

GENETIC POLYMORPHISM OF BLOOD GROUPS AND ERYTHROCYTES ENZYMES IN POPULATION GROUPS OF THE REPUBLIC OF MACEDONIA

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A b s t r a c t: This study presents the results of an examination of 3 blood-group systems (ABO, Rhesus, and P1) and erythrocyte enzymes (ADA, AK, ALADH, PGD, SAHH, PGM1, PGM3, GPT, GOT, ACP, UMPK, ESD and GLO) in populations that reside in R. Macedonia. Four population samples from the Republic of Macedonia (129 Macedonians from Skopje, 98 Albanians from Skopje, 95 Aromanians from Kruševo, 102 Aromanians from Štip) were included in the study. A comparison of the obtained results with data from literature on other Balkan populations has been made. The results of the comparison of the studied alleles indicate relatively small genetic distances among the studied populations. The obtained dendograms indicate a larger homogeneity in the large Balkan populations, and a manifest trend of separating the Aromanian population of the Štip region. A larger separation is characteristic in the Greek population of Thrace.

Key words: Macedonia, genetic structure, blood groups, erythrocyte enzymes.

Introduction

The Republic of Macedonia is a multiethnic country, where a variety of different ethnic groups live in a small area. According to the 2002 census of the population, 2,022,547 inhabitants live in Macedonia: 64.18% Macedonians (most Orthodox Christian, speaking Macedonian, a language from the Slavic group of languages), 25.17% Albanians (most Muslim, speaking Albanian, a language belonging to the Indo-European group of languages, and deriving from an Illyrian language) and other populations with percentages lower than 5%. The Aromanians (Orthodox Christian, speaking a Romance language closely related to classical Latin) represent only 0.48% of the population. In the Republic of Macedonia, the settlement areas of Aromanians are the regions of Ovče Pole (Štip), Kruševo, Bitola and Struga. The diversity of the ethnic structure is a result of the tempestuous historical past of this region [1].

In the works published to date relating to classical genetic markers in the populations that reside in Macedonia, the Aromanians and the Macedonians have been analysed for serum protein polymorphisms [2, 3, 4], whereas the Aromanians from Štip have been analysed for blood groups and polymorphisms of certain erythrocyte enzymes [5]. Blood group systems MNSs and Kk have also been published for the Macedonians, the Aromanians from Kruševo and Štip, and the Albanians [6].

The present study presents the results of an examination of 3 blood-group systems (ABO, Rhesus, and P1) in Macedonians, Albanians from Macedonia and Aromanians from Kruševo. We also present unpublished data for erythrocyte enzymes from Macedonians, and Aromanians from Štip and Kruševo.

Material and methods

Four population samples from the Republic of Macedonia were included in the study (129 Macedonians from Skopje, 98 Albanians from Skopje, 95 Aromanians from Kruševo, 102 Aromanians from Štip) (Fig. 1). The individuals in the study were not family-related.

The affiliation with a certain population group was determined based on statements given during the filling-in of demographics cards. Through them, the subjects confirmed their affiliation to their population group at least as far back as three previous generations, following the pedigrees of both parents.

The blood-group systems ABO, Rhesus, and P1 were studied in population groups using agglutination reaction on plates and in tubs. Results for Aromanians from Štip are published [5].

Five polymorphisms of erythrocyte enzymes (ADA, AK, ALADH, PGD, and SAHH) were tested (using standard techniques) in Macedonians and Aromanians from Štip and Kruševo. Systems PGM1, PGM3, GPT, GOT, ACP, UMPK,

ESD and GLO were studied in Macedonians and Aromanians from Kruševo. These systems have already been published for Aromanians from Štip [5].



Figure 1 – Geographical location of the sample analysed in this study
Слика 1 – Географска локација на местата од каде {то се земани примероците за оваа студија}

To show the genetic relationship between the studied and other Balkan populations, neighbour-joining trees have been created using the PHYLIP program [7], based on Reynolds genetic distances. The data for other Balkan populations came from literature (Albanians, Greeks, Romanians [8, 2, 9]; Serbs: Efremovska data in press)

Results

The phenotype frequencies of the blood groups and the erythrocyte enzyme polymorphisms studied are shown in Tables 1 and 2. In some cases, a test for Hardy-Weinberg equilibrium was impossible due to missing degrees of freedom ($df = 0$), but mostly the observed frequencies corresponded well to the expected values. Generally, the populations are subject to HW conditions.

Table 1 – Tabela 1

Observed and expected phenotype frequencies for blood groups
Најдена и очекувана фенотипска фреквенција по испитуваните
крвно-групни системи

Macedonians		Albanians		Aromuns (Krusevo)	
Obs.	Exp.	Obs.	Exp.	Obs.	Exp.

A1	59	54,14	21	19,96	25	22,47
A2	0	1,62	13	15,20	11	10,19
Macedonians Albanians Aromuns (Krusevo)						
Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
B	26	22,44	20	20,99	27	23,51
O	37	39,85	35	33,96	27	29,84
A1B	5	10,55	3	4,13	3	5,81
A2B	2	0,40	6	3,76	2	3,18
n	129	129	98	98	95	95
	df = 0		$\chi^2 = 0,6082$ df = 1		$\chi^2 = 2,1786$, df = 1	
			30 < p < 50		10 < p < 20	
ccdee	11	9.81	10	11.45	9	10.85
ccDee	4	3.57	0	0	2	2.41
ccDEe	13	12.04	10	11.28	11	10.84
ccDEE	3	2.71	2	2.78	1	2.21
Ccddee	1	0.91	3	3.46	0	0.00
CcDee	37	41.52	33	27.65	36	31.76
CcDEe	17	19.66	16	15.32	14	13.43
CcDEE	1	0.26	1	0.00	0	0.00
CCddee	0	0.02	0	0.27	0	0.00
CCDee	37	33.62	18	20.86	18	20.35
CCDEe	1	0.91	0	0	0	0.00
C ^W cDee	2	0.97	2	1.71	1	0.75
C ^W cDEe	0	0.44	1	0.84	0	0.31
C ^W Dee	1	1.56	2	2.38	1	0.90
CcD ^U ee	0	0	0	0	1	1.12
ccD ^U ee	1	0	0	0	0	0
n	129	129.01	98	98	95	94.96
	df = 0		$\chi^2 = 1,7516$, df = 1		df = 0	
			10 < p < 20			
P1+	95		63		65	
P1-	14		35		30	
n	129		98		95	

Table 2 – Табела 2

Observed and expected phenotype frequencies for erythrocyte enzymes

Најдена и очекувана феноотурска фреквенција по испитувањите еритроцитарни ензими

Phenotype	Macedonians		Aromuns (Krusevo)		Aromuns, Stip	
	Obser.	Expec.	Obser.	Expec.	Obser.	Expec.
ADA 1	118	118,23	87	87,17	86	87,12
ADA 2-1	11	10,53	8	7,66	22	19,76

ADA 2	0	0,23	0	0,17	0	1,12
n	129	129	95	95	108	108
Macedonians		Aromuns (Krusevo)		Aromuns, Stip		
Phenotype	Obser.	Expec.	Obser.	Expec.	Obser.	Expec.
AK 1	116	116,33	90	90,07	87	86,23
AK 2-1	13	12,35	5	4,87	19	20,55
AK 2	0	0,33	0	0,07	2	1,23
n	129	129	95	95	108	108
ALADH 1	91	90,42	77	75,16	98	97,28
ALADH 2-1	34	35,16	15	18,68	9	10,44
ALADH 2	4	3,42	3	1,16	1	0,28
n	129	129	95	95	108	108
PGD A	121	121,12	92	92,02	104	104,04
PGD B	8	7,75	3	2,95	4	3,93
PGD AB	0	0,12	0	0,02	0	0,04
n	129	129	95	95	108	108
SAHH 1	122	122,1	86	86,21	79	79,23
SAHH 2	0	0,07	0	0,17	2	1,02
SAHH 3	0	0	0	0	0	0,23
SAHH 2-1	6	5,84	8	7,62	17	17,99
SAHH 3-1	1	0,97	1	0,95	10	8,25
SAHH 3-2	0	0,02	0	0,04	0	0,97
n	129	129	95	95	108	108
PGM1 1	45	47,16	25	28,46		
PGM1 2	11	7,94	10	8,85		
PGM1 3	0	1,21	0	0,52		
PGM1 4	1	0,33	0	0,52		
PGM1 2-1	37	38,7	34	31,75		
PGM1 3-1	22	15,12	12	7,66		
PGM1 3-2	2	6,2	0	4,27		
PGM1 4-1	7	7,86	8	7,66		
PGM1 4-2	3	3,23	4	4,27		
PGM1 4-3	1	1,26	2	1,03		
n	129	129	95	95		
$\chi^2 = 1.9567, \text{ df} = 2$		$\chi^2 = 0.7630, \text{ df} = 2$				
$30 < p < 50$		$50 < p < 70$				
PGM3 1	82	78,30	58	54,57		
PGM3 2-1	37	44,41	28	34,86		
PGM3 2	10	6,30	9	5,57		
n	129	129	95	95		
$\chi^2 = 3.5842, \text{ df} = 1$		$\chi^2 = 3.6778, \text{ df} = 1$				
$5 < p < 10$		$5 < p < 10$				
GPT 1	32	30,77	19	19,92		
GPT 2	35	33,26	27	27,92		
GPT 2-1	59	61,99	48	46,16		
GPT 3-1	1	0,47	0	0		
GPT 3-2	0	0,51	0	0		
GPT 2-1M	2	1,99	1	1		
GPT 3-1M	0	0,02	0	0		
n	129	129	95	95		
GOT2 1	128	128	93	93		

GOT2 2-1		1 n	1 129	1 129	1 94	1 94	
Phenotype		Macedonians		Aromuns (Krusevo)		Aromuns, Stip	
		Obser.	Expec.	Obser.	Expec.	Obser.	Expec.
ACP A		14	12,72	14	15,6		
ACP B		53	50,86	30	28,46		
ACP C		0	0,44	0	0,21		
ACP AB		47	50,86	42	42,15		
ACP AC		6	4,71	7	3,65		
ACP BC		9	9,42	2	4,93		
	n	129	129	95	95		
		$\chi^2=0.6708$, df = 2		$\chi^2=0.2529$, df = 1		$70 < p < 80$	
UMPK 1		119	119,19	89	89,07		
UMPK 2		0	0,19	0	0,07		
UMPK 2-1		10	9,61	5	4,87		
	n	129	129	95	95		
ESD 1		105	104,31	82	80,59		
ESD 2		2	1,21	2	0,45		
ESD 5		0	0	0	0,01		
ESD 2-1		21	22,48	9	11,97		
ESD 5-1		1	0,9	2	1,84		
ESD 5-2		0	0,1	0	0,14		
	n	129	129	95	95		
GLO 1		21	20,56	14	14,8		
GLO 2-1		61	61,88	47	45,4		
GLO 2		47	46,56	34	34,8		
	n	129	129	95	95		
		$\chi^2=0.0261$, df = 1		$\chi^2=0.1180$, df = 1		$80 < p < 90$	
		$70 < p < 80$					

The allele and haplotype frequencies are shown in Tables 3 and 4.

Table 3 – Табела 3

Allele and haplotype frequencies in the sample studied (blood groups)
Алелна и харлотирна фреквенција во испитуваниот примерок
(крвно-групни системи)

	Macedonians n = 129	Albanians n = 98	Aromuns (Kruševo) n = 95
A1	0.2938	0.1315	0.1620
A2	0.0112	0.1196	0.0886
B	0.1391	0.1601	0.1889

O	0.5558	0.5886	0.5605
cde	0.2769	0.3418	0.3380
	Macedonians n = 129	Albanians n = 98	Aromuns (Kruševo) n = 95
cDe	0.0464	0	0.0357
Cde	0.0128	0.0516	0
CD ^e	0.4998	0.4126	0.4457
cDE	0.1455	0.1683	0.1526
CDE	0.0069	0	0
C ^w De	0.0117	0.0255	0.0105
CD ^u e	0	0	0.0174
P1+	0.4866	0.4023	0.4381
P1-	0.5134	0.5976	0.5620

Table 4 – Табела 4

*Allele and haplotype frequencies in the sample studied
(erythrocyte enzymes)*
*Алелна и харлотирна фреквенција во испитуваниот примерок
(еритроцитарни ензими)*

	Macedonians n = 129	Aromuns (Kruševo) n = 95	Aromuns (Štip) n = 108
ADA*1	0.9574	0.9578	0.8981
ADA*2	0.0426	0.0421	0.1019
AK*1	0.9496	0.9737	0.8935
AK*2	0.0504	0.0267	0.1065
PGD*A	0.9690	0.9842	0.9815
PGD*B	0.0310	0.0158	0.0185
ALADH*1	0.8372	0.8895	0.9491
ALADH*2	0.1628	0.1105	0.0509
SAHH*1	0.9729	0.9526	0.8565
SAHH*2	0.0232	0.0421	0.0972
SAHH*3	0.0039	0.0053	0.0463
PGM1*1	0.6047	0.5474	
PGM1*2	0.2481	0.3053	
PGM1*3	0.0969	0.0737	
PGM1*4	0.0504	0.0737	
PGM3*1	0.7791	0.7579	
PGM3*2	0.2209	0.2421	
GPT*1	0.4732	0.4482	
GPT*1M	0.0152	0.0097	

GPT*2	0.5078	0.5421
GPT*3	0.0039	0.0000
	Macedonians n = 129	Aromuns (Kruševo) n = 95
GOT2*1	0.9961	0.9947
GOT2*2	0.0039	0.0053
ACP*A	0.3140	0.4053
ACP*B	0.6279	0.5474
ACP*C	0.0581	0.0474
UMPK*1	0.9612	0.9734
UMPK*2	0.0388	0.0266
ESD*1	0.8992	0.9211
ESD*2	0.0969	0.0684
ESD*5	0.0038	0.0105
GLO*1	0.3992	0.3947
GLO*2	0.6008	0.6053

Genetic distances between the population groups included in this study, as well as other Balkan populations (data from literature), were calculated with respect to the polymorphic loci studied (Tables 5 and 6).

Table 5 –Табела 5

*Reynolds genetic distances between the populations studied,
calculated of blood groups: ABO, RH, MNSS, PI, Kk*
*Reynold-ова генетска дистанца одредена во однос на крвно-групните системи
ABO, RH, MNSS, PI, Kk*

	1	2	3	4	5	6	7
1. Romanians (South, Ploiesti)	***						
2. Albania (Alba- nians, Tirana)	0.0119	***					
3. Greeks (Thrace)	0.0114	0.0152	***				
4. Macedonians (Skopje)	0.0067	0.0056	0.0132	***			
5. Aromuns, Gra- mostians (Mace- donia, Stip)	0.0079	0.0159	0.0226	0.0063	***		
6. Aromuns, Moskopoliants (Macedonia, Kruševo)	0.0079	0.0097	0.0123	0.0089	0.0172	***	
7. Serbs	0.0021	0.0141	0.0083	0.0098	0.0127	0.0073	***

8. Albanians Macedonia, Skopje)	0.0090	0.0148	0.0106	0.0148	0.0200	0.0042	0.0065
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Table 6 –Табела 6

Reynolds genetic distances between the populations studied, calculated of: blood groups (ABO, RH, MNSS, PI, Kk), erythrocyte enzymes (PGM1, PGM3, GPT, GOT2, GLO I, ESD, ACP, UMPK, ADA, AK, PGD, ALADH, SAHH), serum proteins (GC, HPA, CP, TF, BF, PI, AMY2)

Reynold-ова генетска дистанца одредена во однос на крвно групните системи (ABO, RH, MNSS, PI, Kk), еритроцитарните ензими (PGM1, PGM3, GPT, GOT2, GLO I, ESD, ACP, UMPK, ADA, AK, PGD, ALADH, SAHH) и серумските протеини (GC, HPA, CP, TF, BF, PI, AMY2)

	1	2	3	4	5
1. Romanians (South, Ploiesti)	***				
2. Albania (Albanians, Tirana)	0.0076	***			
3. Greeks (Thrace)	0.0072	0.0085	***		
4. Macedonians (Skopje)	0.0056	0.0067	0.0095	***	
5. Aromuns, Gramostians (Macedonia, Stip)	0.0080	0.0140	0.0146	0.0106	***
6. Aromuns, Moskopolians (Macedonia, Krusevo)	0.0063	0.0106	0.0092	0.0077	0.0111

Figure 2 shows the neighbour-joining tree, based on the five blood groups. Figure 3 shows the same, but based on blood groups, erythrocyte enzyme and serum polymorphisms.

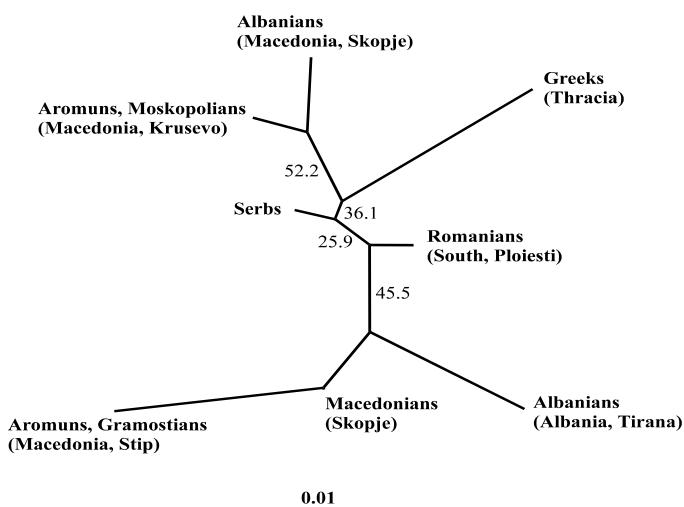


Figure 2 – Neighbour joining tree of the populations considered, based on Reynolds genetic distances (ABO, RH, MNSS, PI, Kk). The bootstrap supports of the branches are given beside the branches

Slika 2 ≠ Дендрограм конструиран врз база на пресметана Reynold-ова генетска дистанца од алелите за ABO, RH, MNSs, P1, Kk

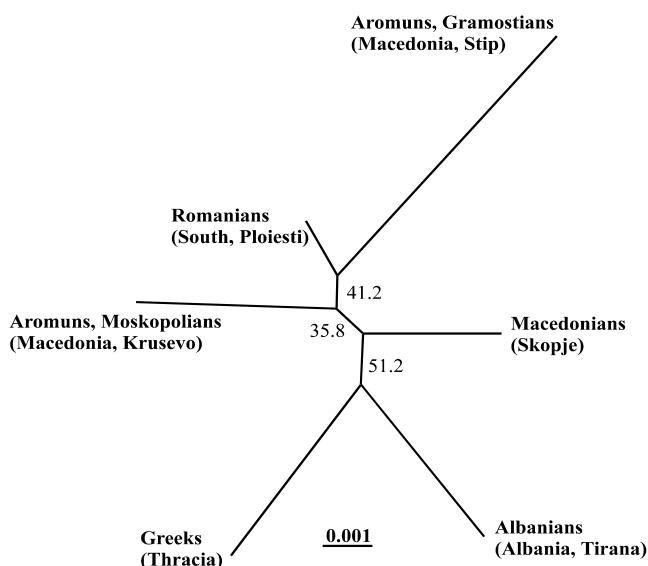


Figure 3 – Neighbor joining tree of the populations considered, based on Reynolds genetic distances (5 blood groups: ABO, RH, MNSs, P1, Kk, 13 erythrocyte enzyme polymorphisms: PGM1, PGM3, GPT, GOT2, GLO I, ESD, ACP, UMPK, ADA, AK, PGD, ALADH, SAHH, 7 serum protein polymorphisms: GC, HPA, CP, TF, BF, PI, AMY2). The bootstrap supports of the branches are given beside the branches

Slika 3 ≠ Дендрограм конструиран врз база на пресметана Reynold-ова генетска дистанца од алелите за 5 крвно-групни системи: ABO, RH, MNSs, P1, Kk 13; ролиморфизми на еритроцитарни ензими: PGM1, PGM3, GPT, GOT2, GLO I, ESD, ACP, UMPK, ADA, AK, PGD, ALADH, SAHH; 7 ролиморфизми на серумски протеини: GC, HPA, CP, TF, BF, PI, AMY2

Discussion

The distribution of allele frequencies of the ABO system in Macedonians showed the type as in the majority of the European population [10, 11]: O > A1 > B > A2. Aromanians from Kruševo and Albanians showed the type O > B > A1 > A2. A relatively high frequency of A1 has been found in Macedonians (0.2938)

The most frequent haplotypes of Rhesus are CDe and cde in all populations studied. The variation is mostly due to the variations of these two haplotypes. Higher values of CDe correspond to lower values of cde and vice

versa. CDe shows the highest frequency (0.4988) in Macedonians. The frequencies of P1+ varied from highest in Macedonians (0.4866) to lowest in Albanians (0.4024).

The allele frequencies of erythrocyte enzyme polymorphisms studied are mostly within the range of European populations [10, 12]. The allele frequencies of ALADH*2 in the Aromanians of Kruševo and in the Macedonians and the frequencies of ADA*2 and AK*2 in the Aromanians of Štip are relatively high.

The genetic distances between the populations are rather small (Tables 5 and 6).

Figure 2 shows the neighbour-joining tree for the eight populations studied, based on the five blood groups. The two Aromanian populations do not show a closer relationship. Altogether, the Aromanians from the Štip region are more separated from the other populations. The two samples of Albanians from Tirana and Skopje do not show a closer relationship either. While the Romanians and the Serbs are characterised by relatively small distances, the other larger populations such as the Greeks from Thrace, Albanians and Macedonians are more separated in the tree. However, the bootstrap values of the branches are mostly small.

The neighbour-joining tree of Figure 3, based on blood groups, erythrocyte enzyme and serum polymorphisms, resembles the tree in Figure 2 to a certain degree. Again, the two Aromanian populations do not show any closer relationship. The Albanians from Tirana and the Greeks from Thrace are more separated. As in the tree in Figure 2, the bootstrap values are small.

Results of this study are in agreement with results concerning mtDNA and y-chromosome haplogroup frequencies in the Balkans [13]. MtDNA and y-chromosome haplogroup frequencies in the Balkans were found to be similar to those elsewhere in Europe. MtDNA sequences and y-chromosome STR haplotypes revealed decreased variation in some Aromanian populations. Variation within Aromanian populations was the primary source of genetic differentiation. Y-chromosome haplotypes tended to be shared across Aromanians, but not across non-Aromanian populations.

Conclusion

The results of the comparison of the researched alleles indicate relatively small genetic distances among the researched populations. The obtained dendograms indicate a larger homogeneity in the large Balkan populations, and a manifest trend of separating the Aromanian population of the Štip region. A larger separation is characteristic in the Greek population of Thrace.

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Rezime

**ГЕНЕТСКИ ПОЛИМОРФИЗАМ НА КРВНИТЕ ГРУПИ
И ЕРИТРОЦИТАРНИТЕ ЕНЗИМИ КАЈ ОДРЕДЕНИ ПОПУЛАЦИИ
ВО РЕПУБЛИКА МАКЕДОНИЈА**

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Во оваа студија се прикажани резултатите од истражувањата на 3 крвно-группни системи (ABO, RESUS и P1) како и полиморфизмот на еритроцитарните ензими (ADA, AK, ALADH, PGD, SAHN, PGM1, PGM3, GPT, GOT, ACP, UMPK, ESD и GLO) кај популации кои живеат во Р. Македонија. Во студијата беа вклучени примероци од 4 популации: 129 Македонци од Скопје, 98 Албанци од Скопје, 95 Власи од Крушево и 102 Власи од регионот на Штип. Направено е компарирање на добиените резултати со податоци од литературата за другите балкански народи. Резултатите од компарирањето на испитуваните алели укажуваат на релативно мала генетска дистанца помеѓу испитуваните популации. Добиените дендрограми укажуваат на поголема хомогеност на големите балкански народи, а манифестиран тренд на сепарирање на влашката популација од регионот на Штип. Поголема сепарираност е карактеристика и на Грците од регионот на Тракија.

Klu~ni zborovi: Makedonija, geneti~ka distanca, крвно-группни, еритроцитарни ензими.

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