

## A comparison of the HLA database of the Romanian population with the Balkan populations

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**Abstract:** The antigens of the HLA system represent a distinctive marker in identifying racial and population groups by means of an anthropological and relational characterization. Building a database is beneficial to forensic identification, to implement transplant programs and to determine a predisposition to certain illnesses.

We performed the HLA class I serotyping on the antigens on HLA A and HLA B loci for 1250 unrelated individuals from the Romanian population; based on this we calculated the allelic frequency. The most frequent antigen on the HLA A locus was HLA A2, and on the HLA B locus was HLA B35. We subsequently compared the results we obtained for the Romanian population with similar data from several Balkan populations.

**Key Words:** serotyping, HLA antigens, allelic frequency, unrelated individuals, Balkan populations.

The present study contributes to creating a database which allows one to catalogue and hierarchize the antigens and the alleles of the HLA system in the Romanian population and to compare them with the antigenic and allelic constellation of the populations from the East and South-East of Europe. Due to its polymorphism, the HLA system represents a distinctive factor among the racial and population groups and can be used as an anthropological marker in the study of population migration and in the study of associated illnesses [1,2].

### Materials and methods

The study group consists of 1250 unrelated individuals (625 women and 625 men), adults, within 16 and 68 years old, who are from all the regions of the country and confirmed as healthy; blood samples were drawn from them for the HLA A and B phenotyping in order to determine paternity, during 1993-2010, in the Serology Laboratory of Mina Minovici INML (National Forensic Institute) Bucharest. The informed

consent was received from a part of the subjects.

The phenotyping of HLA class I A and B loci was performed via the standard serological method, which is based on the microlymphocytotoxicity test; it was used a set of specific commercial antisera, produced by the Biotest and Inntrain companies - Germany. The persons with whom both antigens failed to show on one of the loci were considered homozygotes; this was then confirmed with the HLA SSP Low resolution - Inntrain - Germany.

Serotyping is based on the microlymphocytotoxicity test. The HLA lymphocyte antigen adheres to the specific antibody from the serum, forming antigen – antibody complexes, in the presence of the complement, which permeabilizes the cell membrane and allows different dyes (eosin, ethidium bromide, acridine orange) to enter the cell.

The evaluation of the percentage of lytic and non-lytic cells from each cup is done by using the inverted phase contrast or fluorescence microscope, depending on the technique of lymphocytes separation

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used. The result is quantified in positive/negative for that specific antigen, evaluating the percentage of lytic and non-lytic cells [2,3].

**Data analysis:**

The frequency of every antigen (A) was calculated based on the following formula:  $A = n / N$ , where n is the number of individuals with the given antigen, and N, the total number of individuals in the study. The frequency of every allele was determined using the following formula:

$$p = 1 - \sqrt{1 - A}$$

where p represents the allelic frequency, and A the antigenic frequency [4].

In order to determine the allelic relations between the Romanian population and other 8 European populations, data regarding the frequency of the alleles for each of the two loci in the study, that is, HLA A locus and HLA B locus, were selected from reference literature [5]. The populations we considered are: Albania (n=160), Bosnia and Herzegovina (n=134), Bulgaria (n=55), Croatia (n=100), Northern Greece (n=500), Macedonia (n=216), Serbia (n=386) and Turkey (n=228) [6].

The antigens investigated in the Romanian population were:

- for the HLA A locus - A1, A2, A3, A23, A24, A25, A26, A34, A66, A11, A68, A69, A29, A30, A31, A32, A33, A74 and A36,
- for the HLA B locus - B51, B52, B7, B8, B44, B45, B13, B14, B15, B38, B39, B57, B58, B18, B49, B50, B54, B55, B56, B27, B35, B53, B37, B40, B41, B42, B72, B73, B48, B78 and B47.

In order to evaluate the relations and the distances among the populations, for each locus it was calculated, based on the data registered with the AFND (Allele Frequency Net Database) [6] the allelic frequency at the two digits level and subsequently the Euclidian distance using the following formula:  $distance(x,y) = \{ \sum Si (xi - yi)^2 \}^{1/2}$ .

**Results**

Table no. 1 presents the frequency of the HLA alleles and antigens for the two loci in the study, HLA A and HLA B, found with the Romanian population.

In figure 1 there are represented the antigens frequencies on the HLA A locus, the most frequent antigens being: A2, A3, A24 and A1, and in figure 2 there

are represented the antigens frequencies on the HLA B locus, where the most frequent antigens were: B35, B51, B18, B44 and B8.

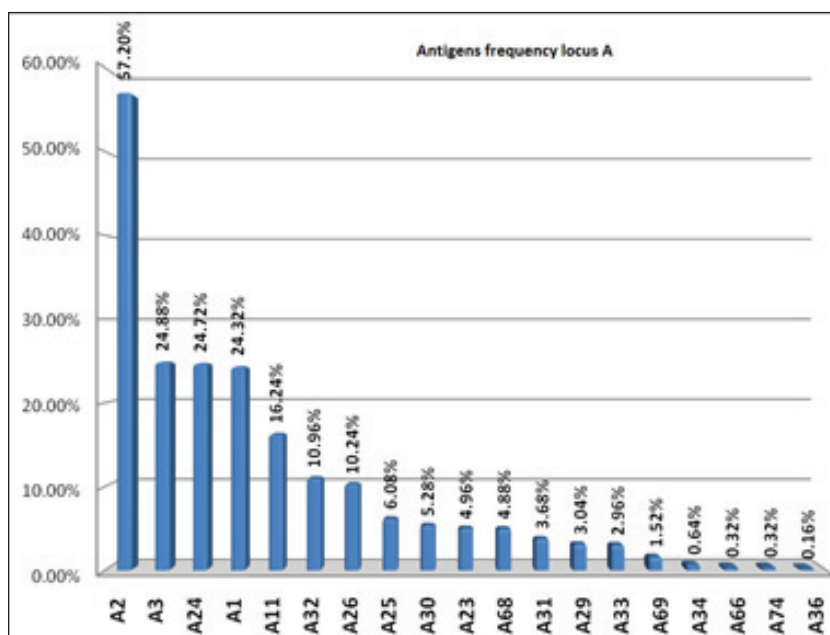
The diversity and the allelic frequencies calculated for Romania and the other 8 populations taken into consideration [6] are represented in table no. 2 for the HLA A locus and in table no. 3 for the HLA B locus.

Following the calculation of the Euclidian distance between the selected populations, taken two by two, we obtained the dendrograms for each locus, represented in figure 3 for the HLA A locus and in figure 4 for the HLA B locus.

They summarize the kinship relations among all the populations taken into consideration.

**Table 1.** The distribution of the HLA class I antigens and the frequency of the alleles in the Romanian population

	Antigenic frequency (%)	Allelic frequency
<b>HLA-A</b>		
A1	24.32	0.13
A2	57.2	0.3457
A3	24.88	0.1337
A23	4.96	0.0251
A24	24.72	0.1323
A25	6.08	0.0308
A26	10.24	0.0525
A34	0.64	0.0032
A66	0.32	0.0016
A11	16.24	0.0847
A68	4.88	0.0247
A69	1.52	0.0076
A29	3.04	0.0153
A30	5.28	0.0267
A31	3.68	0.0185
A32	10.96	0.0563
A33	2.96	0.0149
A74	0.32	0.0016
A36	0.16	0.0008
<b>HLA-B</b>		
B51	20.96	0.1109
B52	4.24	0.0214
B7	12.88	0.0666
B8	15.36	0.08
B44	18.48	0.0971
B45	0.56	0.0028
B13	6.8	0.0345
B14	4.72	0.0238
B15	6.64	0.0337
B38	6.4	0.0325
B39	6.16	0.0312
B57	3.76	0.0189
B58	1.02	0.006
B18	20.48	0.1082
B49	4.72	0.0238
B50	1.76	0.0088
B54	0.24	0.0012
B55	2.96	0.0149
B56	3.2	0.0161
B27	11.28	0.058
B35	28.96	0.1571
B53	0.32	0.0016
B37	2.24	0.0112
B40	9.12	0.0466
B41	2.88	0.0145
B42	0.16	0.0008
B72	0.16	0.0008
B73	0.56	0.0028
B48	0.08	0.0004
B78	0.24	0.0012
B47	0.32	0.0016



**Figure 1.** The graphic representation of the antigen frequency on the HLA A locus

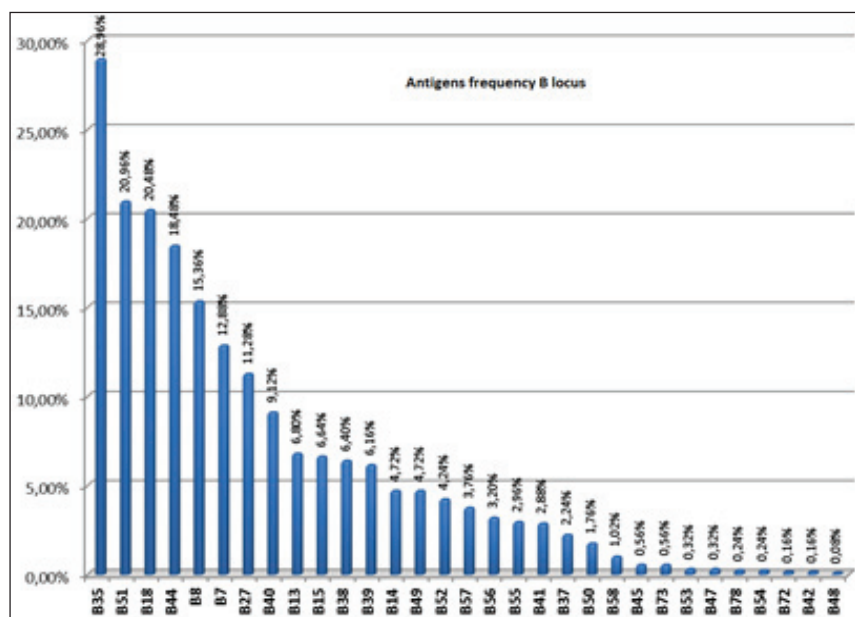


Figure 2. The graphic representation of the antigen frequency on the HLAB locus

On the HLA A locus the Romanian population is situated in a main cluster together with Macedonia, Albania and Northern Greece; yet in the dendrogram obtained for the HLA B locus, Romania can be found next to Serbia and Croatia.

Table 2. Allelic frequency for the HLA A locus

Country Alleles	Romania =1250	Albania = 160	Bosnia and Herzegovina = 134	Bulgaria = 55	Croatia = 100	Northern Greece = 500	Macedonia = 216	Serbia = 386	Turkey = 228
A*01	0.1300	0.1000	0.1343	0.0730	0.1570	0.1000	0.1130	0.1320	0.0660
A*02	0.3457	0.3000	0.3470	0.3450	0.3190	0.2700	0.3220	0.2500	0.2190
A*03	0.1337	0.0910	0.1576	0.0460	0.0980	0.0800	0.1120	0.1110	0.1090
A*23	0.0251	0,0220	0.2230	0.0550	0.0440	0.0300	0.0280	0.0230	0.0530
A*24	0.1323	0,1590	0.1007	0.1180	0.0980	0.1400	0.1350	0.0860	0.2130
A*25	0.0308	0,0090	0.0223	0.0270	0.0390	0.0100	0.0210	0.0480	0.0130
A*26	0.0525	0,0500	0.0410	0.0730	0.0640	0.0600	0.0390	0.0500	0.1350
A*34	0.0032	-	0	0	0	0	0	-	0.0040
A*66	0.0016	-	0.0074	0	0	0.0070	0.0020	0.0040	-
A*11	0.0847	0.0750	0.0485	0.0730	0.0490	0.0800	0.0490	0.0650	0.0610
A*68	0.0247	0.0470	0.0671	0.0180	0.0250	0.0480	0.0490	0.0290	0.0350
A*69	0.0076	0.0030	0	0.0180	0	0.0030	0	0.0040	-
A*29	0.0153	0.0130	0.0074	0.0090	0.0050	0.0200	0.0300	0.0020	0.0150
A*30	0.0267	0.0030	0.0037	0.0180	0.0140	0.0280	0.0240	0.0360	0.0130
A*31	0.0185	0.0130	0.0186	0.0180	0.0290	0.0160	0.0140	0.0210	0.0290
A*32	0.05638	0.0780	0.0149	0.0820	0.0250	0.0700	0.0490	0.0460	0.0280
A*33	0.0149	0.0130	0.0037	0.0180	0.0290	0.0250	0.0050	0.0230	0.0110
A*74	0.0016	0.0060	0.0037	0	0	0.0020	0	-	-
A*36	0.0008	-	0	0	0	0	0	-	-

Discussions and conclusions

The objective of this study is to characterize the Romanian population from the point of view of the frequency of the HLA A and B antigens by creating a study group that represents the entire country, and the characteristics of which were previously presented.

We compared data obtained after the typification of the study group from Romania with those from the AFND (Allele Frequency Net Database) for 8 reference populations from Region 8 as per the European Federation of Immunogenetics mapping. We calculated the antigenic and allelic frequencies of the reference population.

The data we obtained were used to analyse the relations with several Balkan populations by calculating the Euclidian distance.

The antigenic and allelic frequencies calculated for the Romanian population partially overlap with the ones identified in the 8 populations included in the study. The data we obtained following the calculations showed that the most frequent alleles identified for the two loci in the study are close to

**Table 3.** Allelic frequency for the HLA A locus

Country Alleles	Romania = 1250	Albania = 160	Bosnia and Herzegovina = 134	Bulgaria = 55	Croatia = 100	Northern Greece = 500	Macedonia = 216	Serbia = 386	Turkey = 228
<b>B*51</b>	0.1109	0.1720	0.1082	0.2090	0.0830	0.1460	0.1530	0.1190	0.1580
<b>B*52</b>	0.0214	0.0310	0.0298	0.0360	0.0200	0.0180	0.0230	0.0190	0.0170
<b>B*07</b>	0.0666	0.0430	0.0671	0.0450	0.0390	0.0400	0.0450	0.0400	0.0390
<b>B*08</b>	0.0800	0.0560	0.0820	0.0180	0.0880	0.0460	0.0530	0.0910	0.0350
<b>B*44</b>	0.0971	0.0780	0.1492	0.0810	0.0540	0.0570	0.0720	0.0860	0.1340
<b>B*45</b>	0.0028	0.0060	0	0	0	0.0010	0.0020	0.0020	0.0040
<b>B*13</b>	0.0345	0.0190	0.0111	0.0270	0.0540	0.0240	0.0270	0.0420	0.0440
<b>B*14</b>	0.0238	0.0090	0.0111	0.0090	0.0340	0.0250	0.0090	0.0440	-
<b>B*15</b>	0.0337	0.0330	0.0335	0.0360	0.0290	0.0300	0.0320	0.0380	0.0350
<b>B*38</b>	0.0325	0.0250	0.0410	0.0360	0.0740	0.0300	0.0480	0.0500	0.0330
<b>B*39</b>	0.0312	0.0190	0.0480	0	0.0440	0.0300	0.0250	0.0150	0.0110
<b>B*57</b>	0.0189	0.0130	0.0298	0.0180	0.0200	0.0110	-	0.0340	0.0130
<b>B*58</b>	0.0060	0.0220	0.0074	0.0180	0.0250	0.0200	0.0090	0.0200	0.0080
<b>B*18</b>	0.1082	0.1160	0.0820	0.1000	0.1030	0.1030	0.1340	0.0840	0.0310
<b>B*49</b>	0.0238	0.0090	0.0261	0.0180	0.0200	0.0310	0.0190	0.0210	0.0660
<b>B*50</b>	0.0088	0.0030	0.0074	0.0270	0.0290	0.0150	0.0070	0.0060	0.0500
<b>B*54</b>	0.0012	0.0030	0.0037	0	0	0.0010	0	0.0020	-
<b>B*55</b>	0.0149	0.0250	0.0298	0.0180	0.0150	0.0270	0.0460	0.0120	0.0710
<b>B*56</b>	0.0161	0.0030	0.0110	0	0.0150	0.0040	0.0020	0.0150	0.0020
<b>B*27</b>	0.0580	0.0250	0.0485	0.0730	0.0490	0.0250	0.0310	0.0310	0.0280
<b>B*35</b>	0.1571	0.1420	0.0932	0.1270	0.1230	0.2000	0.1350	0.1360	0.1400
<b>B*53</b>	0.0016	0.0160	0	0	0.0050	0.0040	0.0090	-	-
<b>B*37</b>	0.0112	0.0360	0.0074	0.0090	0.0150	0.0180	-	0.0150	0.0040
<b>B*40</b>	0.0466	0.0410	0.0559	0.0450	0.0390	0.0470	0.0490	0.0340	0.0580
<b>B*41</b>	0.0145	0.0030	0.0149	0.0270	0.020	0.0170	0.0180	0.0120	0.0150
<b>B*42</b>	0.0008	-	0	0	0	0	0	-	-
<b>B*72</b>	0.0008	-	-	-	-	-	-	-	-
<b>B*73</b>	0.0028	0.0060	0	0.0090	0	0.0020	0.0020	-	-

**Table 3.** Allelic frequency for the HLA B (ctd)

Country Alleles	Romania = 1250	Albania = 160	Bosnia and Herzegovina = 134	Bulgaria = 55	Croatia = 100	Northern Greece = 500	Macedonia = 216	Serbia = 386	Turkey = 228
<b>B*48</b>	0.0004	0.0030	0	0	0	0.0010	0	-	-
<b>B*78</b>	0.0012	0.0060	0	0	0	0	0.0050	-	-
<b>B*47</b>	0.0016	0.0170	0	0.0090	0.0500	0.0040	0	0.0110	-

the Balkan population; the Romanian population is most closely connected to the populations from Albania, Macedonia, Northern Greece, Serbia and Croatia, as it is also shown in the two dendrograms.

On the HLA A locus the most frequent is the A2 antigen (57,2%), followed by A3 (24,88%), A24

(24,72%) and A1 (24,32%). The calculated allelic frequencies do not modify the order of the antigenic frequency.

The antigens on the A locus of the populations found in the same cluster with Romania (figure 3) that is, Macedonia, Albania and Northern Greece are

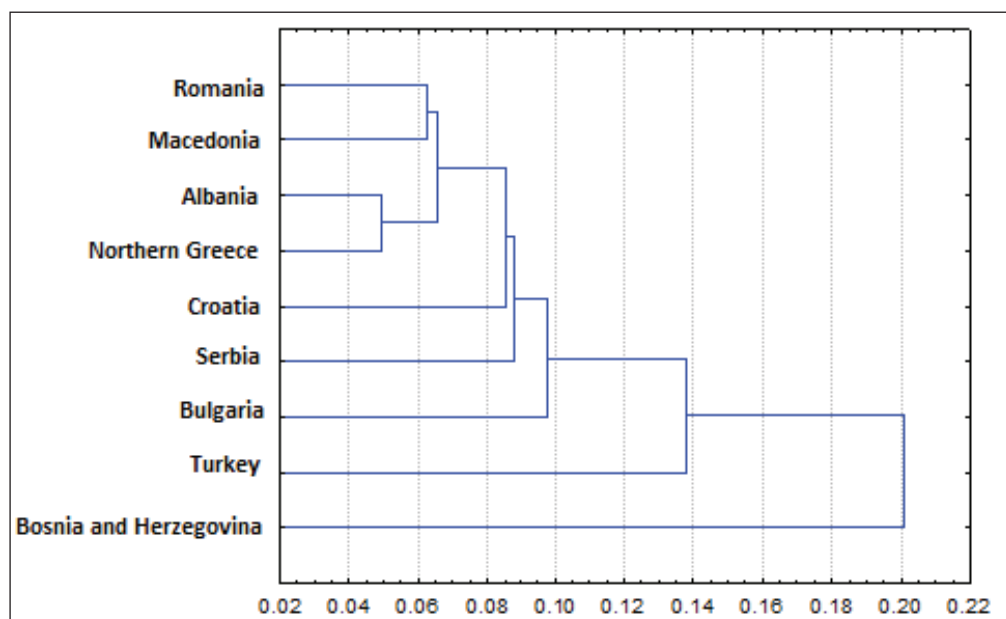


Figure 3. The dendrogram for the HLA A locus summarizes the relations between the Romanian population and the other 8 populations considered in the study

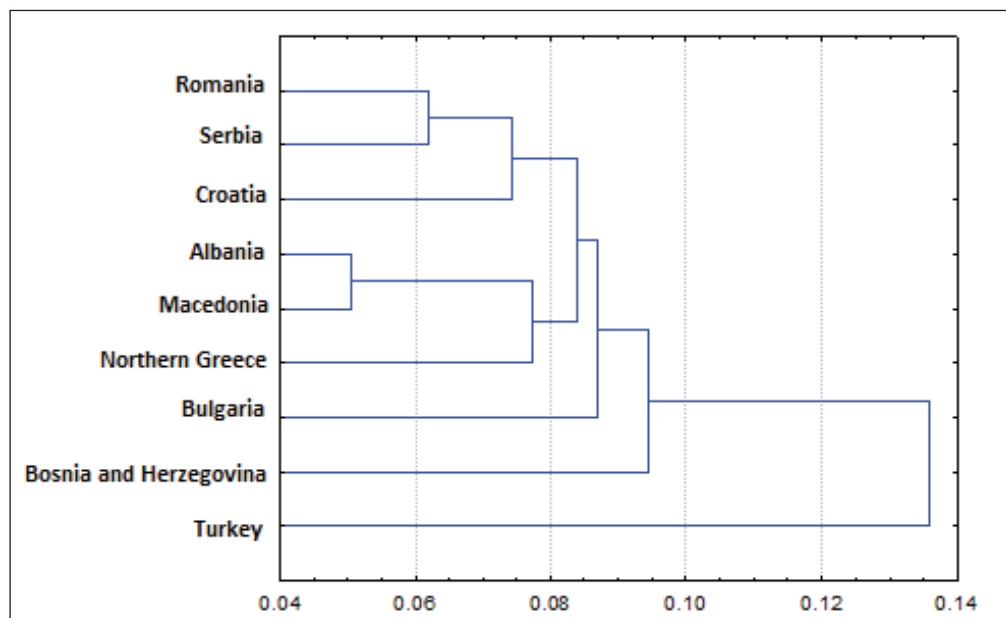


Figure 4. The dendrogram for the HLA B locus summarizes the relations between the Romanian population and the other 8 populations considered in the study

the same, but the antigenic and allelic distribution is different. Thus, for Macedonia, Albania and Northern Greece the most frequent are HLA A2, A24, A1 and A3. For all the 9 populations in the study the most frequent antigen on the HLA A locus is A2.

In contrast we have the antigens A69 (1,52%), A34 (0,64%), A66 (0,32%), A74 (0,32%) and A36 (0,16%); we noticed that in some populations these are missing.

The A34 and A36 antigens do not appear in the antigenic constellation of any of the three countries in the same cluster with Romania. For the

population from Albania, the A69 and A74 antigens are present with a lower frequency than the one calculated for Romania. For the Northern Greece population, the frequency of those is higher, while they lack in the population from Macedonia. The A66 antigen is present both with the Northern Greece and with the Macedonia populations, with a higher frequency than the Romanian population, but is not present with the Albanian population.

On the HLA B locus the higher frequency is given by the B35 antigen (28,96%), followed by B51 (20,96%), B18 (20,48%) and B44 (18,48%) and B8 (15,36%). The calculated allelic frequencies correspond to the order of the antigenic frequency.

We noticed that the HLA B antigens of the populations in the same cluster as us (fig. 4), that is, Croatia and Serbia are the same, though their antigenic and allelic distribution is different. Thus, we see that compared with Romania, for the population in Serbia the descending order of the frequency is B35, B51, B8,

B44 and B18, while for the Croatia population is B35, B18, B8, B51 and B44. For the two populations found in the main cluster together with Romania, the most frequent antigen on HLA B locus is B35.

In contrast we have the antigens B78 (0,24%), B54 (0,24%), B72 (0,16%), B42 (0,16%) and B48 (0,08%). Except for the B54 antigen which can be found only in the constellation of the population from Serbia, where it has a higher frequency than in the Romanian population, the rest of the antigens cannot be found in any of the populations that are in the same cluster as Romania.



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