The Distribution of Red Cell Enzyme and Serum Protein Types in a Sample from Iwate, Northern Japan, with the Description of Geographical Cline of Gc Subtypes*

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Abstract Blood samples from 500 healthy blood donors of Iwate Prefecture, northern Japan, were examined for polymorphism of the following red cell enzyme and serum protein systems by starch-gel electrophoresis and in part by isoelectrofocusing: ACP, PGMI, ESD, GPT, Hp, Tf and subtypes of PGMI and Gc. The distribution of phenotypes and allele frequencies estimated for each system was essentially similar to those reported in other areas of Japan. However, when the data of Gc subtypes thus far reported in Japan excluding Okinawa Islands are compared, statistically significant geographical cline was detected for GC*1F and GC*2 alleles.

Keywords Population genetics, Blood genetic markers, Polymorphism, Geographical cline, Japanese.

Introduction

The geographical difference in gene frequencies among Japanese is an interesting anthropological question. However, aside from the well known case for ABO blood group genes (TANAKA, 1959; NEI and IMAIZUMI, 1966; FUJITA *et al.*, 1978; AOKI and OMOTO, 1980), few systematic regional differences such as geographical cline have been established in the Japanese main islands for the frequency of polymorphic alleles.

The simplest explanation for the paucity of cline in the polymorphic systems other than ABO blood group may be that the number of population samples for these systems are still insufficient at present. Since the publication of the gene frequency data of Japanese collected by the Japanese IBP team in 1975 (AKAISHI and KUDO, 1975; ISHIMOTO, 1975; OMOTO, 1975), the number of polymorphisms examined in Japan, particularly those of enzymes and proteins, may have substantially increased, although the number

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of population samples for these systems are far from sufficient to give a clear picture of the geographical cline such as that obtained for ABO blood group genes. Nevertheless, the geographical cline has been suggested for gene frequencies of red cell GPT (ISHIMOTO and KUWATA, 1974; UEDA, *et al.*, 1979) and of Gc (YUASA *et al.*, 1983a).

In the course of accumulating new regional data of genetic polymorphism of proteins detected by electrophoresis, a blood sample from Iwate Prefecture, northern Japan, was recently examined for several red cell enzyme and serum protein polymorphisms. The study of genetic markers in Tohoku district appears to be important anthropologically because of its rather peripheral situation in relation to the continental east Asia, the center of postagricultural migration, as well as its geographical closeness to Hokkaido, where the Ainu dwell as the native inhabitant. However, only a few gene frequency data of proteins have been reported from this district. In this paper, the gene frequency data for several protein systems in the Iwate sample will be described together with the finding that the geographical cline is statistically highly significant for the frequencies of both GC*1F and GC*2 alleles in the Japanese main islands.

Material and Methods

The blood specimens totalling 500 were obtained from healthy blood donors living in the central part of Iwate Prefecture: 130 from Hananomaki City, 140 from Mizusawa City, 120 from Ichinoseki City and 110 from Morioka City. Since these cities are closely located to each other, the samples were combined into a single population sample.

The blood specimens, red cell clots and sera, were sent to Tokyo with dry ice within

a few days after collection and stored at -30°C up to about 6 months until tested. The following polymorphic systems were examined by starch-gel electrophoresis with the specific enzyme staining and other staining methods conventionally used (HARRIS and HOPKINSON, 1976; GIBLETT, 1969). Red cell enzyme systems: acid phosphatase (ACP), phosphoglucomutase 1 (PGM1), esterase D (ESD) and glutamate-pyruvate transaminase (GPT). Serum proteins: Hp and Tf. Isoelectrofocusing in thin-layer polyacrylamide slab was used for subtyping PGM1 (KUHNLE *et al.*, 1977; MANEYAMA *et al.*, 1978) and Gc, a vitamin-D binding protein in serum (CONSTANS and VIAU, 1977; OMOTO and MIYAKE, 1978). In order to compare rare variant Gc samples, isoelectrofocusing in the Immobiline system (CLEVE *et al.*, 1982) was applied with slight modifications (WATANABE, personal communication).

The search for geographical cline was performed using the allele frequency data obtained in the present study and those previously reported in the Japanese main islands. If two or more data were available in one locality, the data with the largest sample size was used. The data were plotted against the railway distances from Aomori City, and the resulting linear regression was statistically tested for the significance.

Results

The distribution of phenotypes and allele frequencies for 4 red cell enzyme polymorphic systems examined by starch-gel electrophoresis are shown in table 1.

In ASP, only 3 common phenotypes were found as is usually the case in Japanese. However, 6 samples showed no ACP activity on the gel after electrophoresis and excluded

Table 1. Distribution of phenotypes and allele frequencies for 4 red cell enzyme systems examined by starch-gel electrophoresis.

System	Phenotype	N. Obs.	N. Exp.	Gene Frequency	S. E.
ACP	A	18	17.70	ACP*A : 0.1893	0.0124
	B A	151	151.62	ACP*B : 0.8107	0.0124
	B	325	324.68		
	Total	494	494.00		
$X^2=0.0557$, 1df, $P>0.80$					
PGM1	1	284	285.77	PGM1*1 : 0.7560	0.0136
	2-1	177	174.64	PGM1*2 : 0.2310	0.0133
	2	26	26.68	PGM1*7 : 0.0100	0.0031
	7-1	8	7.56	PGM1*5 : 0.0020	0.0014
	7-2	2	2.31	PGM1*6 : 0.0010	0.0009
	5-1	2	1.51		
	6-1	1	0.76		
	Total	500	499.23		
$X^2=0.1274^*$, 3df, $P>0.90$					
ESD	1	207	213.86	ESD*1 : 0.6540	0.0150
	2-1	240	226.28	ESD*2 : 0.3460	0.0150
	2	53	59.86		
	Total	500	500.00		
$X^2=1.8381$, 1df, $P>0.10$					
GPT	1	177	171.70	GPT*1 : 0.5860	0.0155
	2-1	232	242.60	GPT*2 : 0.4140	0.0155
	2	91	85.70		
	Total	500	500.00		
$X^2=0.9545$, 1df, $P>0.30$					

*) In calculation of chi-square of PGM1 type frequencies, the phenotype 5-1 and 6-1 were excluded.

from the allele frequency calculation. The cause for this unusual, apparently silent phenotype was not known. In PGM1, two relatively uncommon phenotypes, PGM1 7-1 and PGM1 7-2, were encountered rather frequently besides the common three phenotypes. The frequency of the allele PGM1*7 was estimated to be approximately 0.01, indicating the occurrence of this allele at polymorphic frequency. Furthermore, two kinds of rare variants were found and tenta-

tively typed as PGM1 6-1 and PGM1 5-1. Judging from the electrophoretic mobility of variant isozymes, it is likely that they are in fact PGM1 6J-1 and PGM1 5J-1, respectively (OMOTO and HARADA, 1970; BLAKE and OMOTO, 1975; SATOH *et al.*, 1976). In ESD, three usual phenotypes only were observed. Also, three common phenotypes and no variants were detected in GPT.

In the PGM1 subtype system, a total of 17 phenotypes could be distinguished (Table 2).

Table 2. Distribution of phenotypes and allele frequencies for the PGM1 subtype examined by isoelectrofocusing.

Phenotype	N. Obs.	N. Exp.	Allele Frequency	S. E.
1+	218	211.90	PGM1*1+ : 0.6510	0.0151
1+1-	61	68.35	PGM1*1- : 0.1050	0.0097
1-	5	5.51	PGM1*2+ : 0.1710	0.0119
1+2+	110	111.32	PGM1*2- : 0.0600	0.0075
1+2-	35	39.06	PGM1*7+ : 0.0060	0.0024
1-2+	26	17.96	PGM1*7- : 0.0040	0.0020
1-2-	6	6.30	PGM1*6 : 0.0010	0.0010
2+	12	14.62	PGM1*5 : 0.0020	0.0014
2+2-	10	10.26		
2-	4	1.80		
1+7+	3	3.91		
1+7-	3	2.60		
1-7+	2	0.63	$X^2=8.231^*$, 6df, $P>0.20$	
2+7-	1	0.68		
2-7+	1	0.36		
1+6	1	0.65		
1+5	2	1.30		
Total	500	497.21		

*) Chi-square was calculated for the common phenotypes determined by four common alleles.

Table 3. Distribution of phenotypes and allele frequencies for 2 serum protein systems examined by starch-gel electrophoresis.

System	Phenotype	N. Obs.	N. Exp.	Allele Frequency	S. E.
Hp	1-1	26	26.22	Hp*1 : 0.2290	0.0133
	2-1	177	176.56	Hp*2 : 0.7710	0.0133
	2-2	297	297.22		
	Total	500	500.00		
$X^2=0.0031$, 1df, $P>0.90$					
Tf	C	494	494.02	Tf*C : 0.9940	0.0024
	C B	2	1.99	Tf*B : 0.0020	0.0014
	C D	4	3.98	Tf*D : 0.0040	0.0020
	Total	500	499.99		
$X^2=0.0002^*$, 1df, $P>0.95$					

*) Two variant alleles were combined in the calculation of the chi-square.

Apart from the 10 phenotypes ascribed to 4 common alleles denoted PGM1*1+, PGM1*1-, PGM1*2+, PGM1*2-, 5 phenotypes were

distinguishable for heterozygotes of PGM1*7, confirming the existence of two subtypes of this allele, denoted PGM1*7+ and PGM1*7-

Table 4. Distribution of phenotypes and allele frequencies for the Gc subtype examined by isoelectrofocusing.

Phenotype	N. Obs.	N. Exp.	Allele Frequency	S.E.
1F	130	115.20	Gc*1F : 0.4800	0.0158
1F-1S	100	114.24	Gc*1S : 0.2380	0.0135
1S	41	28.32	Gc*2 : 0.2420	0.0135
2-1F	99	116.16	Gc*1A2 : 0.0300	0.0054
2-1S	50	57.6		
2	41	29.28	Rare alleles :	
1A2-1F	15	14.40	Gc*1A9 : 0.0030	0.0017
1A2-1S	5	7.14	Gc*J+ : 0.0010	0.0009
1A2-2	8	7.26	Gc*1S- : 0.0010	0.0009
1A2	1	0.45	Gc*2A4 : 0.0020	0.0014
Rare variants :			Gc*2VF : 0.0010	0.0009
1F-J+	1	0.48	Gc*2V+ : 0.0010	0.0009
1F-1A9	2	1.44	Gc*2V- : 0.0010	0.0009
2-1A9	1	0.73		
1S-1S-	1	0.24		
1F-2A4	2	0.96	$X^2=14.10^*$, 6df, $0.05>P>0.02$	
1F-2VF	1	0.48		
2-2V+	1	0.24		
2-2V-	1	0.24		
Total	500	494.86		

*) Chi-square was calculated on the common phenotypes only.

Fig. 1. Diagram showing relative isoelectrofocusing position (pH 4-6) of 11 Gc subtypes found in a survey of 500 sera from Iwate Prefecture, northern Japan. The nomenclature used are tentative. Those subtypes occurring in polymorphic frequency are indicated with a circle.

(TAKAHASHI *et al.*, 1982).

As to the two serum protein systems, Hp and Tf, conventional starch-gel electrophoretic methods yielded only three common phenotypes in Hp, and a common and two relatively infrequent variant phenotypes in Tf (Table 3). The subtyping of common Tf phenotypes by isoelectrofocusing and the identification of variants are underway.

The result of the Gc subtyping is shown in Table 4. In addition to 6 phenotypes ascribed to 3 common alleles, Gc*1F, Gc*1S and Gc*2, at least 12 uncommon phenotypes were observed, indicating the existence of further 8 variant alleles at the Gc locus in northern Japan. Among the rare variants shown in the diagram (Figure 1), four were Gc 2 variants tentatively denoted 2V—, 2Y, 2V+, and 2VF, while another four were Gc 1 variants, 1S—, 1J, 1J+ and 1TKI. According to the international Gc nomenclature, Gc 2Y, Gc 1J and Gc 1TKI are known as Gc 2A4, Gc 1A2 and Gc 1A9, respectively (OMOTO and MIYAKE, 1978; CONSTANS *et al.*, 1979; CLEVE *et al.*, 1981). The variants were confirmed by isoelectrofocusing in the Immobiline system to be distinct, except Gc 2V+ which was indistinguishable in this system with Gc 2A4 (WATANABE, personal communication).

Discussion

Relatively few studies of protein polymorphisms have been reported from Tohoku district, comprising six prefectures, namely, Aomori, Akita, Iwate, Yamagata, Miyagi and Fukushima. Concerning the polymorphic systems dealt with in the present study, Harada and his colleagues reported their results of ACP and PGMI from Aomori, Iwate and Miyagi prefectures (HARADA *et al.*, 1971). A large scale study of ESD

polymorphism was carried out in Miyagi and Yamagata prefectures (SUZUKI *et al.*, 1978). Studies of GPT polymorphism have been published from Aomori, Akita and Miyagi prefectures (KITA *et al.*, 1975; UEDA *et al.*, 1979). Hp types were examined by KUDO (1973) for all the six prefectures. Recently, the result of a survey of red cell enzyme and serum protein polymorphisms in Fukushima Prefecture was published (ABE, 1983).

The frequencies of common alleles and the distribution of rare variants obtained in the present study are essentially similar to those in the previous reports mentioned above. It may be worth pointing out, for example, that two kinds of rare PGMI variants reported previously from Iwate and Miyagi prefectures (HARADA *et al.*, 1971) may be identical to PGMI 5J and PGMI 6J found in the present study, judging from the electrophoretic pattern. Also, it was confirmed by the present study that the two uncommon isozymes of GPT, GPT 3 and GPT 6 which have thus far been found mainly in the western part of Japan (UEDA *et al.*, 1979), seem to be extremely rare in Tohoku district, if not absent. As to Gc subtypes, at least 6 variants in addition to the common subtypes (Gc 1F, Gc 1S and Gc 2) were found in a survey of 893 sera from Fukushima Prefecture: Gc 1A2, Gc 1A3, Gc 1A8, Gc 1A9, Gc 1C2 and Gc 1C4 (ABE, 1983). Gc 1A2, originally known as Gc J (OMOTO *et al.*, 1972), is widespread in Japanese including the Ainu with a polymorphic frequency, e.g. higher than 0.01 (OMOTO and MIYAKE, 1978; ISHIMOTO *et al.*, 1979; OMOTO and MIYAKE, 1979; MATSUMOTO *et al.*, 1980). Other variants reported by ABE (1983) may also have wide distribution in Japanese and certainly not confined to Tohoku district (SHIBATA, 1983). In the present

Fig. 2. The regressions between the frequencies of Gc*1F and Gc*2 and the distances from Aomori City, indicating geographical cline. For data see Table 5.

Table 5. Frequency data of Gc subtypes (Gc*1F and Gc*2) from 11 localities, mostly prefectures, of Japanese Main Islands. Distances are from Aomori City to the prefectural capitols.

Locality	Distance*	N	Gc*1F	Gc*2	References
Akita	185.8	732	0.4693	0.2582	SHINDO & TAKAHASHI, 1983
Iwate	203.9	500	0.4800	0.2420	This study
Fukushima	466.4	893	0.4854	0.2469	ABE, 1983
Tokyo	735.6	1057	0.4782	0.2450	NAKAJIMA & NAKAZAWA, 1980
Yamanashi	869.7	530	0.4642	0.2547	KIDO <i>et al.</i> , 1984
Mie	1168.0	510	0.4650	0.2630	ISHIMOTO <i>et al.</i> , 1979
Osaka	1292.0	342	0.4560	0.2540	SHIBATA, 1983
Izumo	1635.4	300	0.4600	0.2750	YUASA <i>et al.</i> , 1983a
Koryo	1670.9	225	0.4411	0.2645	YUASA <i>et al.</i> , 1983b
Yamaguchi	1775.9	400	0.4422	0.2802	YUASA <i>et al.</i> , 1983a
Nagasaki	2066.0	1057	0.4410	0.2910	YUASA <i>et al.</i> , 1983a

*) Railway distance from Aomori City to the prefectural capitol.

study, six rare Gc variants other than Gc 1A2 were found including several kinds of the Gc 2 variants not recorded by ABE (1983). Further studies are clearly needed to identify the Gc variants detected in the present study.

When the allele frequencies of 8 polymorphic systems obtained in the present study were compared with those reported previously in Japanese main islands, statistically highly significant geographical cline was found for two of the Gc subtypes. Data of Gc subtypes of 11 localities in the Japanese main islands were available (Table 5). The linear regression equations obtained between allele frequencies (x) and railway distances from Aomori (y) were: $y = -0.023x + 0.488$ for Gc*1F; $y = 0.020x + 0.239$ for Gc*2 (Figure 2). In both cases, the regression was statistically highly significant ($P < 0.001$). This finding confirms the cline for Gc*2 pointed out recently (YUASA *et al.*, 1983a), and further indicates that the cline is also statistically significant for Gc*1F frequency, while it may not be the case for Gc*1S. The Gc*1F frequency tends to be lower, while the Gc*2 frequency higher, in the western

part than in the north-eastern part of Japan.

It should be pointed out that the frequency of Gc*1F is known to be high (0.521–0.579) in the Ainu (OMOTO and MIYAKE, 1979; MATSUMOTO *et al.*, 1980), while that for Chinese may be similar to Japanese (data from Hong Kong: 0.494; KWOK and LEWIS, 1981) or much lower than in Japanese (data from Taiwan: 0.397; MATSUMOTO *et al.*, 1980). It is suggested, therefore, that the Gc cline in Japan may have arisen by gene flow.

A clinal tendency was also noted in Hp allele frequencies, where data for Hp*1 were available for 20 population samples from the wide area between Aomori and Kagoshima, the northernmost and the southernmost capitol of Japanese main islands, respectively (Table 6). The regression $y = 0.012x + 0.239$ was found to be statistically at the borderline for the significance ($F = 4.419$, $P = 0.05$) as shown in Figure 3. Although not convincing as in the Gc cline, it is probable that the Hp*1 frequency tends to become higher as one goes from northeast to southwest in Japanese main islands. It is interesting that

Table 6. Data of the Hp allele frequency from 20 localities, mostly prefectures. Distances are from Aomori City to the prefectural capitols.

Locality	Distance*	N	Hp*1	Reference
Aomori	0.0	2401	0.2320	MURAKAMI & KITA, 1972*
Akita	185.8	1142	0.2390	KUDO, 1973*
Iwate	203.9	500	0.2290	This study
Yamagata	397.4	991	0.2550	KUDO, 1973*
Miyagi	387.4	2000	0.2440	KUDO, 1973*
Niigata	458.8	123	0.2300	WATANABE, 1970*
Fukushima	466.4	980	0.2320	KUDO, 1973*
Chiba	774.3	2791	0.2660	ISHIMOTO <i>et al.</i> , 1967*
Shizuoka	915.8	693	0.2510	SHINODA, 1966*
Nagoya	1101.6	630	0.2930	OHARA, 1965*
Gifu	1131.9	1235	0.2530	OHYA <i>et al.</i> , 1971*
Mie	1168.0	545	0.2670	ISHIMOTO, 1973*
Nara	1235.5	343	0.2540	YADA & FUNAKI, 1965*
Osaka	1292.0	822	0.2750	MATSUMOTO, 1964*
Okayama	1468.5	600	0.2600	ISHIZU & ANDO, 1972*
Hiroshima	1630.4	2073	0.2490	FERRELL <i>et al.</i> , 1977
Koryo	1670.9	173	0.2183	YUASA <i>et al.</i> , 1983b
Fukuoka	1912.1	311	0.2570	YAMAGUCHI, 1961*
Nagasaki	2066.0	1374	0.2550	FERRELL <i>et al.</i> , 1977
Kagoshima	2229.2	560	0.2770	ISHIMOTO & KUWATA, 1973*

* Cited in OMOTO, 1975

*) Railway distance from Aomori City to the prefectural capitol.

Fig. 3. The regression between the frequencies of Hp*1 and the distances from Aomori City. For data see Table 6.

this allele frequency is known to be extremely low in the Ainu, while it tends to be higher in Chinese than in Japanese (OMOTO, 1975). Therefore, it is likely that the cline of Hp allele frequencies, if it really exists, may also be ascribed to gene flow as in the Gc cline.

The findings of geographical cline in the present study are consistent with the idea that there were considerable migration from the continent to the western part of Japan in prehistoric and protohistoric times, affecting to the great extent the genetic composition of the native inhabitants of Japan, who may have been related to the Ainu (OMOTO, 1973). A migration model was mathematically applied for the ABO gene frequency cline in Japan (AOKI and OMOTO, 1980).

In the present study, the data from South-western (Okinawa) Islands were not taken into consideration, since the distribution of genes in small island populations may be affected by much more complicated factors such as isolation and founder effect than in that of mainland populations. That there may be two independent clines existing in Japan due to the migrations in the past, an east-west cline in Japanese main islands and a north-south cline in South-western Islands, are indicated by such examples as ear-wax types (TERAWAKI, 1975) and red cell GPT (ISHIMOTO and KUWATA, 1974; UEDA *et al.*, 1979). In both systems, apparent clines were found in South-western Islands but not in the Japanese main islands.

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抄 録

岩手県の一試料における赤血球酵素・血清蛋白型の分布ならびに Gc 亜型の地理勾配

尾 本 恵 市

岩手県在住の健康な献血者 500 名よりえた血液試料につき、デンプンゲル電気泳動ならびに一部は等電点分画により下記の赤血球酵素ならびに血清蛋白の多型を検査した: ACP, PGM 1, ESD, GPT, Hp, Tf, PGM 1 亜型および Gc 亜型, 各形質に関し推定された表現型および対立遺伝子の分布は, 日本の他地域より従来報告されていたものと基本的に同様であった。しかし, Gc 亜型の頻度を沖縄を除く日本各地のデータ間で比較したところ, Gc*1F および Gc*2 の頻度に統計上高度に有意な地理勾配が検出された。

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NOTES ADDED IN PROOF

After the completion of this study the recent paper by YOSHIOKA and his colleagues on the distribution of Gc subtypes in northeastern Japan has come under the author's notice. In this paper, data of all the six prefectures of Tohoku District are presented, the total number of samples examined being 4,109. The estimated gene frequencies are as follows:

	Aomori	Akita	Iwate	Miyagi	Yamagata	Fukushima	Total
N	593	732	697	647	547	893	4109
Gc*1F	0.4545	0.4693	0.4699	0.4359	0.4360	0.4854	0.4611
Gc*1S	0.2470	0.2473	0.2461	0.2666	0.2431	0.2402	0.2480
Gc*2	0.2664	0.2582	0.2518	0.2589	0.2761	0.2469	0.2583
Gc*1A2	0.0245	0.0137	0.0194	0.0247	0.0311	0.0196	0.0215
others	0.0076	0.0115	0.0128	0.0139	0.0137	0.0079	0.0111

The regional variation of allele frequencies in this data is considerable, particularly for Gc*1F. Thus, if these figures were added to those used for analysis of geographical cline in the present study (Table 4), the statistical significance for Gc*1F cline disappears ($F=2.943$, $0.10 < P < 0.20$),

while that for Gc*2 cline remains significant at 1% level ($F=7.925$, $0.01 < P < 0.02$). The disappearance of Gc*1F cline seems to be due mainly to the data of Miyagi and Yamagata, in which quite low Gc*1F frequencies are observed. As to Gc*2 frequencies, however, regional variation

appears to be less marked.

Such an observation seems to indicate the difficulty to establish geographical cline for any gene frequencies when the number of population samples are relatively small. Clearly, much more data are needed to show the Gc cline in Japan to be unequivocal.

YOSHIOKA, N., S. SHINDO and T. KUDO, 1984: Distribution of group specific component subtypes among Japanese. *Hō-igaku no Jissai to Kenkyu (Research and Practice in Forensic Medicine)*, 27: 39-45 (In Japanese with English summary).

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