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HAPTOGLOBIN, GROUP-SPECIFIC COMPONENT, TRANSFERRIN AND α_1-ANTITRYPSIN SUBTYPES AND NEW VARIANTS IN JAPANESE

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Summary The extended polymorphisms of haptoglobin (Hp), group-specific component (Gc), transferrin (Tf) and α_1-antitrypsin (Pi) among Japanese by means of IEF (for Gc, Tf and Pi) and PAGE (for Hp) were presented. The distribution of these phenotypes provides a good fit to the Hardy-Weinberg equilibrium. The subtype allele frequencies obtained in this study were in good agreement with the data thus far presented by other investigators. The new and rare variants of these systems observed in this study were also described. The new variant of Gc allele was designated as GcIC18.

INTRODUCTION

During the last few years, the determining methods for serum protein and red cell enzyme polymorphism based on the electrophoresis have received great technical improvements such as isoelectrofocusing (IEF) on polyacrylamide or agarose gel, bidimensional electrophoresis and polyacrylamide gel electrophoresis (PAGE) containing 6 M urea.

These new electrophoretic procedures have demonstrated the existence of unknown polymorphisms in the serum protein and red cell enzyme thus far investigated. Phosphoglucomutase locus 1 and 2, haemoglobin, haptoglobin, α_1-antitrypsin, group-specific component and transferrin are the most significant examples of the molecules benefited by these technical developments. The new methods have become increasingly important for the determination of rare variants and are indispensable for a reliable classification of the subtypes.

The data on the serum protein polymorphisms (haptoglobin, group-specific component, transferrin and α_1-antitrypsin) among Japanese have been so far reported by several investigators (Toyomasu and Tate, 1979; Omoto and Harada, 1968; Omoto and Miyake, 1978; Ishimoto *et al.*, 1979; Matsumoto *et al.*, 1979; Nakajima and Nakazawa, 1980; Matsumoto *et al.*, 1980; Ferrell *et al.*, 1977;

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Miyake *et al.*, 1979; Sebetan and Akaishi, 1981; Matsumoto and Sugimoto, 1982).

This study aimed not only at applying the new electrophoretic procedures to these samples in order to show the existence of the new protein mutants but also at presenting the subtype gene frequencies of the mentioned proteins.

Then the data were discussed in order to determine in which way they might contribute to the estimation of the rare variants among the populations, and also to enlight the importance of the qualitative factor such as the nature of the amino acid substitutions in the mutants.

MATERIALS AND METHODS

The blood samples were collected from 342 unrelated normal individuals living in the Osaka area. The serum samples were prepared from whole venous blood without addition of anticoagulants. The sodium azide solution was added to all of them, and they were stored at $-20°C$ until used.

IEF was used for the detection of group-specific component, transferrin and α_1-antitrypsin and PAGE containing urea was used for haptoglobin.

Haptoglobin (Hp)

The crude purification and the reductive cleavage of the disulfate bonds of haptoglobin were performed substantially according to the original methods by Smithies *et al.* (1962) previous to the electrophoresis. The serum was mixed with the DEAE-cellulose equilibrated in the 0.01 M sodium acetate buffer pH 4.7 for 1 min. The absorbed haptoglobin was eluted by the 0.125 M ammonium acetate. The purified haptoglobin solution was added to the equal volume of the reductive reagent (0.1 M boric acid, 0.04 M NaOH pH 8.8 buffer and β-mercaptoethanol) containing 8 M urea. The mixture was kept for 15 min at room temperature, then the reaction was terminated by adding the 0.5 M iodoacetamide solution.

The electrophoresis with the polyacrylamide gel containing urea was performed with the buffer system modified from the methods by Constans and Viau (1975). The migration gel containing 8% acrylamide, 0.20% bisacrylamide and 6.25 M urea was prepared in the acetate buffer pH 3.2 and polymerized in the presence of TEMED and ammonium persulfate. The stacking gel containing 3.1% acrylamide, 0.75% bisacrylamide and 6.25 M urea was prepared in the acetate buffer pH 4.2 and polymerized in the presence of TEMED, ammonium persulfate and riboflavin under fluorescence light.

The electrophoresis was performed in the glycine-acetic acid buffer pH 3.7 at 80 V for 45 min then at 160 V until the fuchsin basic added to the sample solution reached the bottom of the column. The gel was stained for 30 min with the amido black in the 7% acetic acid, and destained in the 7% acetic acid.

Group-specific component (Gc)

Gc polymorphism was examined by IEF and the immunofixation with the anti-Gc antiserum on cellulose acetate strips according to the methods by Constans *et al.* (1978).

The 1 mm thick polyacrylamide gel containing the ampholyte solution (pH 3.5–10, 4–6) was prepared.

The electrophoretic conditions were 1,200 V, 20 mA and 15 W. The migration time was for 5.5 hr at 10°C. Paper strips saturated with 0.2 M NaOH as cathode and with 1 M H_3PO_4 as anode were used.

After IEF, the immunofixation with the anti-Gc antiserum was performed. For classification, sera were also examined by the polyacrylamide gel electrophoresis with the buffer system as described by Kitchin (1965).

Transferrin (Tf)

Tf polymorphism was examined by IEF according to the methods by Constans *et al.* (1980a). Before IEF, each serum was diluted 1 : 5 by 0.25% ferrous ammonium sulfate $6H_2O$ solution and kept overnight at 4°C.

The 0.5 mm thick polyacrylamide gel containing the ampholyte solution (pH 5–7, 5–5.5) was prepared.

The electrophoretic conditions were 1,200 V, 10 mA and 7.5 W. The migration time was for 5 hr at 10°C.

Paper strips saturated with 1 M ethanolamine as cathode and 1 M H_3PO_4 as anode were used.

After IEF, the gel was stained with the Coomassie brilliant blue R-250 in the ethanol solution. The immunofixation with the anti-Tf antiserum on cellulose acetate strips was performed to identify the electrophoretic pattern of the Tf protein.

α_1-Antitrypsin (Pi)

Pi polymorphism was examined by the separator IEF procedure as described by Constans *et al.* (1980b).

The 0.5 mm thick polyacrylamide gel containing the ampholyte solution (pH 4–5, 4–6.5) and ACES (*N*-(2-acetamide)2-aminoethanesulfonic acid) was prepared.

The electrophoretic conditions were 1,700 V, 10 mA and 10 W. The migration time was for 4.5 hr at 10°C.

Paper strips saturated with 1 M NaOH as cathode and with 1 M H_3PO_4 as anode were used.

After IEF, the gel was stained with the Coomassie brilliant blue R-250 in the ethanol solution.

The immunofixation with the anti-Pi antiserum on cellulose acetate strips was performed to identify the electrophoretic pattern of the Pi protein.

RESULTS AND DISCUSSION

Table 1 shows the phenotype distribution and the subtype gene frequencies of Hp, Gc, Tf and Pi obtained in this study. The photographs and figures (1–4) show their electrophoretic separation patterns.

Haptoglobin (Hp)

In the previous studies it was shown that Hpα^1S chain was the only subtype present among Asiatic populations. This observation was confirmed again in this study, but Hpα^1F chain was detected as a rare mutant (gene frequency : 0.003).

Hp2 gene frequency obtained was in the range of the values already reported on Japanese population.

In all the samples, the Hpα^2 chain observed was corresponding to the Hpα^2FS mobility, one Hpα^2 mutant was detected as with a slower mobility than that of the

Table 1. Phenotype distribution and subtype gene frequencies.

Haptoglobin (Hp)		Group-specific component (Gc)		Transferrin (Tf)		α_1-Antitrypsin (Pi)	
Hp 1F-1F	= 0	Gc 1F-1F	= 77	Tf C1-C1	=196	Pi M1-M1	=175
1F-1S	= 2	1F-1S	= 85	C1-C2	=125	M1-M2	=118
1S-1S	= 25	1F-1A2	= 6	C1-Dchi	= 4	M1-M3	= 19
2FS-1F	= 0	1S-1S	= 23	C1-B2	= 1	M1-M4	= 1
2FS-1S	=126	1S-1A2	= 4	C2-C2	= 15	M1-Nhamp	= 2
2FS-2FS	=185	1S-1A9	= 4	C2-C8	= 1	M2-M2	= 15
2FS-α_2 var	= 2	1A2-1C18	= 1			M2-M3	= 10
		2-1F	= 64			M2-Nhamp	= 1
Total	340	2A4-1F	= 1				
		2-1S	= 36			Total	341
		2-1A2	= 6				
		2-1A9	= 2				
		2-1C2	= 1				
		2-2	= 32				
		Total	342				
Hp1F	=0.003	Gc1F	=0.456	TfC1	=0.765	PiM1	=0.719
Hp1S	=0.263	Gc1S	=0.258	TfC2	=0.227	PiM2	=0.234
Hp2FS	=0.734	Gc1A2	=0.023	TfDchi	=0.006	PiM3	=0.043
		Gc1A9	=0.009	TfB2	=0.002	PiNhamp	=0.004
		Gc2	=0.254				

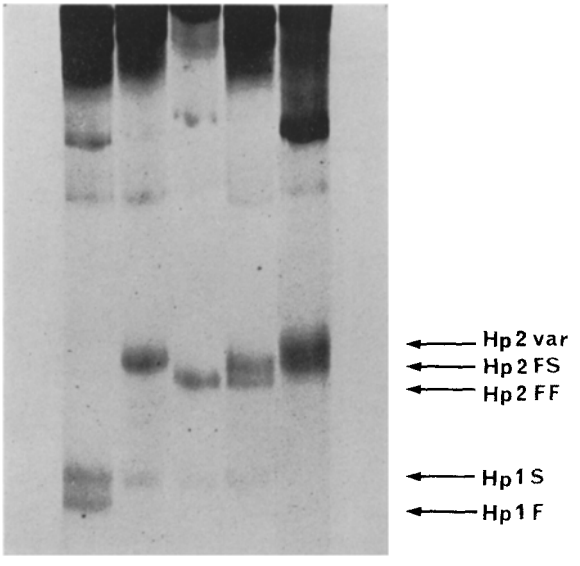


Fig. 1. Hp subtype and Hpα^2 mutant patterns after PAGE.
Samples: 1 Hp1F/1S
2 Hp2FS/1S
3 Hp2FF/1S
4 Hp2FF/2FS + Hp2FS/1S
5 Hp2FS/2var

Hpα^2SS chain. The presence of this mutant could not be suspected if the Hp chain subtyping was not performed.

Group-specific component (Gc)

The IEF polymorphism of this protein is well known among Japanese and Asiatic populations.

The Gc1F and Gc2 frequencies observed in this study were in good agreement with the data obtained by other investigators. Five rare Gc mutants were also observed in this study. Gc1A2 was the most frequent of them. Gc1A9, Gc1C2 and Gc2A4 were also detected. The new Gc variant was observed in this study. This mutant was characterized after IEF by a set of two bands with the cathodal mobility in comparison with the Gc1S bands which were used as IEF reference mobility. In routine screening, these protein bands might be misclassified with Gc1C14 bands (this allele was observed for the first time in Europe). But after PAGE, these bands were distinctly different from those of the Gc1C14 protein. The new variant allele was designated as Gc1C18 according to the new nomenclature adopted at the first International Workshop 1978 in Paris.

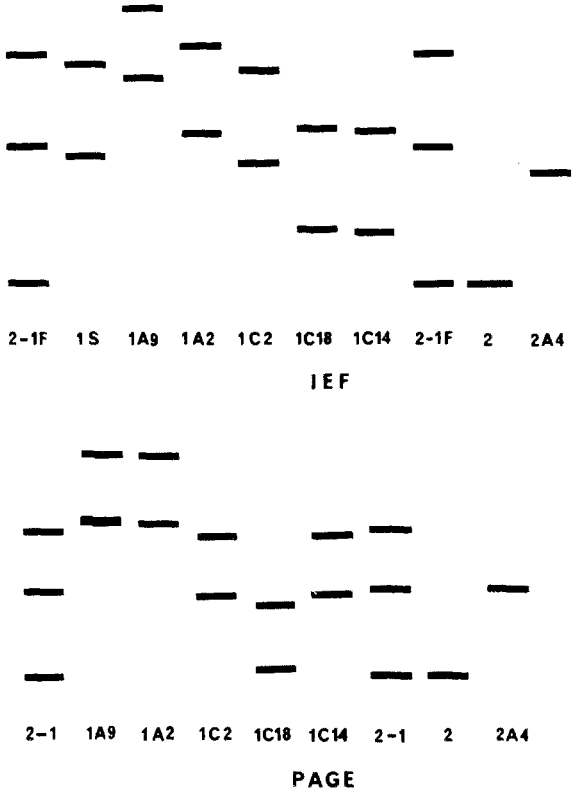


Fig. 2. Gc patterns after IEF and PAGE.

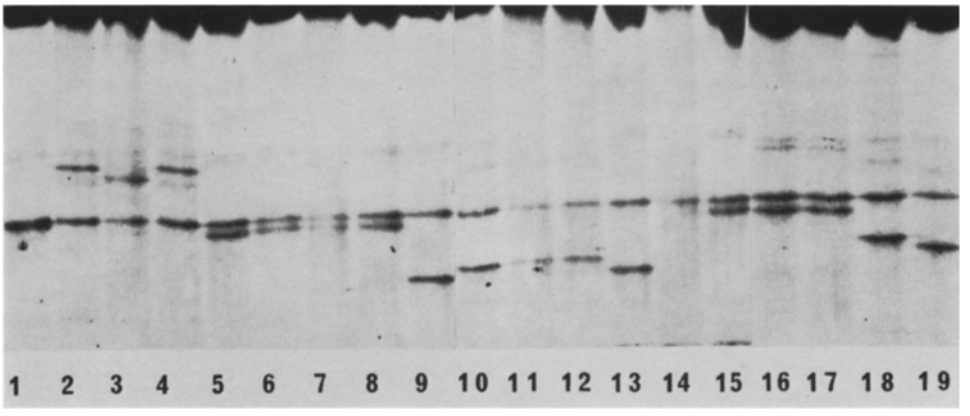
Transferrin (Tf)

TfC1 was the most frequent allele in this sample. This is a general pattern of the Tf subtype distribution among human populations. TfC2 was present with a high frequency in comparison with the values obtained among African or European populations. TfC3 was not observed in this study. According to the data published by Beckman *et al.* (1980), TfC2 gene frequencies were about 26% among Japanese population and 20% among Chinese population. These data were confirmed by this study. A new TfC8 mutant was detected. The IEF mobility of this protein which is shown in the Fig. 3 was very similar to the TfC1 band.

TfDchi was the only mutant with a cathodal mobility observed in this study and TfB2 was also the only mutant with an anodal mobility. Several anodal and cathodal Tf mutants were reported by Ferrell *et al.* (1977). In this study such different mutants were not detected. This difference between the two reports can be explained as follows.

a) The sample size: In this study 342 sera were studied while 3,900 samples have been screened in their study.

b) The geographical origin of the sample: The samples of this study were



Samples:	Tf:
1	C1 C3
2,4	C1 B2
3	C1 B3
5,8,15	C1 C2
6,7	C8 C2
9,13,19	C1 D1
10, 11, 12, 18	C1 Dchi
14	C1
16,17	C1 C6

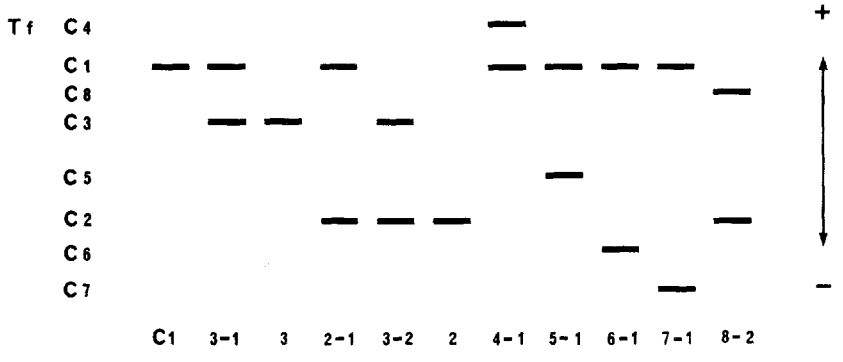


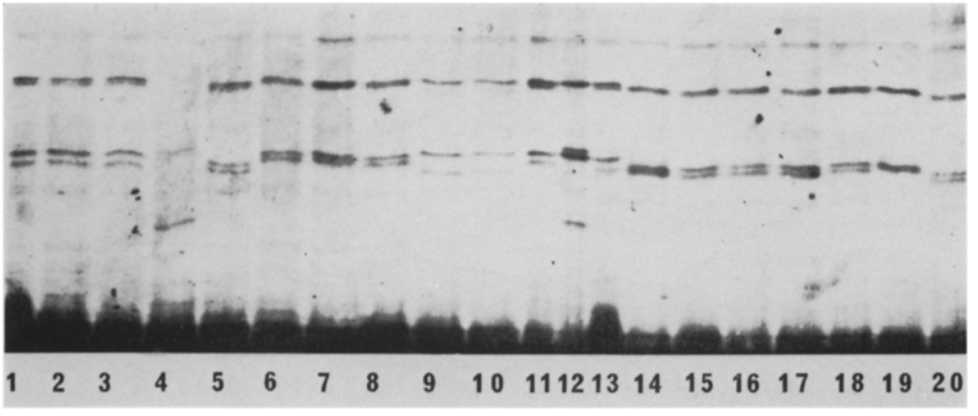
Fig. 3. TfC subtype and Tf mutant patterns after IEF pH 5–7.

from the Osaka area. The samples of their study belonged to the populations from the Hiroshima and Nagasaki areas.

This protein is given as an example of the variability of the rare mutant frequencies in a population. This variability is first under the influence of the sample size as demonstrated by Bhatia (1980). An important factor to be considered is also the heterogeneity of the samples used in the comparisons.

α_1-Antitrypsin (Pi)

Only limited studies have been published for the Pi polymorphism among



Samples:		Pi:
	1,2,3,11,13	M1 M2
	4	S
	5	M2 Nhamp
	6,8,15,16,17,20	M3 M2
	7,18	M1 M4
	9,10	M1 Nhamp
	12	M1 S
	14	M4 M2
	19	M1 M3

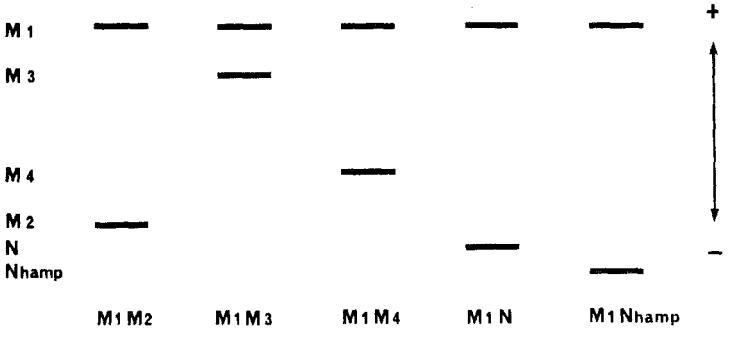


Fig. 4. Pi patterns after separator (A.C.E.S.) IEF pH 4–6.5.

Asiatic samples examined by using the more accurate IEF procedure. The most recent data were published by Miyake *et al.* (1979).

In this study four PiM subtypes were observed. The frequency of PiM4 allele was not estimated because the presence of this allele was the only one heterozygous phenotype as PiM1/M4. This result shows that this allele is also present among Japanese population. In European populaions it was described for the first time as a non rare mutant by Constans *et al.* (1980b).

The fifth allele was detected in this sample. After electrophoretic comparison with PiN described by Cox and Celhoffer (1974), the protein bands of this mutant

presented the more cathodal mobility than those of the PiN protein. This allele could be similar to PiNhampton described by Arnaud *et al.* (1978), though no direct comparison has been made. The frequency of this mutant was very similar to the data published by Miyake *et al.* (1979). This experience enables us to consider this allele to be present in other Asiatic populations.

Table 2. Serum protein polymorphism, subtype and rare mutant according to electrophoretic procedure.

	MW	Subtype	Mutant	Usual procedure	Starch or PAGE 6 M urea	IEF
Haptoglobin (Hp)						
α_1 Chain Hpα^1	9,000	HpαF HpαS	2-3	3 phenotypes	Hp1 subtypes	
α_2 Chain Hpα^2	18,000	Hp2FS	Hp2SS Hp2FF Hp2SF +2 or 3	Hp1-1 Hp2-1 Hp2-2	Hp2FS and mutants	
α_3 Chain Hpα^3	27,000	HpJ	2			
Group-specific component (Gc) no subunit	51,000	Gc1F Gc1S Gc2	49	3 phenotypes Gc1-1 Gc2-1 Gc2-2 10 mutants		subtypes and 49 mutants
Transferrin (Tf)	77,000	TfC1 TfC2 TfC3	17	TfC and most of the mutants		subtypes TfC1 to TfC8
α_1-Antitrypsin (Pi)	54,000	PiM1 PiM2 PiM3 PiM4	26	PiM and most of the mutants		PiM subtypes

Table 3. Geographical distribution of the Gc rare mutants.

		Gc^{1A} (Anodic form)	Gc^{1C} (Cathodic form)	Gc^{2A}	Gc^{2C}	Gene frequency (Mean value)
Europe		4	10	4	6	0.0013
	%	16.66	41.68	16.66	25.0	
Africa		3	3	3	0	0.020
	%	33.33	33.33	33.33	0	
Asia		5	3	3	0	0.014
	%	45.46	27.27	27.27	0	
South American Indians		6	1	0	0	0.010
	%	87.71	12.29	0	0	

Table 2 shows the numbers of the subtypes and mutants of haptoglobin, group-specific component, transferrin and α_1-antitrypsin detected by the different electrophoretic procedures and also their molecular weights and the presence or absence of their subunits.

From this table it is obvious that the number of the subtype and mutant does not invariably increase due to the number of the subunit, but increases due to the improvement of the electrophoretic procedures.

Table 3 shows the geographical distribution of the Gc rare mutants. As to the Gc polymorphism, Gc1 mutants are detected more than Gc2 mutants in any human populations studied. The frequencies of rare mutants are different between European populations and other populations. Gc mutants with increasing isoelectric points seem to be more frequent in European populations, but Gc mutants with decreasing isoelectric points are predominant in African and Asiatic populations.

As to the serum protein polymorphism, "rare mutant" seems to be a more appropriate denomination rather than "private variant." The distributions of the subtype gene frequencies are observed as related to the geographical factors. Rare mutants have been useful for the anthropological investigation (historical migrations or marriage between groups).

The use of the new electrophoretic procedures, such as isoelectrofocusing or more elaborated methods like bidimensional electrophoresis and polyacrylamide gel electrophoresis containing 6 M urea, enables us to detect the presence of the serum protein subtypes and the several mutants.

For the estimation of mutation rates, the following points are to be considered.

a) The first factor to be considered is the electrophoretic procedures to study the serum protein polymorphism.

b) The influence of the sample size is not trivial, but its influence is variable according to the protein studied and from population to population, where the condition of migration or genetic drift might be quite different in the past.

c) The presence or absence of subunits among serum protein is not necessarily correlated to the number of the mutants.

d) The nature of the amino acid substitutions in a polypeptide chain is different from population to population. This qualitative factor not included in the estimation of the mutation rate may reflect the physicochemical constraints in relation with the biological activity of the molecule.

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