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HLA Class I and Class II Polymorphism in the Population of Rijeka, Croatia

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A B S T R A C T

The aim of the study was to examine frequencies of HLA-A, -B, -DR antigens and haplotypes in population of Rijeka and to compare them with general Croatian and European populations. The subjects were 117 unrelated healthy blood donors. The antigens with the highest frequencies were: A2 (27.2%), A9 (16.3%), B5 (14.8%), B12 (11.8%), B18 (11.8%), DR5 (21.6%) and DR6 (13.8%). Comparison of HLA antigens frequencies has shown statistically significant difference in 1 antigen with Croatian population and in 8 antigens with European population. The HLA haplotypes with high frequencies included HLA-A2, B5 (6.84%), HLA-A2, B12 (6.84%), HLA-A2, B18 (6.84%), HLA-B12, DR2 (9.78%) and HLA-B18, DR5 (6.84%). The antigen B5 showed strongest association with DR5 (6.41%; LD = 1.30) as in general Croatian and in some European populations. The results have shown great diversity of HLA haplotypes in Rijeka population which can be the result of admixture with neighborhood immigrating populations during the history.

Introduction

Due to extreme polymorphism of HLA system and differences in HLA alleles and haplotype frequencies in various human populations and ethnic groups, HLA molecules are useful genetic markers for studies of inheritance, ancestry and genetic history of populations[1–6].

Croatian population is a very interesting one for investigation, because there is no consensus whether Croats are of German, Iranian or Slavic origin. The most probable is that Croats are of Slavic origin with one or two migrations of Slavs in VI and VII century to South East Europe,

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but the name »Hrvat« (Croat) is of Iranian origin[7]. Also, during their history Croats were admixtured by other populations: by other Slavic and Baltic populations (in pre-Slavic time), by autochthonous populations Illyrians and Thracians and recently by immigrated populations of Hungarians, Austrians, Italians, Germans, Turks etc.[8].

HLA polymorphism in different regions of Croatian population (Dalmacija, Lika, Posavina, Zagorje and Zagreb) as well as in isolated population of Adriatic islands (Hvar and Krk) was previously described[9–12], but the northern Adriatic region – Primorje was not investigated.

With the aim to enrich and contribute to the existing knowledge about HLA polymorphism in Croatian population, in this study the authors examined frequencies of HLA-A, -B, -DR antigens and haplotypes in population of Rijeka and compared them with general Croatian and European populations.

Rijeka is the biggest Adriatic port in Primorje with about 200,000 inhabitants. During the history inhabitants of Rijeka were admixtured with Italians (especially with Venetian), Hungarians and Austrians[13].

Material and Methods

The population study consisted of 117 unrelated healthy blood donors (65 males and 52 females) from Rijeka. Tissue typing for HLA -A, -B antigens was performed on T and -DR on B lymphocyte, according to the standard microlimphocytotoxicity method (using monoclonal and polyclonal alloantibodies)[14]. The typing sera were obtained from commercial sources. The significance of differences in antigen and gene frequencies among populations of Rijeka and general Croatian and West European population was evaluated using the chi-square test, while Fisher's exact test was used if any value in 2x2 table was less than 5. Gene and haplotypes frequencies were calculated using maximum likelihood method. Probable two-locus haplotypic associations were assigned on the basis of known linkage disequilibrium [15]. In order to compare phenotype and haplotype HLA frequencies with other populations, the obtained data of the 11th and 12th International HLA Workshops were used [15,16]. Dendrogram was constructed from the allele frequencies by using the Neighbor-Joining (NJ) method[17] with the standard genetic distances (SGD)[18], by using the Genetic Distance and Phylogenetic Analysis (DISPAN) software designed by T. Ota (Pennsylvania State University, Philadelphia, PA, USA), which contains the GNKDST and TREEVIEW programs[19,20].

Results and Discussion

The antigen and gene frequencies of the HLA-A, -B and -DR loci are presented in Table 1. Eight HLA-A antigens were found in Rijeka population. The common HLA-A antigens were A2 (27.2%), A9 (16.3%), and A19 (12.3 %). Among the 17 HLA-B antigens found in our population, the most frequent ones were B5 (14.8%), B12 (11.8%), B18 (11.8%) and B35 (10.4%). Among 10 HLA-DR antigens detected in Rijeka population the most frequent were DR5 (21.6%), DR6 (13.8%) and DR7 (12.3%). Results were compared with the results of the general Croatian population (Table 2). The analysis of the HLA -class I and class II antigen frequencies revealed similarity between the population of Rijeka and general Croatian population. The only frequency of DR 3 antigen in the population of Rijeka (9.4%) was significantly lower ($p<0.05$) than in general Croatian population (19.4%), whereas the comparison of all other HLA-A, -B and -DR frequencies did not reveal any significant differences.

TABLE 1
HLA ANTIGENS AND GENES FREQUENCIES IN RIJEKA POPULATION (N = 117)

A locus	af	gf	B locus	af	gf	DR locus	af	gf
A1	0.1624	0.0848	B5	0.2735	0.1477	DR1	0.1996	0.1037
A2	0.4701	0.2721	B7	0.1111	0.0572	DR2	0.3590	0.1994
A3	0.2051	0.1084	B8	0.0940	0.0482	DR3	0.0940	0.0482
A9	0.2991	0.1628	B12	0.2222	0.1181	DR4	0.1453	0.0755
A10	0.2051	0.1084	B13	0.0769	0.0392	DR5	0.3846	0.2155
A11	0.1624	0.0848	B14	0.0598	0.3040	DR6	0.2564	0.1377
A28	0.0769	0.0392	B15	0.0769	0.0392	DR7	0.2308	0.1230
A19	0.2308	0.1230	B16	0.1282	0.0663	DR8	0.0684	0.0348
			B17	0.0769	0.0392	DR9	0.0171	0.0086
Bla	0.1795	0.0165	B18	0.2222	0.1181	DR10	0.0342	0.0172
			B21	0.0513	0.0260			
			B22	0.0769	0.0392	Bla	0.2051	0.0365
			B27	0.0684	0.0348			
			B35	0.1966	0.1037			
			B37	0.0256	0.0129			
			B40	0.1111	0.0572			
			B41	0.0171	0.0086			
			Bla	0.1111	0.0142			

af = antigen frequencies; gf = genes frequencies; Bla = blank

TABLE 2
COMPARISION OF HLA-A, -B, -DR ANTIGEN FREQUENCIES IN RIJEKA POPULATION AND IN GENERAL CROATIAN AND EUROPEAN POPULATIONS

Antigen	Rijeka		Croatia			Europe		
	N	af	N	af	p	N	af	p
A1	117	0.162	175	0.263	ns	2,163	0.264	<0.005
A9		0.229		0.251	ns		0.220	ns
A10		0.205		0.166	ns		0.115	<0.0005
B5	117	0.274	175	0.223	ns	2,132	0.157	<0.005
B7		0.111		0.126	ns		0.217	<0.025
B8		0.094		0.149	ns		0.183	<0.025
B18		0.222		0.200	ns		0.107	<0.0005
DR3	117	0.094	175	0.194	<0.05	1,926	0.226	<0.05
DR4		0.145		0.211	ns		0.238	<0.05

af = antigens frequency

Although that comparison of HLA class I and class II frequencies between Croatian and European population did not reveal significant differences[10,21], we found statistically significant difference in 8 antigens with European population. Among HLA-A antigens this two populations were different in HLA-A1 and A10. HLA -A1 antigen had higher frequency in the European population whereas HLA-A10 in population of Rijeka. No difference was observed in the distribution of HLA-A9 antigen between two populations. HLA-B antigens -B5 and -B18 showed significantly higher frequency in the Rijeka population, and HLA-B7 and -B8 in the European population. Significant difference between two populations was ob-

TABLE 3
THE MOST FREQUENT TWO-LOCUS HAPLOTYPES HLA-A-B IN POPULATION OF RIJEKA (N = 234)

Haplotype	hf (%)	LD x 100
A2, B5	6.84	1.85
A2, B12	6.84	5.50
A2, B18	6.84	5.00
A2, B40	5.13	2.74
A9, B5	4.27	2.48
A9, B35	3.85	2.15
A19, B12	3.85	3.42
A1, B8	3.42	2.29
A3, B7	3.42	2.82
A9, B12	3.42	2.67

hf = haplotype frequency; LD = linkage disequilibrium

TABLE 4
THE MOST FREQUENT TWO-LOCUS HAPLOTYPES HLA-B-DR IN POPULATION OF RIJEKA (N = 234)

Haplotype	hf (%)	LD x 100
B12, DR2	9.70	9.27
B18, DR5	6.48	6.05
B5, DR5	6.41	1.30
B5, DR5	5.56	3.53
B12, DR7	5.56	5.13
B18, DR2	5.13	4.53
B16, DR6	3.85	3.37
B35, DR6	3.85	2.47
B5, DR2	3.42	1.84
B5, DR6	3.42	1.98
B8, DR3	3.42	2.71

hf = haplotype frequency; LD = linkage disequilibrium

served for HLA-DR3 and -DR4 antigens which were more frequent in the European population (Table 2).

Analysis of the HLA-A, -B haplotypes showed that among 162 examined haplotypes, three most frequent ones were HLA-A2, B5, HLA-A2, B12 and HLA-A2, B18 with frequency of 6.84% in population of Rijeka (Table 3). The most frequent haplotype HLA-A1, B8 in many European populations (5.9%) was also present in general Croatian population (6.0%), while in population of Rijeka it was present at lower frequency (3.42%) like in Italians (4.2%) and Romanians (4.4%)[15,21]. One of the common Caucasoid haplotypes HLA-A2, B5 in Rijeka population has been observed at a higher frequency (6.84%) than in general Croatian population (5.75%)[10], in Albanians (4.3%)[22], Spaniards (4.8%) and Basques (5.5%)[23]. Another typical Caucasoid haplotype HLA-A3, B7 in our population was present with lower frequency (3.42%) than in Croats (5.0%)[10], French (4.1%)[24], while in Albanians it was 1.1%[22].

Two-locus haplotypes analysis between HLA class I and class II alleles were also performed. The 11 most frequent HLA -B, -DR haplotypes found in population of Rijeka are presented in the Table 4.The most frequent haplotype in Rijeka population HLA-B12, DR2 (9.78%) and HLA-B18, DR5 (6.84%) were present in general Croatian population with a frequency of 1.2% and 2.1%, respectively. On the other side, a haplotype HLA-B8, DR3, which has been detected as the most frequent haplotype in Croats (5.3%; LD = 4.7) in our population was present at 3.42% (LD 2.71). This haplotype was found with similar frequency in many other Caucasoid European populations[25], but in Basques it was present at 6.4% (LD = 4.3)[23]. The second most frequent haplotype in Croats HLA-B7, DR2 (4.3%) was found in Rijeka population (3.42%), which was similar to that of Germans, Belgians, Italians, Hungarians and Spaniards (< 3.8%), while in Basques it was present at 7.1% (LD = 5.6)[23,26]. The antigen B5 showed strongest association with -DR5 (6.41%; LD = 1.30) as in general Croatian population and in some European populations (German, Sardinian and other populations from Mediterranean area).

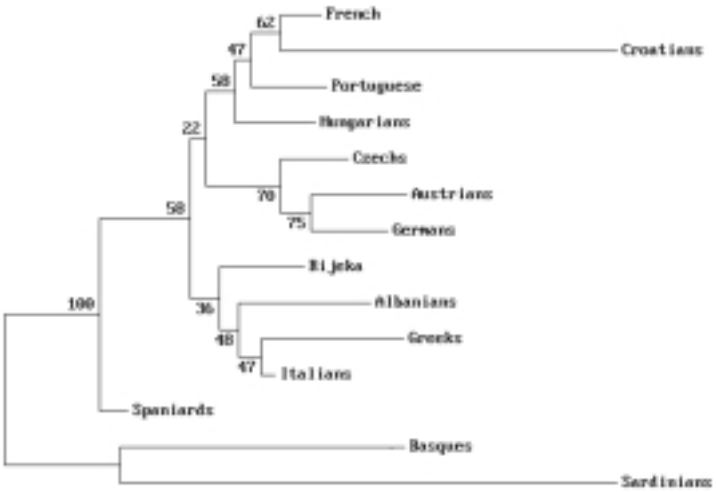

Fig. 1. Neighbor-joining dendrogram based on HLA-A, -B and -DR allele frequency data showing relatedness between Rijeka population and other European populations. Bootstrap values from 1,000 replicates are given.

A phylogenetic tree was constructed (Figure 1) and the corresponding genetic distances calculated with HLA-A, -B and -DR allele frequencies from Rijeka and other European populations (Table 5). The closest relationship was observed with Czechs, Albanians, Greeks and Italians, followed by other European people in the following decreasing order: Hungarians, Austrians, Portuguese, French, Germans, Croatians (general population) and Spaniards. The greater genetic distances from our population showed Basques and Sardinians, known as examples of isolated genetic pools with their genetic, social and ethnographic characteristics.

On the basis of our results we can conclude that population of Rijeka has shown great diversity of HLA haplotypes, which can be the result of admixture with neighborhood and immigrated populations during the history (especially with Italians, Hungarians, Austrians, Czechs). Our results will be confirmed in further studies using molecular genotypic methods with increased resolution and accuracy. The utilization of those methods will add to refined data facilitating a more accurate comparative population-based analysis.

TABLE 5
GENETIC DISTANCES (GD) BETWEEN RIJEKA POPULATION AND OTHER EUROPEAN POPULATIONS

Population	Genetic distance
Czechs	0.0128
Albanians	0.0151
Greeks	0.0237
Italians	0.0345
Hungarians	0.0392
Austrians	0.0448
Portuguese	0.0475
French	0.0503
Germans	0.0622
Croatians	0.0675
Spaniards	0.0808
Basques	0.1474
Sardinians	0.1753

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RAZNOVRSNOST ANTIGENA HLA RAZREDA I I RAZREDA II U POPULACIJI RIJEKE

SAŽETAK

Cilj rada je bio istražiti učestalost HLA-A, -B i -DR antigena i haplotipova u populaciji Rijeke te usporediti ih s općom populacijom Hrvatske i Europskim populacijama. Ispitanici su bili 117 nesrodna dobrovoljna darovatelja krvi. Rezultati su pokazali da su najučestaliji bili sljedeći antigeni: A2 (27.2%), A9 (16,3%), B5 (14.8%), B12 (11.8%), B18 (11.8%), DR5 (21.6%) i DR6 (13.8%). Populacija Rijeke se statistički značajno raz-

likovala od populacije Hrvatske u 1 HLA antigenu i u 8 antigena od Europskih populacija. U populaciji Rijeke najveću učestalost su pokazali haplotipovi: HLA-A2,B5 (6.84%), HLA-A2,B12 (6.84%), HLA-A2,B18 (6.84%), HLA-B12,DR2 (9.78%) i HLA-B18,DR5 (6.84%). Antigen B5 je pokazao najjaču združenost s antigenom DR5 (6.41%; LD = 1.30) kao i u općoj populaciji Hrvatske i nekim Europskim populacijama. Rezultati su ukazali na veliku različitost HLA haplotipova u populaciji Rijeke, koji su vjerojatno posljedica miješanja stanovništva sa susjednim imigrirajućim populacijama tijekom povijesti.