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Immunological studies in an isolate in Hungary: Ivád

Frequency of blood groups, saliva secretion and antibody titers

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The village Ivád is located in Northern Hungary in the county Heves, at the northern margin of the Mátra mountains. About 70 p. c. of the population (561 inhabitants in 1949) belong to the great family of Ivádys, who originate from Pétervására and moved to the neighbouring range Ivád in 1738. From 1759, the Ivádys began to marry Ivádys, the frequency of such marriages was increasing till the 20th century. Inbreeding was caused by geographical and social factors as well. The frequency of consanguineous marriages expressed in p.c. of the total number of marriages changed as follows:

Period	consanguineous marriages in p. c.
1751-1800	3,5
1801-1850	18,0
1851-1900	30,2
1901-1940	36,2

Ivád is undoubtely endogameous as far as European conditions are concerned.

An extended study of the population was made between 1940 and 1952 by a group of anthropological, sociological and medical experts. As regards anthropometric characters a slightly narrowed variation was found. The medical examinations have not revealed special hereditary defects. (10, 11, 12, 13). The purpose of the present study was to determine the frequency of the blood groups and of the saliva secretion in the population of Ivád and to compare the results with corresponding data for other Hungarian populations. Simultaneously immunological investigations were made too. Some preliminary reports were published earlier (1, 2, 4).

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Materials and methods

1. *ABO blood groups.* Determinations were made by the slide test of blood samples taken from the fingertip or from a vein, using human anti-A, anti-B and anti-A + B grouping sera of at least 1:64 titre. The results were confirmed by isoagglutinin titrations in about 50 p. c. of the cases. Gene frequencies were determined by *Fisher's* formula as quoted by *Khérumian* (7).

$$\text{O—gene} = \frac{s}{v};\ \text{A—gene} = \frac{t-s}{v};\ \text{B—gene} = \frac{u-s}{v};\ \chi^2 = \frac{t.u}{w.x}\,z^2;\ \text{where}$$

$$s = \sqrt{O};\quad t = \sqrt{O+A};\quad u = \sqrt{O+B};\quad v = t+u-s;\quad w = v^2;$$
$$x = w - /O + A + B/;\ y = AB;\ z = x - y.$$

A, B, AB and O mean the absolute number of individuals belonging to the corresponding blood group. If the value of χ^2 is higher than the limiting value computed at one degree of freedom ($\chi^2_{0,05} = 3{,}841$), it means that the population tested is not in genetical equilibrium or that the blood group determinations were erroneous.

2. *MN blood groups.* Determinations were made by the slide test using two anti—M and two anti—N absorbed rabbit sera. Gene frequencies were computed from the formula of *Wiener* and *Waisberg* (8).

$$\text{M—gene} = M + \frac{MN}{2};\qquad \text{N—gene} = N + \frac{MN}{2};\ \text{where}$$

M, MN and mean the frequency of the respective group in p.c.

The frequencies expected and χ^2 were determined as follows (15):

expected frequency of group M = (gene-M)²
expected frequency of group MN = (gene-M) . (gene-N)
expected frequency of group N = (gene-N)²

$$\chi^2 = n\,\frac{\left[\overline{MN}^2 - (4\cdot\overline{M}\cdot\overline{N})\right]^2}{\left[(2\cdot\overline{M}) + \overline{MN}\right]^2 \cdot \left[\overline{MN} + (2\cdot\overline{N})\right]^2},\ \text{where}$$

$\overline{M}$, $\overline{MN}$ and $\overline{N}$ mean the absolute number of individuals belonging to the respective group and n the total number examined.

3. *Factor D.* Factor D (Rh_0) was determined by the slide method by means of human anti-D sera of at least 1:64 titre containing incomplete haemagglutinins. In some cases an anti-C+D serum of complete type was employed too. About 50

p. c. of the D-negative bloods were examined by means of indirect antiglobulin test too. D- and d-gene frequencies were calculated as follows (9):

d-gene $= 1 - \sqrt{d}$; D-gene $= 1 -$ d-gene, where

d means the frequency of D-negative individuals.

4. *Saliva secretion.* Salivas of individuals of blood groups A, B and AB were boiled at 100 C_0 and their inhibiting capacity on complete anti-A and anti-B haemagglutinins was measured. (Having no anti-H serum or lectin this time, blood group O subjects could not be tested).

5. *Isoagglutinins.* Titres of anti-A and anti-B complete haemagglutinins were determined in test tubes, adding equal volumes of a 2 p. c. erythrocyte suspension to the serial dilutions of the serum to be examined. Heamagglutination was read after 4 hours incubation at room temperature.

6. *Bacterial agglutinins.* Titres of bacterial agglutinins were determined by serial dilutions of the sera to be tested in serological tubes. As agglutinogens the following bacterial suspensions were used: S. typhi O, S. typhi H, Sh. sonnei, Sh. flexneri 2a, Sh. dysenteriae I. (Sh. shigae), Ps. pyocyanaea (6 strains).

7. *Diphtheria antitoxin.* Serum titres were determined by the method of *Jensen* (6).

Results

1. *ABO blood groups.* 577 individuals were tested, 414 of them belonged to the *Ivády* family (this group included about 50 *Ivádys* who lived outside the village e. g. at *Pétervására*). As comparative data we present the results for the people living in *Eger* (*Eger* a town to 30 Km to *Ivád*) and in villages near *Eger* and *Ivád* (Table I). In none of the populations examined was the χ^2 higher, than the limiting value ($\chi^2_{0,05} = 3{,}841$), which means, that the population seems to be in a genetical equilibrium. The A-

Table 1. Frequency of ABO blood groups and genes in Ivád, Heves County and Budapest

Population tested	N. obs.	Frequency of blood groups				Frequency of genes			χ^2
		O	A	B	AB	A	B	O	
Ivády family	414	26,57	48,79	15,70	8,94	35,14	13,43	51,40	0,069
Non-Ivádys in Ivád	163	29,45	42,23	19,69	8,59	30,32	15,67	54,01	0,096
Eger	1160	31,89	42,41	16,72	8,96	29,90	13,35	56,78	0,951
Villages near Eger	175	32,58	39,43	19,49	8,57	27,48	15,06	57,06	0,011
Budapest, series I	1041	29,87	45,24	17,00	7,88	31,86	13,74	54,40	0,639
Budapest, series II	2966	32,87	42,04	17,76	7,32	29,11	13,79	57,12	0,968

gene is higher and the O-gene is lower in frequency among the members of the Ivády family than among the other three populations tested, but the differences are not significant. The limiting value of $\chi^2_{0,05}$ with 3 degrees of freedom is 7, 815, while corresponding values are as follows:

groups compared	χ^2
Ivádys — non-Ivádys at Ivád	2,722
Ivádys — Eger population	5,839
Ivádys — population of villages near Eger and Ivád	6,079

2. *MN blood groups.* 219 subjects were tested. 164 of them were *Ivádys.* The number of non-Ivádys being very small, earlier data for Hungary (*Budapest*) served as a basis of comparison (8). In none of the two groups exceeded the χ^2 the limit $\chi^2_{0,05} = 3,841$. The M-gene is 4,13 p. c. higher in frequency among the *Ivádys* than among the inhabitants of *Budapest* but the difference is not significant, χ^2 beeing 2,637, which at 2 degrees of freedom is lower than the limiting value ($\chi^2_{0,05} = 5,991$). Results are shown in Table 2.

Table 2. Frequency of MN blood groups and genes in Ivád and Budapest

	Ivády family		Budapest population	
	observed	expected	observed	expected
N. obs.	164		624	
M group	66	62,17	209	205,92
MN group	70	77,58	229	305,07
N group	28	24,19	116	113,00
M group p. c.	40,24	37,92	33,49	33,00
MN group p. c.	42,68	47,31	47,92	48,89
N group p. c.	17,07	14,75	18,59	18,11
M-gene p. c.	61,58		57,45	
N-gene p. c.	38,42		42,55	
χ^2	1,330		0,00003	

3. *Factor D.* 523 tests were done, including 387 made on members of the *Ivády* family (Table III). D-negative blood type and d-gene is lower in frequency among the *Ivádys* than among the other populations serving as controls, but the differences

Table 3. Frequency of D-positive and D-negative blood types and genes in Ivád, Heves County and Budapest

Population tested	No. obs.	D-pos.	D-neg.	D-gene	d-gene
		p. c.			
Ivády family	387	84,50	15,50	60,62	39,38
Non-Ivádys in Ivád	136	80,88	19,12	56,27	43,73
Eger	1160	83,28	16,72	59,01	40,99
Villages near Eger	175	81,16	18,84	56,57	43,43
Budapest	3349	83,43	16,57	59,29	40,71

are not significant. The limiting value of $\chi^2_{0,05}$ at 1 degree of freedom is 3,841, while corresponding values are following:

groups compared	χ^2
Ivádys — non-Ivádys at Ivád	0,937
Ivádys — Eger population	0,886
Ivádys — population of villages near Eger and Ivád	0,961

It may be noted that among 30 D-negative *Ivádys* examined by anti-C + D serum none was C-positive and indirect antiglobulin tests performed with these bloods using incomplete anti-D sera were negative. It can be stated therefore that chromosome Cde and factor D^u was lacking among the *Ivádys tested.*

4. *Saliva secretion.* Secretion of AB-substances was investigated in a total of 102 cases. The results were compared with those for *Budapest* and *Szeged* (town in South Hungary). No significant difference could be demonstrated among the three groups, χ^2 beeing 0,050, while at 2 degrees of freedom the limiting value is $\chi^2_{0,05} = 5,991$ (Table 4).

Table 4. Frequency of the saliva AB-secretion in Ivád, Budapest and Szeged

Population tested	No. obs	secretors p. c.	non-secretors p. c.
Ivád	102	80,95	19,05
Budapest	139	82,00	18,00
Szeged	114	81,58	18,42

6. *Serum antibodies.* Isoagglutinin titres were determined in sera of 110 individuals. The most frequent are the isoagglutinins of titres 1:32 — 1:256, which is in a good agreement with those obtained by *Thomsen* and *Kettel* (17), and by *Szathmáry* and *Molnár* [16]. (Table 5).

Table 5. Isoagglutinin titres of the Ivád population

Blood groups	Antibody tested	Isoagglutinin titres, number and frequency of cases					Total
		$1:2\text{-}2^2$	$1:2^3\text{-}2^4$	$1:2^5\text{-}2^6$	$1:2^7\text{-}2^8$	$>1:2^8$	
B	anti-A	1 4,2	1 4,2	9 37,5	11 45,8	2 8,3 per cent	24
O		0 —	6 19,3	13 41,9	12 38,7	0 — per cent	31
O+B		1 1,8	7 12,7	22 40,0	23 41,8	2 3,6 per cent	55
A	anti-B	1 1,9	10 18,9	25 47,2	16 30,2	1 1,9 per cent	53
O		1 3,5	5 17,2	14 48,3	9 31,0	0 — per cent	29
O+A		2 2,4	15 18,3	39 47,5	25 30,5	1 1,2 per cent	82

Frequencies of four bacterial agglutinins are shown in Table 6, using our own data obtained in *Budapest* for comparison. Titres of Sh. flexneri and Sh. dysenteriae I agglutinins were higher at *Budapest*, those of S. typhi O at *Ivád*. It is noteworthy that antibody titres showed a narrower variation among the inhabitants of *Ivád* than among those of *Budapest*. Sh. dysenteriae I infections are very rare in Hungary, Sh. dysenteriae I agglutinins can be regarded as " natural antibodies ". (In 1951 only 0,2 p. c. of all Shigella infections were caused by Sh. dysenteriae I. [14]). In the great majority of cases S. typhi H, and Ps. pyocyanaea agglutinins were not demonstrable, in other their titres were very low (1:10 or 1-20), so they are not shown in Table 6.

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Table 6 - Frequency of bacterial agglutinins in Ivád and Budapest

Antibody titre 1:	Sh. flexneri		Sh. sonnei		Sh. shigae		S. typhi o	
	Ivád	Budapest	Ivád	Budapest	Ivád	Budapest	Ivád	Budapest
$<2^3$	26,9	23,1	90,0	87,3	23,9	26,8	2,1	25,2
2^3-2^4	60,5	45,8	10,0	12,7	64,5	53,8	31,4	35,6
2^5-2^6	12,7	28,3	—	—	11,5	18,7	65,0	34,3
2^7-2^8	—	2,3	—	—	—	0,6	2,1	5,0
$<2^8$	—	0,5	—	—	—	—	—	—
Number of observations	134	441	110	250	138	310	137	140

According to the results of immunological and clinical investigations, inheritable serological disorders e.g. agammaglobulinaemia were not observed.

Maternal isoimmunization against D-factor was demonstrable in one case, therefore it can be concluded, that this phenomenon did not show a greater frequency among married D-negative women in Ivád (29 wives), than mentioned in the literature (about 5 p.c. [15]). 140 tests have been made for diphtheria antitoxin titres with the following results:

less than 0,005 I.U.	7,1 p. c.
0,005 — 0,05 I.U.	7,9 p. c.
0,05 — 0,5 I.U.	13,6 p. c.
more than 0,5 I.U.	71,4 p. c.

Discussion

Evaluating our data concerning the frequencies of the A-, B-, O-, d-, M-, N- and Secretor-genes, no significant difference is demonstrable at the 0,05 level between the members of the *Ivády* family and the control groups. Similar investigations made by us in isolated populations in North-East Hungary (Bodrogk$^{\ddot{o}}$z) showed significantly increased frequencies of the A- and d-genes in *Alsóberecki* and *Kisrozvágy* as compared with those for the populations of the neighbouring villages. It seemed then the most likely that the original gene frequencies of the immigrating families which significantly differed from those of the neighbouring populations were preserved by means of endogamy and isolation maintained by geographical, social and religious factors. On the contrary, although the Ivády family seems to form a comparatively homogenous community, inbred for a considerable time, before the beginning of the endogamy they had incorporated most of their genes from the populations surrounding them. The separation of the family could give rise to a difference in gene-fre-

quencies both from natural selection and in view of the relaive small number, from genetic drift.

As regards the mechanism of their separation, there is a close similarity between the *Ivády* family and the *Dunker* isolate (5). The later derived from ancestors whose gene frequencies were presumably similar to those of the environment, but inbreeding is undoubtely the cause of significant differences in the frequencies of some genes (A, M, D) between this isolate and the original German population. It is notable that a higher frequency of the *same genes* is observable among the members of the *Ivády* family, though the differences at a level of 0,05 are not significant.

Antibody titres showed a minor variation around the mean in Ivád, than in Budapest. The evidence we have obtained at Ivád as to the inheritability of the individual antibody producing capacity does not rule out such a possibility, but the number of examinations is insufficient to enable us to make a final statement. Perhaps the narrower variation of antibody titres and of anthropometric characters can be explained by a similar machanism caused by endogamy. The greater uniformity of antibody titres may be due enviomental factors too. Our epidemiological investigations showed that the course of some epidemics was similar to that observed among other isolated populations. Between two outbreaks of measles 12 years elapsed, but the last one affected nearly all children under 12 years. A similar behaviour of cholera in the 19th century and of pertussis was observable. Uniformity in antibody titres could be caused by simultaneous infection of the susceptible population. Variations found concerning diphtheria antitoxin titres speak however against the possibility of such generalisation and the role of genetic factors seems to be more likely.

Summary

Data are presented concerning the distribution of the ABO, MN and Rh blood groups and the saliva AB-secretion in the endogameous population of *Ivád* (Ivády family). Although a greater frequency of the A-, M-, and D-genes was observable, the differences between the *Ivády* family and the surrounding populations from where the family originated, were not significant. Isoagglutinin and bacterial agglutinin titres showed a narrower variation at *Ivád* than in *Budapest*. No agammaglobulinaemia or a higher frequency of Rhesus isoimmunization was observable. The most prominent feature of the *Ivády* isolate was the conservation of the original gene frequencies. The effect of random genetic drift or of selection was not demonstrable as far as the genes investigated are concerned.

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RIASSUNTO

Lo studio riporta dati sulla frequenza dei gruppi sanguigni ABO, MN, Rh e della secrezione salivare relativamente alla popolazione endogamica di Ivád (famiglia Ivády). Benchè si fosse potuta osservare una maggiore frequenza dei geni A, M e D, le differenze tra la famiglia Ivády e la popolazione circostante — dalla quale detta famiglia discende — non erano considerevoli. I titoli delle isoagglutinine e delle agglutinine batteriche mostravano una variazione meno rilevante a Ivád che non a Budapest. Non si poteva osservare una qualche frequenza di agammaglobulinemia o dell'isoimmunizzazione Rh. La caratteristica più notevole dell'isolato Ivády è la conservazione della originale frequenza dei geni. Nei riguardi dei geni esaminati non si è potuto dimostrare l'effetto della selezione o del « random genetic drift ».

RÉSUMÉ

Cette étude rapporte des données sur la fréquence des groupes sanguins ABO, MN et Rh, et de la secrétion salivaire concernant la population endogamique d'Ivád /famille Ivády/.

Bien que nous y ayons observé une plus grande fréquence de gènes A, M et D; la différence qui se montre entre la famille Ivády et la population environnante — d'où la famille en question descend — n'est pas considérable.

Les titres des isoagglutinines et des agglutinines bactériennes montrent une variation moins grende à Ivád qu'à Budapest. On n'a pas observé de fréquence importante d'agammaglobulinaemie ou d'isoïmmunisation Rh.

Le trait le plus caractéristique de l'isolat Ivády se présente dans la conservation de la fréquence originale des gènes. Quant aux gènes observés, on n'a pu y démontrer ni l'effet de la sélection, ni celui du « random genetic drift ».

ZUSAMMENFASSUNG

Die Studie enthält Daten über die Häufigkeit der Blutgruppen ABO, MN und Rh und der Sekretion in der endogamen Bevölkerung von Ivád /Familie Ivády/. Obgleich eine grössere Häufigkeit der Gene A, M und D beobachtet werden konnte, war der Unterschied zwischen der Familie Ivády und der umliegenden Bevölkerung — von welcher die Familie abstammt — unwesentlich.

Die Titel der Isoagglutinine und bakteriellen Agglutinine wiesen in Ivád eine geringere Variation auf, als in Budapest. Eine grössere Häufigkeit von Agammaglobulinaemie oder von Rh-Isoimmunisation war nicht festzustellen. Das charakteristische Merkmal der Endogamie von Ivád ist die Konservation der Originalhäufigkeit der Gene. In Bezug auf die untersuchten Gene war der Selektionseffekt oder der « random genetic drift » nicht nachweisbar.