GENETIC POLYMORPHISM OF RED CELL ENZYMES (AcP, EsD, GPT, 6-PGD) AND OF SERUM PROTEIN(Gc) IN FUKUSHIMA PREFECTURE

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Summary The polymorphism of red cell enzymes (AcP, EsD, GPT and 6-PGD) and of serum protein (Gc) was examined in samples of unrelated individuals living in Fukushima Prefecture. Gc subtypes were determined by isoelectric focusing and immunofixation. It was found, in the formers, the gene frequencies of AcP^A, EsD¹, GPT¹ and 6-PGD^A to be 0.229, 0.634, 0.623 and 0.921, and in the latter, Gc^{1F}, Gc^{1S}, Gc² and Gc^{others} to be 0.4854, 0.2402, 0.2469 and 0.0276, respectively. Two of rare phenotypic variants of Gc were also described.

INTRODUCTION

The numbers of genetic markers for characterization of human populations are increasing. Polymorphic systems of the red cell enzymes and of the plasma proteins are useful for demonstrating the differences and distinctions among populations. Population studies on phenotypic distribution in the red cell enzyme systems have been performed extensively in Japan (Ishimoto and Kuwata, 1973). Phenotypes of these systems are determined by a starch gel electrophoresis followed by the stainings specific for each of the enzymes. Group-specific component (Gc), the vitamin D binding protein, is one of human plasma proteins showing genetic polymorphism. Recently, an isoelectric focusing technique using acrylamide gel has been developed for the study of inheritance of the protein polymorphism (Viau *et al.*, 1977). Thereby, it has become possible to divide Gc1-1 type into 3 subtypes, Gc1F-1F, 1F-1S and 1S-1S, and to determine additional polymorphic variants of Gc. In the present study, we examined the polymorphic distributions and gene frequencies of the 4 red cell enzyme systems and of the Gc system in Fukushima Prefecture, and found rare variants of Gc (1A2-1A9 and 1A9-1A9).

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MATERIALS AND METHODS

Blood samples were obtained from healthy and unrelated blood donors living in Fukushima Prefecture, northeastern part of Japan. In order to study inheritance of the Gc variants, 15 sera were collected from one family.

The genetic variations were examined in the following 4 enzymes; acid phosphatase (AcP), esterase D (EsD), glutamic-pyruvic transaminase (GPT) and 6-phosphogluconate dehydrogenase (6-PGD). The procedures of blood collection, preservation and electrophoretic analysis were in accord with those of Hopkinson *et al.* (1964).

Gc polymorphism was determined by the isoelectric focusing and the immunofixation (Constans *et al.*, 1977, 1978). The former step was performed on a slab acrylamide gel of 0.5 mm thickness containing 5% carrier ampholyte (pH 4-6, Ampholine, LKB), photopolymerized in the presence of riboflavin. After a 4 hr migration period, the immunofixation was carried out on a cellulose acetate strip soaked with monospecific antiserum to Gc(DAKO). The immune precipitates thus obtained were stained with Coomassie Brilliant Blue.

RESULT AND DISCUSSION

Red cell enzyme systems

The gene frequencies of the 4 red cell enzyme systems were shown in Table 1. They were statistically not contradictory to the Hardy-Weinberg law in the all of 4.

Table 1. The gene frequencies of the 4 red cell enzyme systems in Fukushima Prefecture.

Enzyme	Phenotypes	No. observed	No. expected	Gene frequencies
AcP	A	58	54.3	AcP ^A 0.229
	BA	359	365.8	AcP ^B 0.771
	B	619	615.8	$\chi^2=0.40$
	Total	1,036	1,035.9	$0.5 < p < 0.7$
EsD	1	830	848.1	EsD ¹ 0.634
	2-1	1,016	979.2	EsD ² 0.366
	2	264	282.6	$\chi^2=2.99$
	Total	2,110	2,109.9	$0.05 < p < 0.1$
GPT	1	238	226.7	GPT ¹ 0.623
	2-1	252	274.3	GPT ² 0.377
	2	94	83.0	$\chi^2=3.83$
	Total	584	584.0	$0.05 < p < 0.1$
6-PGD	A	730	727.8	PGD ^A 0.921
	AC	120	124.9	PGD ^C 0.079
	C	8	5.4	$\chi^2=1.45$
	Total	858	858.1	$0.2 < p < 0.3$

These frequencies are in general, except in GPT system, similar to the values in the data previously reported for other districts of Japan (in average, AcP^A 0.210 ± 0.051 , EsD¹ 0.635 ± 0.033 , GPT¹ 0.601 ± 0.018 and 6-PGD^A 0.911 ± 0.011 , Ishimoto, 1974). The GPT¹ gene frequency in Fukushima Prefecture is 0.623. On the one hand it is reported to be 0.535 in Okinawa, 0.592 in Kagoshima and 0.612 in Hiroshima. This fact suggests that the GPT¹ gene frequency decreases gradually from the northern part towards the south in Japan.

Gc system

The isoelectric focusing patterns of Gc observed in the present study are shown in Fig. 1, presenting 18 phenotypes. At the International Workshop on the Gc-system

Fig. 1. Isoelectric focusing patterns of Gc observed in this study. 1F-1F: 11, 39. 1F-1S: 1, 8, 10, 15, 16, 22, 29, 36, 38, 40, 42, 45. 1S-1S: 12. 2-1F: 9, 43. 2-1S: 3, 14, 32, 41. 2-2: 13. 1A2-1F: 7, 25, 28, 35. 1A2-1S: 6, 30, 37. 1A2-2: 5, 27, 34. 1A2-1A9: 24, 33, 44. 1A3-1S: 4, 26. 1A8-1S: 17, 18. 1A9-1F: 23. 1A9-1S: 21, 46. 1A9-2: 20. 1A9-1A9: 19. 1C2-1F: 2. 1C4-1F: 31.

Fig. 2. Pedigree of a family illustrating the transmission of the Gc variant (1A2-1A9).

Table 2. Distribution of Gc subtypes in Fukushima Prefecture.

Subtypes	No. Obs.	No. Exp.	Subtypes	No. Obs.	No. Exp.
1F-1F	203	210.4	1A9-1F	4	4.9
1F-1S	214	208.2	1A9-1S	2	2.4
1S-1S	54	51.5	1A9-2	1	2.5
2-1F	222	214.0	1A9-1A9	1	—
2-1S	93	105.9	1C2-1F	1	0.5
2-2	60	54.4	1C4-1F	1	0.5
1A2-1F	19	17.0	Others	—	3.0
1A2-1S	10	8.4			
1A2-2	5	8.6			
1A2-1A9	1	0.2			
1A3-1S	1	0.3			
1A8-1S	1	0.3			
			Total	893	893.0

$\chi^2=16.63$
 $0.50 < p < 0.75$
(d.f.=18)

Table 3. Gene frequencies of Gc in Fukushima Prefecture and other populations in Japanese.

Alleles	Fukushima (Present study)	Tokyo Nakajima (1980)	Osaka Matsumoto (1979)	Nagasaki Iwasaki (1982)
Gc ^{1F}	0.4854	0.4782	0.4209	0.4409
Gc ^{1S}	0.2402	0.2507	0.3006	0.2356
Gc ²	0.2469	0.2450	0.2579	0.2909
Gc ^{1A2}	0.0196	0.0204		
Gc ^{1A3}	0.0006	0.0014		
Gc ^{1A8}	0.0006	0.0009		
Gc ^{1A9}	0.0056	0.0033		
Gc ^{1C2}	0.0006	—		
Gc ^{1C4}	0.0006	—		

(Gc^{variants})

held in 1978, the Gc phenotypes determined by a total of 30 alleles were demonstrated. At that time it was proposed to unite new nomenclatures of variants in place of the designations used independently by investigators. Cleve *et al.* (1981) reported that 6 Gc phenotypic variants, observed in Japanese and called GcJa, Jb, Jd, Mie and Ok, were found to be identical with Gc1A2, 1A3, 1A9, 1A8, 1C4 and 1C2. These represent double band variants and are presumably mutants of the Gc¹ allele. All of these 6 alleles are observed in the present study. Two rare phenotypic variants, 1A9-1A9 and 1A2-1A9, are also observed. The former is a homogeneity of one variant allele and the latter is a heterogeneity of two variant alleles. Family study on 1A2-1A9 indicates that the variant inherits as a codominant character (Fig. 2).

The distribution of Gc subtypes and gene frequencies are shown in the Tables 2 and 3, respectively. There is a good agreement between the observed and the expected numbers of these subtypes (Table 2). The result of Gc gene frequencies in Fukushima Prefecture is summarized in Table 3, comparing with these in the other populations in Japanese. The gene frequencies of Gc in Tokyo are nearly identical with our present study. We observe a higher Gc^{1F} frequency (0.4854) than that found in Osaka (0.4209) and in Nagasaki (0.4409), and a lower Gc^{1S} frequency (0.2402) than that in Osaka (0.3006). The Gc² frequency (0.2469) is in agreement with that reported in Tokyo (0.2450), on the contrary it is lower than that reported in Osaka (0.2579), Mie (0.263, Ishimoto *et al.*, 1979) and Nagasaki (0.2909). This result indicates that the Gc² gene frequency gradually increases from east to west in Japan. The Gc^{variants} frequency is found to be 0.0276, ranging in the values reported in other districts. Therefore, the alleles of Gc^{variants} are distributed widely in Japanese.

Kuwata *et al.* (1978) examined Gc polymorphic frequency in Fukushima Prefecture using an agar gel immunoelectrophoresis, and estimated the Gc frequencies of Gc¹ 0.7285, Gc² 0.2463 and Gc^J 0.0252. There is no significant difference between these values and the ones presented in our study, that is, Gc¹ 0.7256, Gc² 0.2469 and Gc^{others} 0.0276.

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