DQ7(3)	0.274	0.520	0.147	0.276
DR18(3)	0.007		0.003		DQ8(3)	0.021		0.010	
DR4	0.169		0.088		DQ4	0.028		0.014	
DR11(5)	0.345		0.190		DQ blank	0.387		0.217	
DR6	0.028		0.014						
DR13(6)	0.119	0.189	0.061	0.096					
DR14(6)	0.042		0.021						
DR7	0.154		0.080						
DR8	0.021		0.010						
DR9	0.007		0.003						
DR10	0.028		0.014						
DR blank	0.352		0.195						

af=phenotype frequency gf=gene frequency

TABLE 2. HLA-DR/DQ haplotype frequencies in Vojvodina (frequency ≥0.02).

Haplotype	Observed HF	Expected HF	Δ	X^2 values
DR1DQ1	0.0316	0.0234	0.0082	0.570^{ns}
DR2DQ1	0.0475	0.0360	0.0115	1.924
DR15(2)DQ1	0.0228	0.0247	-0.0019	0.029^{ns}
DR3DQ1	0.0299	0.0457	-0.0157	1.229^{ns}
DR3DQ2	0.0528	0.0270	0.0258	3.709^{ns}
DR4DQ3	0.0316	0.0105	0.0212	4.718
DR11(5)DQ2	0.0211	0.0405	-0.0194	1.624^{ns}
DR11(5)DQ7(3)	0.0669	0.0280	0.0389	8.069
DR7DQ2	0.0316	0.0170	0.0146	1.696^{ns}

^{ns}= not significant

TABLE 3. Genetic distances (GD) between the population of Vojvodina and other populations.

Population		Genetic distance
Croats	Crnić-Martinović *et al.* (2002)	0.1563
Hungarians	Gyodi *et al.* (1998)	0.1622
Slovenians	Vidan-Jeras *et al.* (1998)	0.1632
Greeks	Lazidou *et al.* (1998)	0.2039
Serbs (general population)	Lazović *et al.* (1996)	0.2107
Romanians	Reed *et al.* (1992)	0.2144
Irish	Finch *et al.* (1998)	0.2242
Germans	Wernet *et al.* (1998)	0.2447
Austrians	Fisher *et al.* (1998)	0.2532
French	Bignon *et al.* (1998)	0.2622
Czechs	Drabek *et al.* (1998)	0.2783
Dutch	Schreuder *et al.* (1998)	0.2841
Belgians	Huang *et al.* (1998)	0.2928
Russians	Kapustin *et al.* (1997)	0.2998
Turkish	Kuloglu *et al.* (2008)	0.3062
Italians	Mareinetti *et al.* (1998)	0.3136
Finnish	Siren *et al.* (1996)	0.3144
Scottish	Jazwinska *et al.* (1987)	0.4076

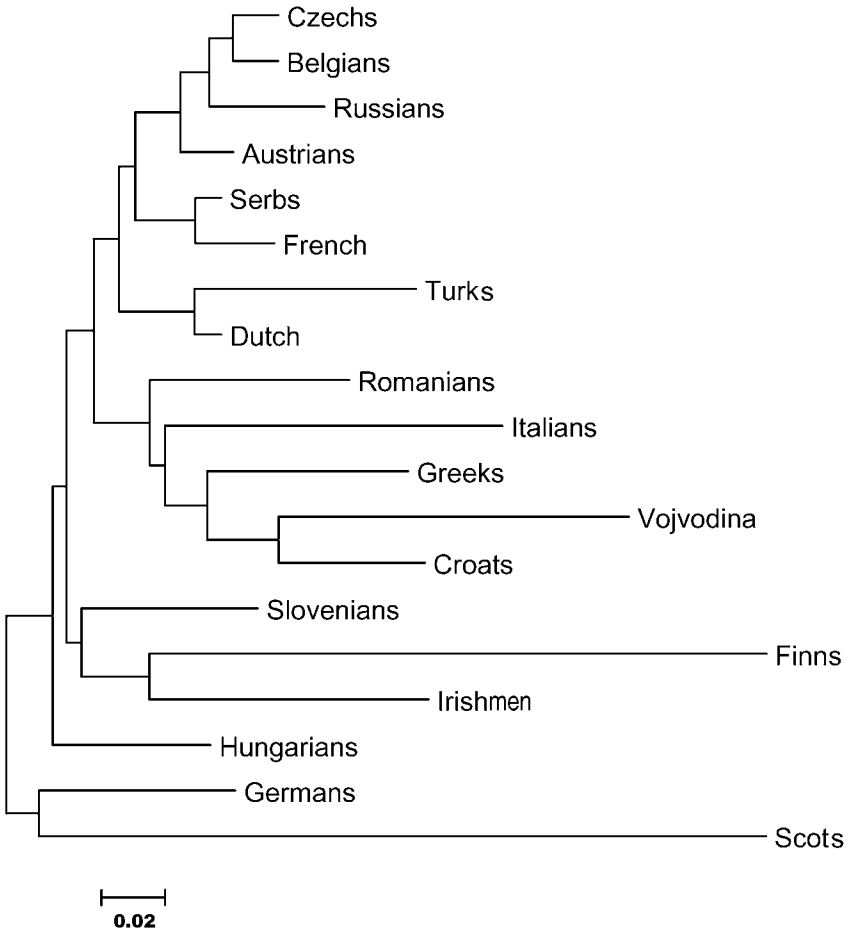

FIGURE 2. Dendrogram constructed by the neighbour-joining method using MEGA 4.0 software, reflecting relationships between the population of Vojvodina and other 18 populations.

population, the most frequent ones were DQ1 (68.2%), DQ3 (52%) and DQ2 (38%).

Analysis of the HLA-DR-DQ haplotype associations showed that the three most frequent ones were: DR11(5)DQ7(3) (6.69%), DR3DQ2 (5.28%) and DR2DQ1 (4.75%). Calculations of the linkage disequilibrium coefficient were also performed. The most frequent HLA-DR-DQ haplotype associations (frequency≥0.02) found in the population of Vojvodina and their Δ values are presented in *Table 2*. The antigen DR11(5) showed strongest association with DQ7(3) (Δ=0.0389, X^2=8.069), and the antigen DR4 with DQ3 (Δ=0.0212, X^2=4.718).

Phylogenetic trees (dendrograms) were constructed (*Figure 2*) basing on the corresponding genetic distances calculated between the population of Vojvodina and other populations (*Table 3*).

The closest relationship was observed between the Croats, Greeks, Italians, Romanians followed by the Slovenians, Dutch, Turkish, French and Serbs (general population). Greater genetic distances from the population of Vojvodina showed the Scottish and Finnish as geographically distant populations.

DISCUSSION

A total of 13 antigens at HLA DR locus and 8 antigens at HLA DQ locus (*Table 1*) were found out by this research. At locus DR, the most frequent antigen DR11(5) (0.345) within this study was found. It was also reported by Kuloglu *et al.* (2008), Lazidou *et al.* (1998), Martinetti *et al.* (1998) and Crnić-Martinović *et al.* (2002) as the most common antigen in the populations of Turkey, Greece, Italy and Croatia. At locus DQ, DQ1 (0.682) and DQ3 (0.52) antigens were the most common in our population, and they were also the most common ones in Romanians and Hungarians, as noted by Reed *et al.* (1992) and Gyódi *et al*. (1998).

Tests for linkage disequilibrium (LD) between DR and DQ loci showed two cases of significance (p=0.05) (*Table 2*). We noticed that two locus haplotype associations with strong LD values were among the most common haplotype associations, which are reported in previous studies by Oudshoorn *et al.* (1997) and Vojvodić *et al.* (2007). For example, two haplotype associations with the strongest LD were DR11(5)DQ7(3) (Δ=0.0389) and DR4DQ3 (Δ=0.0212) with frequencies of 0.0669 and 0.0316, respectively. This study also found out that haplotype associations such as DR3DQ2 exhibit a relatively high

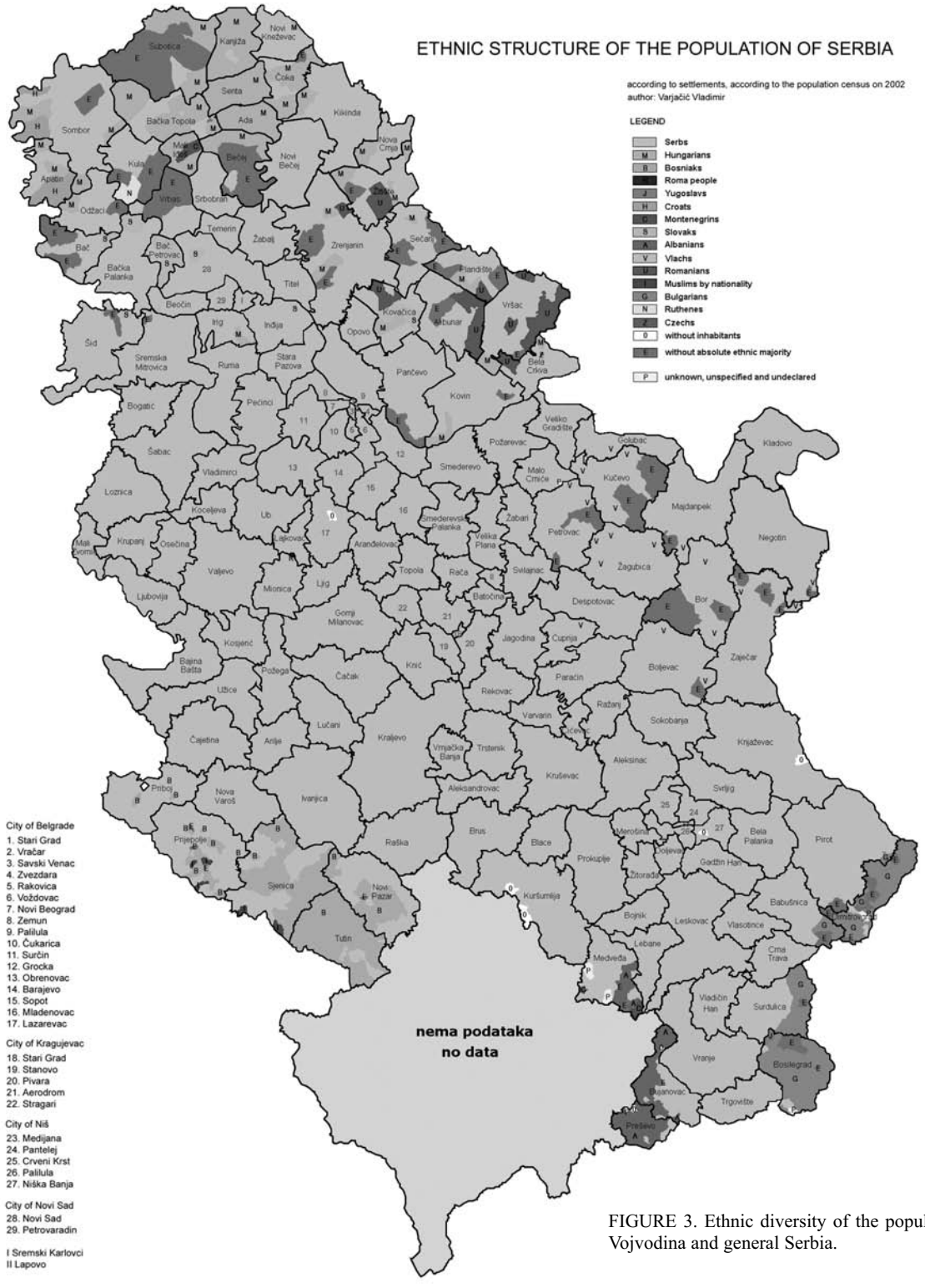

FIGURE 3. Ethnic diversity of the population of Vojvodina and general Serbia.

frequency (0.0528) and strong LD (D value is 0.0258), but no statistical significancy of the difference between the observed and expected haplotype frequencies (X^2=3.709 is lower than the border value of 3.841, for p=0.05).

Except for the population of Vojvodina, the gene frequencies data of compared populations were taken from previous studies announced by Gyódi *et al*. (1998), Kuloglu *et al.* (2008), Lazidou *et al.* (1998), Fischer *et al.* (1998), Lazović *et al.* (1996), Mareinetti *et al.* (1998), Reed *et al.* (1992), Wernet *et al.* (1998), Crnić-Martinović *et al.* (2002), Siren *et al.* (1996), Jazwinska *et al.* (1987), Vidan-Jeras *et al.* (1998), Drábek *et al.* (1998), Bignon *et al.* (1998), Finch *et al.* (1998), Huang *et al.* (1998), Schreuder *et al*. (1998), and Kapustin *et al.* (1997).

The phylogenetic trees (*Figure 2*) show that the Vojvodina population is approximately at the same distance from the populations of Croatia, Greece and Italia, and even closer to these than to the general (inner) population of

Serbia. This could be explained by the differences in historical influence of neighbouring populations on the region of Vojvodina and on the overall region of Serbia, as described by Bihalji-Merin *et al*. (1968), Fotić *et al.* (1991) and Grubač (1961). The Turkish conquest of the Balkan Peninsula went as far as the Danube River, while Vojvodina was in recent history part of Austro-Hungarian Monarchy. Also, according to the last population census in 2002, in Vojvodina there were 29 ethnic groups, the absolute majority among them constituted by the Serbs (*Figure 3*). There were 65% Serbs, 14.5% Hungarians, 2.79% Slovaks, 2.78% Croats, 2.45% Yugoslavs, 1.57% Montenegrins, 1.5% Romanians, 1.43% Roma people, 0.97% Bunjevci, 0.77% Ruthenians, 0.58% Macedonians, 0.23% Ukrainians, 0.18% Muslims (by nationality), 0.16% Germans, 0.1% Slovenians, 0.08% Albanians, Bulgarians and Czechs, 0.05% Russians, 0.03% Gorani, 0.02% Bosniaks and 0.26% others (Vlachs, Šokci, Jews, Ashkali, Egyptians, Greeks, Poles and Chinese). In contrast to such a great ethnic diversity present in Vojvodina, in general Serbia there were 6 ethnic groups, and among them Serbs constitute 89.5% of all population, as announced by the Republic Institute of Statistics of Serbia – Population Census 2002 (2002) and http://en.wikipedia.org/wiki/Ethnic groups in Vojvodina.

The dendrogram based on genetical distances among 19 populations (including the population of Vojvodina) shows that the relationship among populations in the dendrogram is in accordance to their geographical distances. The population of Vojvodina turns out similarities to the geographically and ethnically related south-eastern populations in Europe that might be a result of admixture with populations in recent history. The greatest genetic distances from our population showed geographically distant populations such as the Scots, Irishmen and Finns who have no historical connections to the populations at the Balkan Peninsula.

CONCLUSIONS

This study provides a description of HLA class II antigens in the population of Vojvodina that could be used for various applications: anthropological studies, organ and tissue transplantation and disease association studies. Our results will be confirmed by future studies using molecular methods that will provide a more accurate comparative population-based analysis.

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