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PHENOTYPE AND GENE FREQUENCIES OF SOME BLOOD GROUPS SYSTEM IN BULGARIANS FROM SMOLYAN REGION

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ABSTRACT. For the study of blood group, serum and enzyme systems we made parallel investigations. We took blood samples from 540 individual with Bulgarian origin, living in the Smolyan region of Bulgaria, males and females, at the age from 18 to 45, clinically healthy and without family relationships among them.
ABO, Rh, MNSs, P1, GM1 blood grouping were done by conventional methods
The phenotypes of ACP, PGM, ESD and HPA were determined through horizontal electrophoresis of haemolysates on starch gel.
During all surveys we found only the classic types of blood group systems.
In the contingent surveyed the greatest distribution belongs to the phenotypes respectively in erythrocyte systems – A_1 (43,33%), CcDee (33,33%), MNS (34,44%) and $P1^+$ (56,67%); in serum systems – HPA 2-2 (58,89%), $GM1^-$ (66,67%) and in enzyme systems - ACP B (37,78%), EsD1 (85,56%) and PGM 2-1 (54,44%).
The frequency of the blood group, serum and enzyme systems variation in the studied Bulgarian population from Smolyan do es not differ substantially from the other Bulgarian population. We observe a tendency of West-East and North-South geographic distribution.

KEY WORDS. blood group, phenotype, genotype, enzyme, polymorphism.

INTRODUCTION

The survey of blood system polymorphism gives answers to some general biological problems such as the micro-selective and micro-evolutionary changes taking place constantly in contemporary humans in the process of their interaction with the conditions of the environment in which they live. The stability of blood group factors as well as the possibility to determine them by means of objective and relatively simple methods makes them particularly convenient for population surveys

and provides an opportunity to find mutations, phenomena like isolation, migration and drift of genes.

The various populations are typical with their own aggregates of features that can be predetermined by mixing or isolation, genetic mutations or chromosome combinations, or adaptation capacities to different ecological situations.

After reviewing the available literature sources, we found out that in Bulgaria so far population-genetic research has been conducted concerning blood group systems in big cities – Sofia, Plovdiv, and in the South-central and Southeastern regions.[Калчев, 1980; Пеев,1980; Рупчева,1972; Baltova et.al., 2005; Boev et al., 1969; Ilieva,1956; Karamihova-Tsacheva,1967; Popwassilew et al.,1962].

We still have insufficient data about their phenotype and genotype distribution for other towns and regions.

The purpose of the present survey is to determine the phenotype distribution and gene frequencies of blood group systems aiming at clarification the genetic status of Bulgarian population from the district of Smolyan, which constitutes a part of the south-central region; then we would like to make comparisons with other populations. There is no data in literature references about surveys conducted of serum and enzyme systems in this region.

MATERIAL AND METHODS

For the study of blood group, serum and enzyme systems we made parallel investigations. We took blood samples from 540 individual with Bulgarian origin, living in the Smolyan region of Bulgaria, males and females, at the age from 18 to 45, clinically healthy and without family relationships among them.

ABO, Rh, MNSs, P1, GM1 blood grouping were done by conventional methods.

The phenotypes of ACP and PGM were determined through horizontal electrophoresis of haemolysates on starch gel, according to the method of Radam G., Strauch.

We performed the development of the phenotypes by using a substrate respectively for PGM – Glucose-1-phosphate with the participation of Glucose-6-phosphate dehydrogenase, NADP, PMS and MTT in Tris-agar buffer solution, and for the ACP – phenolphthaleindiphosphate with subsequent alkalizing of the gel plate with ammonium.

The phenotypes of ESD and HPA were determined through horizontal electrophoresis of haemolysates on starch gel, according to the method of Goedde and Benkmann, Smithies and modification of Prokop and Bundschuh.

The dyeing was conducted respectively for EsD with 4 methyl-umbelliferyl acetate, and for the HPA with benzidine after fixation with 0,5% naphthol yellow. The reading of the phenotypes EsD was performed under ultraviolet lighting at wavelength of 366 nm.

The statistical analysis of the data was mode by the methods of alternative, correlation and non-parametric analyses.

RESULTS AND DISCUSSION

During all surveys we found only the classic types of blood group systems.

In the contingent surveyed the greatest distribution belongs to the phenotypes respectively in erythrocyte systems – A_1 (43,33%), CcDee (33,33%), MNS (34,44%) and $P1^+$ (56,67%); in serum systems – HPA 2-2 (58,89%), $GM1^-$ (66,67%) and in enzyme systems - ACP B (37,78%), EsD1 (85,56%) and PGM 2-1 (54,44%).

The other most rarely found versions are: A_2 (2,22%); Ccddee and CCDEe (1,11%); NS (3,33%); P^- (43,33); HPA 1-1 (7,78%); $GM1^+$ (33,33%); ACP A (4,44%); PGM 2-2 (12,22%); ESD 2-2 (2,22%).

The surveyed contingent of persons without kinship relations amongst them provided an opportunity for us to calculate the incident gene frequencies.(Table 1).

By using the Pearson criterion we compared the distribution between the monitored and expected values and we found that the difference is insignificant /$P>0,05$/. The concordance between the monitored and expected values is good and according to the law of Hardy-Weinberg, it demonstrates that the surveyed population is in genetic equilibrium in terms of the blood group systems.

The present survey is a continuation of our previous studies of blood group systems among Bulgarian population from the South central and the Southeastern regions.[Baltova et al.,2005]. We also compared the results of the tests with the results concerning the Bulgarian population by other authors and from the data in available literature references. (Table 2).

The phenotype and gene frequencies of blood group systems vary in the different populations and on the grounds of the difference thus established between them their serological characteristic is formed. The informative value of a given system depends on the intensity of the existing geographical, racial and national differences in the frequency of its belonging alleles. Not each of them demonstrates identical variability.

It is evident from table 2 that among the persons tested in the district of Smolyan the gene frequencies in the separate blood group systems is slightly increased for alleles A_1, B, MS, Ms, NS, cDe, Cde, ACP*B, PGM*2, a bit higher for cde, cDE, CDE, $P1^-$, ACP*C, EsD*1, HPA 2, $GM1^-$. In alleles A_2, O, Ns, PGM1*1 it is a bit lowered, while in $P1^+$, ACP*A,ESD*2, HPA 1 and $GM1^+$ – the lowering is greater.

The lower values for the frequency of the alleles in the surveyed group of Bulgarians corresponds to the historical and geographical data related to their origins. This population has not yet experienced the migration processes and the people who remained to live there are of native origin. The genetic memory of previous populations has been preserved in them to a certain extent. The genetic changeability exists most of all inside the population unit and the change rate increases, while the effect of the accidental drift of the genes increases.

The values of the allele frequency of different Bulgarian subpopulations vary within narrow limits with a certain tendency of increasing from the south to the north and from the west to the east.

The results of our surveys as a whole also comply with these related to other European populations from literature references. [Pap, 2000; Scheil et al. 2000, 2001; Schmid et al. 2000, 2001, 2003]. The demonstrated tendency of west-east geographical distribution persists.

CONCLUSIONS

The most frequently phenotypes of enzyme systems are homozygote followed by the heterozygous. The genetic frequencies of the alleles from ACP, ESD and PGM are as follows: ACP*A- 0,2278, ACP*B- 0,5278, ACP*C- 0,1444; ESD*1-0,9167, ESD*2-0,0833 and PGM*1- 0,6056, PGM*2- 0,3944.

The frequency of the blood group, serum and enzyme systems variation in the studied Bulgarian population from Smolyan do es not differ substantially from the other Bulgarian population. We observe a tendency of West-East and North-South geographic distribution.

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Table 1. The distribution of phenotypes and gene frequencies in Blood groups from Smolyan regions

Phenotype	*N = 540*			
	Obs.	**%**	**Exp.**	**Allele and Haplotype frequencies**
A_1	234	43,33	218,64	A1 0.2812
A_2	12	2,22	9,96	A2 0.0161
B	114	21,11	95,28	B 0.1394
O	156	28,89	171,36	0 0.5633
A_1B	24	4,45	42,06	
A_2B	-	-	2,7	
			df = 0	
ccddee	72	13,33	58,89	cde 0.3295
ccDee	36	6,67	29,62	cDe 0.0741
ccDEe	24	4,44	61,65	Cde 0.0164
ccDEE	12	2,22	11,03	Cde 0.4245
Ccddee	6	1,11	6,09	cDE 0.1409
CcDee	180	33,33	186,57	CDE 0.0147
CcDEe	114	21,11	73,77	
CCDee	90	16,68	105,09	
CCDEe	6	1,11	7,29	
			df = 0	
MS	138	25,56	151,08	MS 0.3108
Ms	54	10,00	46,92	Ms 0.2948
MNS	186	34,44	159,06	NS 0.0837
MNs	84	15,56	98,94	Ns 0.3107
NS	18	3,33	31,86	
Ns	60	11,11	52,14	
			χ^2 = 2.7094, df = 2, 20 < p < 30	
P1+	306	56,67	-	P1+ 0.3417
P1-	234	43,33	-	P1- 0.6583
HPA 1-1	42	7,78	32,28	HPA*1 0.2444
HPA 2-1	180	33,33	199,44	HPA*2 0.7556
HPA 2-2	318	58,89	308,28	
			χ^2 = 0.8547, df = 1, 30 < p < 50	
GM1+	180	33,33	-	GM1 0.1835
GM1-	360	66,67	-	nonGM1 0.8165
ACP A	24	4,44	28,02	ACP*A 0.2278
ACP B	204	37,78	212,82	ACP*B 0.6278
ACP C	-	-	11,28	ACP*C 0.1444
ACP AB	156	28,89	154,44	
ACP AC	42	7,78	35,52	
ACP BC	114	21,11	97,92	
			χ^2 = 2.7495, df = 2, 20 < p < 30	
ESD 1	462	85,56	453,78	ESD*1 0.9167
ESD 2-1	66	12,22	82,50	ESD*2 0.0833
ESD 2	12	2,22	3,72	
			df = 0	
PGM1 1	180	33,34	198,06	PGM1*1 0.6056
PGM1 2-1	294	54,44	257,94	PGM1*2 0.3944
PGM1 2	66	12,22	84,00	
			χ^2 = 1.7576, df = 1, 10 < p < 20	

"df = 0" means: no degrees of freedom for Hardy-Weinberg testing

Table 2. Allele and haplotype frequencies in the samples studied and from literature

Allele bzw.Haplotyp en	References					
A1	0.2554	0,2812	0,2447	0,2880	0,3058	0,3193
A2	0.0378	0,0161	-	-	-	-
B	0.1285	0,1394	0,1074	0,0783	0,1263	0,1322
O	0.5783	0,5633	0,6479	0,6337	0,5679	0,5485
n	2346	540	1000	500	15422	11000
	Baltova et al.2005	Baltova* 2005	Ilieva 1956	Stojanov 1959	Zographov 1962	Boev&Powas silew 1969
Population	South, central,South-eastern Bulgaria	Smolyan	Sofia	Sofia	Bulgaria	Bulgaria
MS	0.2862	0,3108	0,5545	0,5722		
Ms	0.2641	0,2948	-	-		
NS	0.0544	0,0837	0,4455	0,4278		
Ns	0.3953	0,3107	-	-		
n	2346	540	1000	672		
	Baltova et al.2005	Baltova 2005	Ilieva 1956	Boev&Popva ssilew 1969		
Population	South, central,South-eastern Bulgaria	Smolyan	Sofia	Sofia		
cde	0.2708	0,3295				
cDe	0.0737	0,0741				
Cde	0.0126	0,0164				
CDe	0.5528	0,4245	D :0,6061	D :0,6232		
cDE	0.0818	0,1409	d :0,3939	d :0,3768		
CDE	0.0083	0,0147				
n	2346	540	232	500		
	Baltova et al.2005	Baltova 2005	Ilieva 1956	Stojanov 1959		
Population	South, central,South-eastern Bulgaria	Smolyan	Sofia	Sofia		

P1+	0.4740	0,3417	0,4317	0,4813
P1-	0.5260	0,6583	0,5683	0,5817
n	2346	540	1000	1200
	Baltova et al.2005	Baltova 2005	Popwassilew &Rackwitz	Boev&Popwassilew 1969
Population	South, central,South-eastern Bulgaria	Smolyan	1962 Sofia	Sofia
ACP*A	0.3529	0,2278	0,1596	0,3594
ACP*B	0.5989	0,6278	0,7983	0,5733
ACP*C	0.0482	0,1444	0,0420	0,0673
n	2346	540	119	1440
	Baltova et al.2003-5	Baltova 2005	Ananthakrishnan et al.	Rupcheva 1972
Population	South, central,South-eastern Bulgaria	Smolyan	1972 Sofia	Sofia
PGM1*1	0.6782	0,6056	0,8346	0,7118
PGM1*2	0.3218	0,3944	0,1653	0,2882
n	2346	540	127	1785
	Baltova et al.2005	Baltova 2005	Ananthakrishnan et al.	Kalchev 1980
Population	South, central,South-eastern Bulgaria	Smolyan	1972 Sofia	Bulgaria
ESD*1	0.8497	0,9167	0,9070	0,8976
ESD*2	0.1503	0,0833	0,0930	0,1024
n	2346	540	1161	1660
	Baltova et al.2005	Baltova 2005	Rupcheva 1972	Peev 1980 South
Population	South, central,South-eastern Bulgaria	Smolyan	Sofia	Bulgaria

ESD*2	0.1503	0,0833	0,0930	0,1024				
n	2346	540	1161	1660				
	Baltova et al.2005	Baltova 2005	Rupcheva 1972	Peev 1980				
Population	South, central,South-eastern Bulgaria	Smolyan	Sofia	South Bulgaria				
HPA*1	0.3367	0,2444	0,3702	0,2865	0,3263	0,3452	0,3845	0,3798
HPA*2	0.6633	0,7556	0,6298	0,7135	0,6737	0,6548	0,6155	0,6202
n	2346	540	235	363	213	365	342	682
	Baltova et al.2005	Baltova 2005	Karamihova-Tsacheva 1967(1429	Karamihova-Tsacheva 1967(1428	Karamihova-Tsacheva 1967(1429	Karamihova-Tsacheva 1967(1429	Karamihova-Tsacheva 1967(1429	Karamihova-Tsacheva 1967(1429
Population	South-central,South-eastern Bulgaria	Smolyan	Central Danubian Plain	North-East and Dobruja	North-West	Thrace,Central and Eastern	Thrace,Rhodopen Mountains	South-West and Sofia
GM(1) +	0.2605	0,1835						
GM(1) –	0.7395	0,8165						
n	2346	540						
	Baltova et al.2005	Baltova 2005						
Population	South , central,South-eastern Bulgaria	Smolyan						

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