

STUDIES OF RED BLOOD CELL AND SERUM POLYMORPHISMS AMONG THE MATAGI

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Summary Serum samples from 159 Matagi were tested for nine blood group systems, haptoglobins, transferrins, Gc, 10 red blood cell enzyme polymorphisms and for the Gm and Inv allotypes to obtain evidence concerning their ethnic origin. The data exclude all claims except that they are of Japanese origin.

INTRODUCTION

The Matagi have lived in the Ohu mountains of north eastern Japan for an unknown number of centuries. They differ from the main body of Japanese in two respects; they speak a dialect (Matagi) of Japanese peculiar to them and they were and are professional big game hunters. There was and may still be an involved tradition (religion?) associated with the hunting pattern (Encyclopedia of the World). These two cultural characteristics have kept them isolated from the remainder of the inhabitants of Japan and this has led to much speculation about their origin, especially by cultural anthropologists. The speculations range from the claim by the folklorist Kita (1932) that the Matagi are Ainu to Muto's claim (1970) that they are descended from a tribe in India.

The hypotheses advanced by the cultural anthropologists concerning the origin of the Matagi are so markedly heterogeneous that one would have expected physical anthropologists to have studied them. We know of no such studies, however. While we are not physical anthropologists, it seemed to us that a study of genetic polymorphisms among these people could offer insight concerning their racial ancestry. Accordingly, several serum and red blood cell polymorphisms were determined on 159 blood samples collected from Matagi living in villages in the Ohu mountains.

Table 1. Reagents used to type serum samples from the Matagi for Gm and Inv allotypes.

Allotype	Anti-Allotype	Anti-D
Gm(1)	Mor	Bar
Gm(2)	Ham	Bar
Gm(3)	Coa	Car
Gm(5)	Pay	Jol
Gm(6)	Har	Kre
Gm(13)	Ter	Jol
Gm(14)	Ber	Car
Gm(15)	2624 ¹	Pur ²
Gm(16)	George ³	Pur
Gm(17)	R 15 ⁴	Bar
Gm(21)	Cli	Bar
Gm(26)	Whi	Kre
Inv(1)	Mas	Roe

¹ Gift from Dr. E. van Loghem.

² Gift from Dr. M. Schanfield.

³ Rhesus monkey anti-Gm(16).

⁴ Rabbit anti-Gm(17).

MATERIALS AND METHODS

Blood samples were collected from 159 Matagi residing in the following villages: Nakamura, Shinnaka, Koyawatari, Okashinai, Hitachinai, Utto, Nekko and Totorinai in Kita-Akita County, Akita Prefecture.

Blood groups (ABO, MNSs, Rh, Kell, Duffy, Kidd, Diego, Lutheran, P), haptoglobins, transferrins, Gc, and enzyme polymorphisms (red blood cell acid phosphatase, adenosine deaminase, phosphogluconate dehydrogenase, esterase D, phosphoglucomutase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, phosphohexose isomerase, phosphoglycerate kinase and adenylate kinase) were determined in Japan by standard methods (Giblett, 1969). The sera were tested in Cleveland, by previously published methods (Steinberg, 1962) for Gm (1,2,3,5,6,13, 14,17,21,26), and Inv(1) with the reagents shown in Table 1. Selected samples were tested for Gm(15) and Gm(16). The allotypes, except for Gm(26), are described in Grubb's book (1970). Gm(26) is usually present in a haplotype lacking Gm(15) and, *vice versa*, it is usually absent when the haplotype carries Gm(15) (van Loghem and Grobbelaar, 1971; Steinberg, in press.)

RESULTS

Gm and Inv: The data, analysed without regard to Gm(26), to make comparisons with published data easier, are presented in Table 2 (The data for Gm(26) will be discussed in a later section). The haplotype frequencies were estimated by the

Table 2. Gm and Inv data for serum samples from Matagi tested for Gm(1,2,3,5,6,13,14,17,21).

Gm Phenotype	Numbers		Haplotypes		
	Obs.	Exp.	Gm	Freq.	σ
1,13,17,21	39	42.5	1,17,21	.453	.029
1,17,21	34	32.6	1,13,17	.295	.025
1,2,17,21	27	27.2	1,2,17,21	.161	.021
1,2,13,17,21	17	15.1	1,3,5,13,14	.091	.016
1,13,17	15	13.9			
1,3,5,13,14,17,21	14	13.1			
1,3,5,13,14,17	8	8.6			
1,2,3,5,13,14,17,21	3	4.7			
1,3,5,13,14	2	1.3			
Total	159	159.0			

$$\chi^2_{(5)} = 1.721$$

$$.9 > p > .8$$

Inv	
Phenotype	No.
1	81
-1	78
Total	159

$$Inv^1 = .300 \pm .028$$

maximum likelihood method assuming the Hardy-Weinberg (H.-W.) equilibrium, with the aid of the computer program MAXIM (Kurczynski and Steinberg, 1967). We are aware of nine publications reporting tests of serum samples from eleven Japanese populations for at least Gm(1,2,3,5,13) (Mårtensson *et al.*, 1966; Matsumoto and Takatsuki, 1968a and b; Schanfield *et al.*, 1972; Steinberg and Goldblum, 1965; Steinberg and Kageyama, 1970; Ueno, 1975; van Loghem and Mårtensson, 1967; van Loghem *et al.*, 1970). The haplotype frequencies were determined for the data from these publications with the computer program MAXIM. Each of the 11 gave a satisfactory fit to the H.-W. distribution. Each population had four haplotypes and these were equivalent to haplotype $Gm^{1,17,21}$, $Gm^{1,2,17,21}$, $Gm^{1,3,5,13,14}$, and $Gm^{1,13,17}$ found when tests are done for Gm(1,2,3,5,6,13,14,17,21). Furthermore, the haplotype frequencies among the several samples appeared to be the same, within statistical limits. The data for the 11 samples were treated as though each had been tested for only Gm(1,2,3,5,13) and reanalysed, to permit a test for heterogeneity to be performed. The test gave $\chi^2_{(20)} = 9.212$; $.975 > p > .950$. Clearly, the data may be considered to be homogeneous. Accordingly, the haplotype frequencies as determined for the 2,360 samples in the 11 sets of data are used for comparison with the haplotype frequencies found for the Matagi.

We know of published reports of three sets of samples from Ainu tested for Gm and Inv allotypes (Matsumoto and Miyazaki, 1972; Steinberg, 1966; Steinberg and

Kageyama, 1970). These samples were treated as were those for the Japanese, and these also may be considered to be homogeneous ($\chi^2_{(3)}=0.023$; $.9995 > p > .9990$). The haplotype frequencies as determined for the 753 Ainu samples in the three sets of data are used for comparison with the haplotype frequencies found for the Matagi.

The data for the Ainu, Japanese, and Matagi are presented in Table 3. All of the haplotype frequencies for the Matagi and the Japanese are the same, within statistical limits, while all of the haplotype frequencies for the Ainu, except that for $Gm^{1,13,17}$, are significantly different from those for the Japanese and from those for the Matagi. As far as these data are concerned, the conclusion must be that the Gm patterns of the Matagi are similar to those of the Japanese with no evidence of Ainu ancestry. Similarly, the data exclude origin from India. (See Steinberg (1974) for a review of Gm data for India.)

The homogeneity of the haplotype frequencies among the Japanese is striking. The samples came from populations covering a range of about 1,100 km; from the southern coast of Hokkaido on the north to Osaka on the south. Samples collected in Europe or Africa over a comparable distance show clear-cut heterogeneity (Johnson *et al.*, 1977).

We are aware of six publications that report $Inv(1)$ data for eight Japanese populations (Schanfield *et al.*, 1972; Steinberg and Kageyama, 1970; Steinberg and Matsumoto, 1964; Abe, 1965; Ueno and Yokoyama, 1964; van Loghem *et al.*, 1970). The Inv^1 allele frequencies are homogeneous, therefore we have summed all the data to arrive at a weighted mean frequency.

Three publications have data for $Inv(1)$ for the Ainu (Matsumoto and Miyazaki, 1972; Steinberg, 1966; Steinberg and Kageyama, 1970). These data have also been combined to yield a single estimate.

Table 3. Comparison of haplotype frequencies among Ainu, Japanese and Matagi.¹

Population No.	No.	1,17,21		1,2,17,21		1,3,5,13,14		1,13,17		2,17,21	
		Freq.	s.e.	Freq.	s.e.	Freq.	s.e.	Freq.	s.e.	Freq.	s.e.
Ainu	753	.548	.014	.070	.019	.028	.004	.272	.013	.082	.018
Japanese	2360	.472	.008	.164	.006	.102	.005	.262	.007		
Matagi	159	.453	.029	.161	.021	.091	.016	.295	.025		

¹ See text for details.

Table 4. Inv^1 allele frequencies among Ainu, Japanese and Matagi.¹

Population	No.	Inv^1	
		Freq.	s.e.
Ainu	753	.195	.011
Japanese	2294	.304	.007
Matagi	159	.300	.028

¹ See text for details.

Table 5. Gm phenotypes and probable genotypes for a Matagi family with an unusual pattern for Gm (26).

Individual	Phenotype ¹	Probable genotype
Fa.	1,2,17,21,26	1,2,17,21,26/1,17,26, or 1,26, or 26
Mo.	1,13,15,16,17,21,26	1,13,15,16,17/1,17,21,26
Proband	1,13,15,16,17,26	1,13,15,16,17/1,17,26, or 1,26, or 26
Sib	1,2,17,21,26	1,2,17,21,26/1,17,21,26

¹ All samples were tested for Gm (1,2,3,5,6,13,14,15,16,17,21,26).

The *Inv*¹ allele frequencies (.300 ± .28) is also essentially the same as those for the Japanese and significantly different from those for the Ainu (Table 4).

Gm 26: As mentioned in the MATERIALS AND METHODS section, this antigen is expected to be absent from a haplotype when Gm(15) is present and *vice versa*, it is expected to be present when Gm(15) is absent.

The *Gm*^{1,13,17} haplotype becomes *Gm*^{1,13,15,16,17} when tests are done for Gm(15, 16) on serum samples from Japanese; hence those with the phenotype Gm(1,13,17) (*i.e.* Gm(1,13,15,16,17) and therefore homozygous for *Gm*^{1,13,15,16,17}) are expected to be Gm(-26) and all others are expected to be Gm(26). This expectation was fulfilled in 158 of the 159 samples. The exception is in a sample from a 17 year old girl with the phenotype Gm(1,13,15,16,17,26). The Gm data for this donor and her family are presented in Table 5. Tests of blood samples drawn six months after the first set confirmed the results of the tests of the first samples. The proband's serum is Gm (-21) when tested undiluted with two different anti-Gm(21) antisera. We conclude that the data are correct.

If Gm(26) had not been determined, the father's probable genotype (Table 5) would have been believed to be *Gm*^{1,2,17,21}/*Gm*^{1,2,17,21} or *Gm*^{1,2,17,21}/*Gm*^{1,17,21} and the proband, whose probable genotype would have been believed to be *Gm*^{1,13,15,16,17}/*Gm*^{1,13,15,16,17}, would have been considered to be extra-marital. None of the blood groups, serum factors (other than Gm) or enzyme polymorphisms indicate paternal exclusion.

The most likely interpretation, it seems to us, is that the father and the proband have a rare haplotype that produces Gm(1,17,26), or Gm(1,26), or Gm(26). Unfortunately, samples from the father's relatives are not available for testing.

The Blood Group Data: The blood group data, except for S, Kell, Lu(a), Di(a), and P₁ which are not polymorphic in the population, are summarised in Tables 6A and 6B.

The samples were tested for ABO with anti-A and anti-B only. The fit to H.-W. expectancy is satisfactory. Unfortunately the allele frequencies for the Japanese and Ainu overlap considerably (Mourant, 1954; Mourant *et al.*, 1976), hence this system is not of help in characterizing the Matagi.

The samples were tested with anti-M, anti-N and anti-S, but only three were S positive. Therefore the data are treated as though the samples were tested for M

Table 6A. Observed and expected phenotype numbers for the ABO, MN, Rh, Jk, and Fy blood groups among the Matagi.

ABO			MN			Rh		
Phenotype	No.		Phenotype	No.		Phenotype	No.	
	Obs.	Exp.		Obs.	Exp.		Obs.	Exp.
O	51	52.7	M	34	33.9	Rh ₁ Rh ₁	68	69.3
A	47	45.0	MN	79	79.1	Rh ₁ Rh ₂	70	66.7
B	49	47.0	N	46	46.0	Rh ₂ Rh ₂	14	15.7
AB	12	14.3	Total	159	159.0	Rh ₁ rh	4	4.6
Total	159	159.0				Rh ₂ rh	2	1.9
					$\chi^2_{(1)}=0.0004$	rh"rh	1	0.3
						rh"rh"	0	0.4
						rh rh	0	0.1
						Total	159	159.0
		$\chi^2_{(1)}=0.601$						$\chi^2_{(1)}+0.496$

Kidd		Duffy		
Phenotype	No.	No.		
		Phenotype	Obs.	Exp.
Jk (a+)	70	a	134	130.9
Jk (a-)	88	ab	14	20.3
Total	158	b	4	0.8
		Total	152	152.0
				$\chi^2_{(1)}=14.829$

Table 6B. Frequencies of the alleles for the ABO, MN, Rh, Jk and Fy blood groups among the Matagi.

System	Allele	Freq.	s.e.
ABO	<i>I^A</i>	.208	.024
	<i>I^B</i>	.216	.025
	<i>I⁰</i>	.576	.030
MN	<i>M</i>	.462	.039
Rh	<i>R¹</i>	.660	.027
	<i>R²</i>	.270	.042
	<i>r</i>	.022	.008
	<i>r"</i>	.048	.035
Kidd	<i>Jk^a</i>	.254	.026
Duffy	<i>Fy^a</i>	.928	.021

and N only. The frequency of the *M* allele (.462, Table 6B) is low for a Japanese population (frequencies > .5; Mourant *et al.*, 1976) and high for an Ainu population (~.4; Mourant *et al.*, 1976). The frequency of the *S* allele is $\geq .15$ among the Ainu and < .10 among the Japanese (Misawa and Hayashida, 1972; Nakajima, 1961). Hence the frequency of .010 among the Matagi, while unusually low, is more like that of the Japanese than that of the Ainu.

the Japanese (over .9 in both populations, Mourant *et al.*, 1976). The .928 frequency of Fy^a among the Matagi (Table 6B) fits in with the earlier investigation of Japanese and Ainu.

The P_1 allele frequency is $\sim .15$ in both Japanese and Ainu (Mourant *et al.*, 1976). It is .068 among the Matagi (18/159 were $P_1(+)$), hence distinctly different from both the former populations.

Di^a of the Diego system occurs with a frequency of less than .05 in all three populations. Seven out of 159 samples were $Di(a+)$; Di^a allele frequency = $.022 \pm .008$

Lu^a and K seem to be absent from, or at most very rare among, the Japanese and the Ainu (Nakajuma, 1961; Nakajima *et al.*, 1962). It is not surprising therefore that all 159 samples were $Lu(a-)$ and $K(-)$.

Serum Factors (other than Gm and Inv): The data for the Hp and Gc polymorphisms are presented in Table 7.

Hp^1 occurs with a frequency of about .10 among the Ainu and about .25 among the Japanese (Mourant *et al.*, 1976). The frequency of .181 for Hp^1 among the Matagi is about midway between these values, and is consistent with a postulate of some Ainu admixture. It is, of course, consistent with sampling error and /or drift.

The Gc allele frequencies are essentially identical among the Japanese and Ainu, with Gc^1 equal to about .75 (Mourant *et al.*, 1976). The value of .610 for Gc^1 among the Matagi is lower than that in either of the former two populations and is probably due to chance variation.

The transferrin phenotype of one sample was B_1C and of another B_2C . The remaining 157 samples were Tf C. Since Tf variants are rare, these data are of no use for classifying populations.

Enzyme Polymorphisms: The data for the enzyme systems showing polymorphism among the Matagi are presented in Table 8. None of the systems is useful for distinguishing Ainu and Japanese, because they have essentially the same frequencies in the two populations (Ap and PGD) or there are no data available for comparison (EsD, PGM₁ and GPT).

The PHI, PGK, and AK systems showed no variants, and there was only one variant (3-1) in the GOT system, hence these systems like those mentioned above, do not provide any information concerning the classification of the Matagi.

DISCUSSION

The Gm data clearly exclude any origin of the Matagi other than Mongoloid (see Johnson *et al.*, 1977 for a review of the Gm data among races) and therefore they exclude the Ainu as the ancestors of the Matagi. The reputed similarities between the Matagi and Ainu cultures clearly have nothing to do with a common biological ancestry. These similarities probably have been acquired in the same way that

American cultural patterns are acquired by Europeans or Japanese who live in the U.S. If this is correct, it implies that the Ainu and Matagi were sympatric at one time and provides evidence that the Ainu did occupy at least a portion of Honshu in the past, as has been speculated by anthropologists on other grounds.

The Gm haplotype arrays of the Japanese and the Matagi are identical and their frequencies are very similar, *i.e.* the same within statistical limits. These data, supported by the data for the blood group and the remaining serum polymorphisms, indicate that the Matagi have descended from Japanese ancestry.

The unusual Gm haplotype apparently present in a father and daughter *i.e.*, one that produces only Gm (26) on the γ^3 heavy chain of IgG, is worthy of more extensive study and we shall continue to try to obtain a donation of sufficient blood to permit such a study. The data for the family with this haplotype (Table 5) indicate that great caution should be exercised in using Gm data for paternity tests.

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