

GROUP-SPECIFIC COMPONENT (Gc) POLYMORPHISM IN JAPANESE: AN ANALYSIS BY ISOELECTRIC FOCUSING ON POLYACRYLAMIDE GELS¹

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Summary Using the immunofixation isoelectric focusing method, Gc subtypes were examined in the samples with the Gc J phenotypes previously determined by immunoelectrophoresis, and it was found that the Gc^Js are subdivided into 4 different alleles. A population study in sera from 1,041 individuals confirmed the presence of the 4 variant alleles with the frequencies between 0.020 for the highest allele and 0.001 for the lowest one. The distribution of Gc subtypes in a Japanese population and a pedigree study from 18 families with the propositus of the variant are presented along with a description of the 2 other variants observed in the course of this study.

INTRODUCTION

Recently, Constans and Viau (1977) developed a new technique to detect a genetic variation of the group-specific component(Gc) of human serum and demonstrated the presence of heterogeneity in the Gc 1 protein. Using isoelectric focusing on polyacrylamide gels followed a specific immunofixation, they found 3 subtypes, Gc 1F-1F, 1F-1S and 1S-1S, in Gc 1-1 phenotypes and also 2 subtypes, Gc 2-1F and 2-1S, in Gc 2-1 phenotypes. Later, Constans *et al.* (1978a,b) showed that this technique has a great resolving power to discriminate some Gc variants.

In the previous paper (Kuwata *et al.*, 1978) we observed that a variant allele, Gc^J, attains to be a polymorphic frequency in Japanese populations. As mentioned in that paper, however, there were some cases in which it was somewhat difficult to distinguish the Gc J-1 from Gc 1-1 by agar gel immunoelectrophoresis used at that time. Therefore, we tried to re-examine this protein polymorphism by the immunofixation isoelectric focusing method. Omoto and Miyake (1978) already published their result on the distribution of Gc subtypes in this country. Our observation agrees with theirs as to the frequencies for the common alleles and also

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one of the variant alleles. Furthermore, it appears to show that there exists much complicated variation in Japanese populations, when larger samples are examined.

In this paper we report the result of re-analysis of the Gc J samples previously determined as well as a population survey in Japanese populations. Additional variants observed in the course of this study are also presented.

MATERIALS AND METHODS

The procedures for isoelectric focusing and immunofixation were essentially the same as those described by Constans *et al.* (1978a,b). The polyacrylamide gels of 1 mm thickness (7.5% T, 3% C, 2% Ampholine pH 4-6) were photopolymerized for 2 hr in the presence of riboflavin. Serum samples were applied at the cathodal side of the gel using small rectangles of filter paper. Paper strips saturated with 1% phosphoric acid and 2% ethylenediamine were used for anode and cathode, respectively. Isoelectric focusing was performed with the LKB Multiphor or the apparatus manufactured by Joko Sangyo (Tokyo) under cooling. After 4 hr separation period at 1,000 V, the immunofixation was carried out using the monospecific anti-Gc (DAKO) with 1:3 dilutions. A strip of cellulose acetate membrane was soaked with the diluted antiserum and placed on the Gc region of the gel for a few minutes. Subsequently the strip was removed, washed overnight with saline and stained with Amido black 10B.

For further examinations some sera were also analysed by agar gel immunoelectrophoresis (Hirschfeld, 1962) and antigen-antibody crossed electrophoresis (Laurell, 1965) with the buffer system of polyacrylamide gels described by Kitchin (1965).

The serum samples with Gc J phenotypes previously determined by immunoelectrophoresis (Kuwata *et al.*, 1978) were examined first in order to discriminate the variants from common Gc phenotypes. They were stored for up to 3 years after the initial use. For a population study sera newly collected from: (1) 103 medical students and 407 apparently healthy blood donors in Mie, western-central part of Japan; and (2) 229 medical students and 302 apparently healthy blood donors residing in and around Tokyo, were examined. Some family samples were also analysed, but only those with the variant subtypes are described in this paper.

RESULTS

Heterogeneity of the Gc J

In the previous study which was performed by prolonged immunoelectrophoresis (Kuwata *et al.*, 1978), we identified the phenotypes with Gc J in 69 out of 1,347 individuals. Among them, 52 samples were re-examined by the immunofixation isoelectric focusing, and it was found that the Gc^J previously determined was subdivided into 4 different alleles.

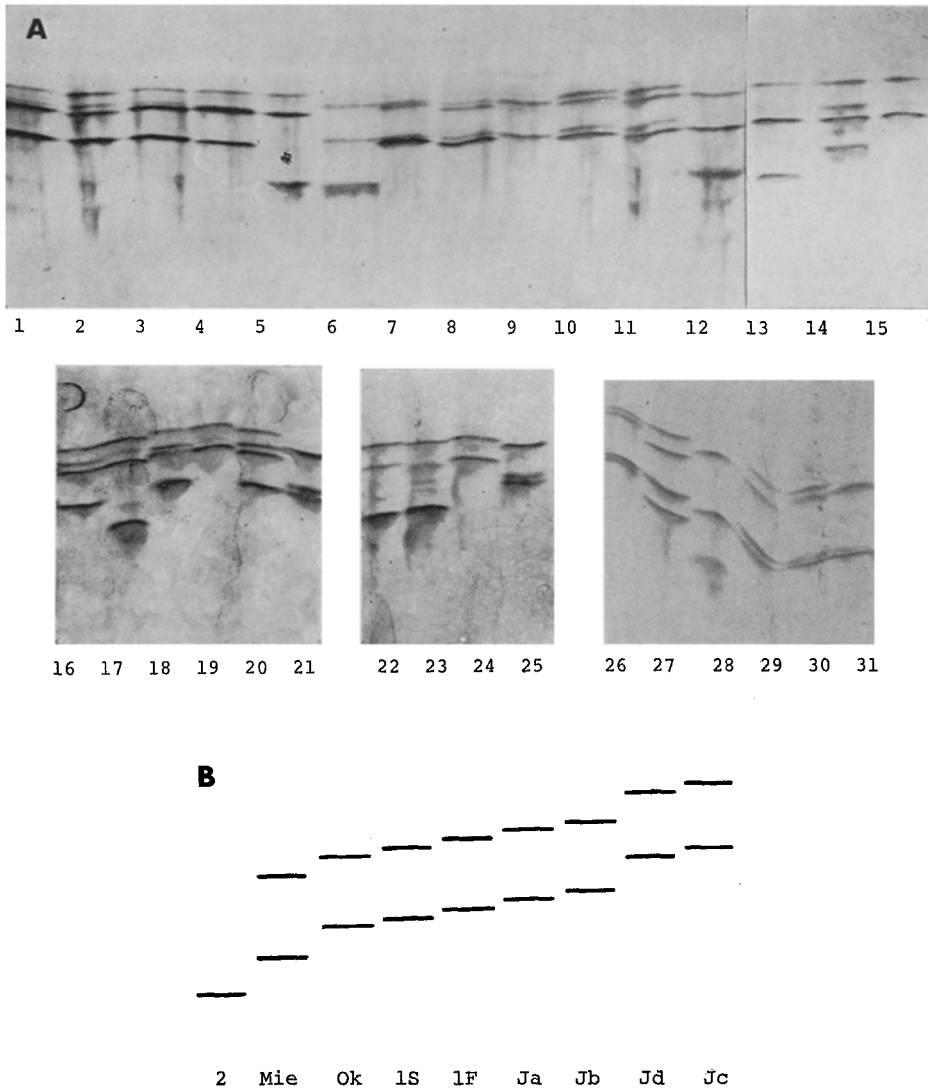


Fig. 1. Immunofixation isoelectric focusing patterns in this study. (A) Photographs of cellulose acetate membranes. Samples —1, 3, 18: Jc-1F, 2: Jd-1F, 4: Jc-1S, 5: Jc-2, 6, 23: Ja-2, 7: Ja-1F, 8: Ja-1S, 9: Ja-Ja, 10: Jb-1F, 11: Jb-1S, 12: 2-1S, 13: 2-1F, 14, 27: Mie-1F, 15: 1F-1F, 16, 20: Jd-1S, 17: Jd-2, 19: Jc-Jc, 21, 25, 26, 30: 1F-1S, 22: Jb-2, 24: Jb-Jb, 28: Ok-2, 29: Ok-1F and 31: 1S-1S. (B) Scheme of the Gc bands produced by the 9 alleles observed in Japanese.

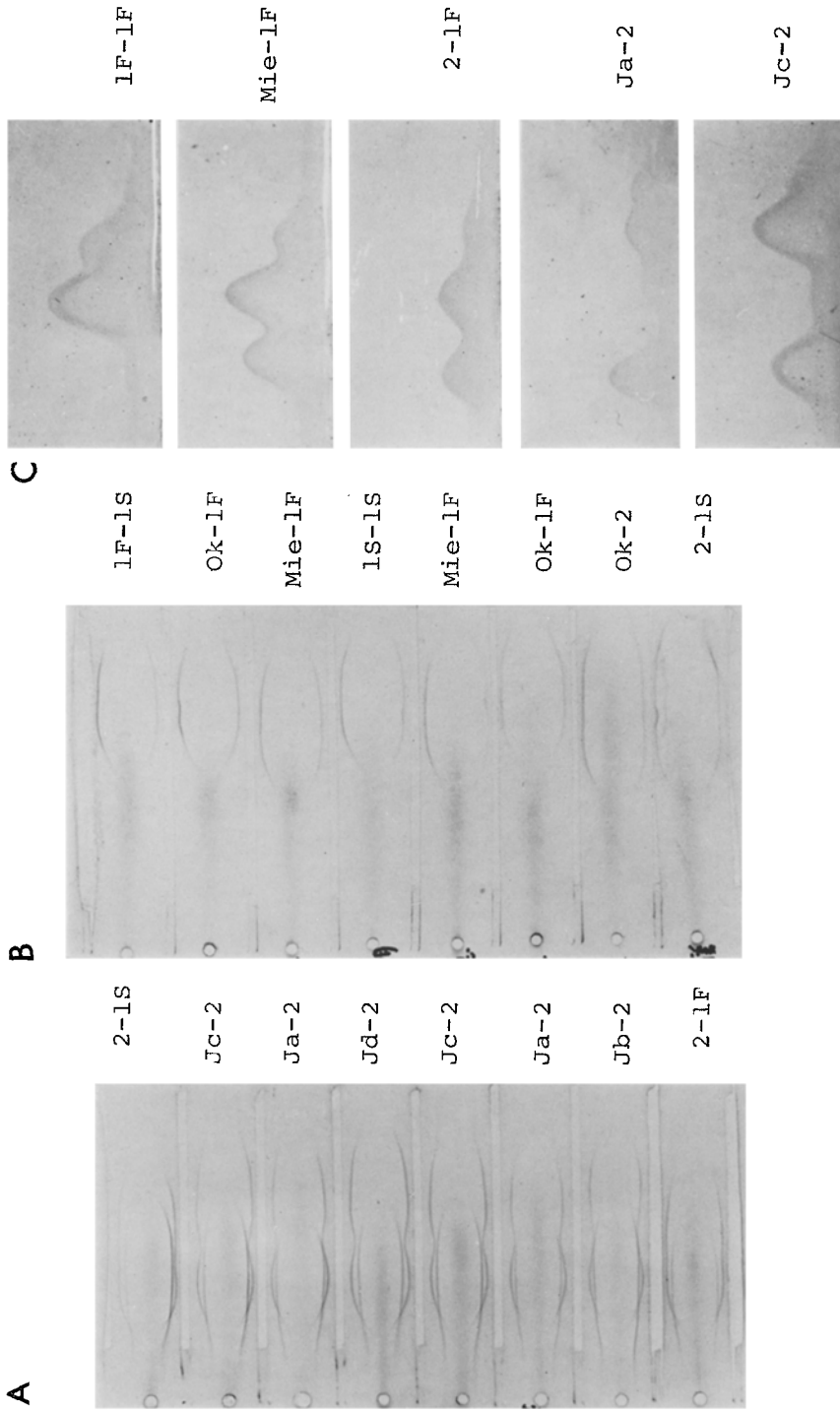


Fig. 2. Electrophoretic patterns of some Gc variants detected by the immunofixation isoelectric focusing. (A) Agar-gel immunoelectrophoresis; developed with anti-Gc (Behringwerke). (B) Agar-gel immunoelectrophoresis; developed with monospecific anti-Gc (DAKO). (C) Crossed electrophoresis. Anodes toward the right.

Table 1. Group-specific component (Gc) subtyping of the Gc J samples previously determined by immunoelectrophoresis.

Previous type	No.	Subtype in this study
J-J	3	Ja-Ja 1, Jb-Jb 1, Jc-Jc 1
J-2	16	Ja-2 12, Jc-2 4
J-1	33	Ja-1F 15, Jb-1F 2, Jc-1F 4, Jd-1F 1 Ja-1S 7, Jb-1S 2, Jc-1S 2

Three samples of the Gc J-J showed double-band patterns, but their isoelectric points (IEP) were different from each other. In one case, the double bands were seen with slightly lower IEP than those of Gc 1F, and in another case the double bands were observed with lower IEP than those of the first one. The third case was found with both bands located much faster toward the anode. These three subtypes were provisionally named Gc Ja-Ja, Jb-Jb and Jc-Jc, respectively.

From 16 samples of the previous Gc J-2 phenotype two subtypes were detected, each having the double bands of Gc Ja or Jc together with a Gc 2 band.

Isoelectric focusing patterns from the previous Gc J-1 phenotype were somewhat complicated because of the presence of 4 bands in each case. However, a careful analysis by repeated and parallel runs enabled us to classify 7 subtypes in 33 samples, in which all but one had the double bands of Gc Ja, Jb or Jc in addition to a set of Gc 1F or 1S proteins. In one case, the double bands of slightly higher IEP than those of Gc Jc were observed together with the double bands of Gc 1F. This type was tentatively called Gc Jd-1F.

The immunofixation patterns obtained in this study are shown in Fig. 1, and the result of subtyping among the selected samples is given in Table 1. It is remarkable that the immunofixation isoelectric focusing permits the distinction of 12 different subtypes among three phenotypes distinguished by the ordinary immunoelectrophoresis. As mentioned later, they are genetically determined by 4 variant alleles, Gc^{Ja} , Gc^{Jb} , Gc^{Jc} and Gc^{Jd} , in combination with one of the common alleles, Gc^{1F} , Gc^{1S} and Gc^2 .

Some electrophoretic patterns by immunoelectrophoresis and crossed electrophoresis are shown in Fig. 2. As seen in the figure, the precipitation lines were almost indistinguishable between the samples of Gc Ja-2, Jb-2, Jc-2 and Jd-2. On the other hand, the profiles of crossed electrophoresis were quite different between Gc Ja-2 and Jc-2: both Gc Ja-2 and Jc-2 had 3 peaks, but the 2 peaks for Gc Ja were approximately equal and less pronounced than the Gc 2 peak, while in the case of Gc Jc-2, the middle peak showed much more pronounced than the faster one and also the Gc 2.

Other variants demonstrated by isoelectric focusing

In the course of this study by the immunofixation isoelectric focusing 2 variants other than those described above were identified, which are also shown in Fig. 1.

One variant had a 4-band pattern, 2 of them corresponding to Gc 1F bands whereas the other 2 were located in a more cathodal position than the Gc 1S bands. This variant subtype was first detected in a student at Mie Univ. during a population study, and called Gc Mie-1F. Later, the same type was encountered in another student in Tokyo, who is not related to the former family. The family examinations revealed that the same variants were shared in other members of both families.

Table 2. Group-specific component (Gc) subtypes and gene frequencies in the Japanese.

Sample group	Mie	Tokyo	Combined		
	No. obs.	No. obs.	No. obs.	%	No. exp.
Gc subtype					
1F-1F	112	120	232	22.29	235.31
1F-1S	120	128	248	23.82	237.93
1S-1S	29	27	56	5.38	60.15
2-1F	117	131	248	23.82	251.78
2-1S	60	68	128	12.30	127.29
2-2	40	28	68	6.53	67.35
Ja-1F	6	14	20	1.92	19.48
Ja-1S	4	3	7	0.67	9.85
Ja-2	7	5	12	1.15	10.48
Ja-Ja	1	0	1	0.10	0.40
Jb-1F	1	0	1	1.10	1.88
Jb-1S	2	1	3	0.29	0.95
Jc-1F	5	2	7	0.67	6.23
Jc-1S	1	1	2	0.19	3.15
Jc-2	4	0	4	0.38	3.33
Jd-1F	0	1	1	0.10	0.99
Jd-2	0	1	1	0.10	0.53
Others	0	0	0	0	1.97
Variant*	1	1	2	0.19	—
Total	510	531	1,041	100.00	1,038.99
Chi²					
	12.24	9.09		10.676	
	15 df	21 df		21 df	
	p>0.50	p>0.975		p>0.95	
Gene					
<i>Gc^{1F}</i>	0.465	0.487		0.4759±0.0110	
<i>Gc^{1S}</i>	0.241	0.241		0.2406±0.0094	
<i>Gc²</i>	0.263	0.246		0.2546±0.0096	
<i>Gc^{Ja}</i>	0.019	0.021		0.0197±0.0030	
<i>Gc^{Jb}</i>	0.003	0.001		0.0019±0.0010	
<i>Gc^{Jc}</i>	0.010	0.003		0.0063±0.0017	
<i>Gc^{Jd}</i>	0	0.002		0.0010±0.0007	

* Mie-1F, excluded for the calculation of gene frequencies.

The immunoelectrophoretic pattern of Gc Mie-1F gave the precipitation line in the region intermediate between those of Gc 1-1 and 2-1, and the crossed electrophoresis showed 3 peaks in which the middle one was more pronounced than the others (Fig. 2).

The other variant was also a double-band variant. They had slightly higher IEP than those of Gc 1S. This variant was found in a family of a paternity case in Tokyo, and tentatively called Gc Okada (Ok) after the family name. The father and son had Gc Ok-1F and Ok-2, respectively (Fig. 1). Gc Ok was observed to be indistinguishable from Gc 1 on immunoelectrophoresis (Fig. 2).

Distribution of Gc subtypes in Japanese population

For a population study 1,041 sera from 2 local groups were examined. As indicated in Table 2, 18 subtypes corresponding to the presence of 8 alleles were identified. The distribution of Gc subtypes and Gc alleles in the 2 local groups is very similar and a good agreement between observed and expected numbers of the phenotypes under Hardy-Weinberg equilibrium is obtained for each local group as well as the total sample.

In the present series 6 common phenotypes were observed in about 94.1% of the samples while so-called rare variant phenotypes were detected in nearly 5.9%. This proportion is compatible with the previous observation (rare phenotypes in 5.1% out of 1,347 individuals) by immunoelectrophoresis (Kuwata *et al.*, 1978). Furthermore, 4 variant alleles determined from the selected samples in the previous study were

Table 3. Data on the families with variant Gc subtypes.

Family	Propositus	Parent	Offspring
Mat	Ja-Ja	Ja-1F × Ja-1S	Ja-Ja*, Ja-1F
77-84	Ja-Ja	Ja-2 × Ja-1S	Ja-Ja*, 2-1S, 2-1S
Mae	Ja-2	2-1F × Ja-1F	Ja-2*
Fur	Ja-2	Ja-1S × 2-1S	Ja-2*
FuAs	Ja-2	Ja-2 × 2-2	Ja-2*
Taka	Ja-2	Ja-2* × 1F-1F	2-1F, Ja-AF
T7752	Ja-1F	Ja-2 × 1F-1F	2-1F, Ja-1F*, Ja-1F
Iso	Ja-1F	Ja-1F* × 2-1S	1F-1S, 1F-1S, Ja-2
T7774	Ja-1S	Ja-2 × 1F-1S	Ja-1S*, Ja-1S
Om	Jb-Jb	Jb-Jb* × 2-1F	Jb-2, Jb-2, Jb-2
77-48	Jb-1F	1F-1F × Jb-1F	Jb-1F*
77-75	Jc-2	Jc-2 × 2-2	Jc-2*
78-13	Jc-1F	1F-1F × Jc-1F	Jc-1F*, 1F-1F
Suzu	Jc-1F	2-1S × Jc-1F*	Jc-2, 2-1F
T7736	Jd-1F	Jd-1S × 1F-1S	Jd-1F, Jd-1F*
77-55	Mie-1F	† × 1F-1F	Mie-1F, Mie-1F*
T7556	Mie-1F	Mie-1F × 2-1F	Mie-1F*, Mie-1F
Okada	Ok-2	Ok-1F × 2-1F	Ok-2*

* Indicates the propositus.

again observed in the new series, indicating that such variant alleles are widely distributed in Japanese populations.

As to the distribution of Gc subtypes in Japan, Omoto and Miyake (1978) already published their result in 310 individuals in Tokyo. The allele frequencies given in Table 2 are very similar to their figures for common alleles as well as Gc^{Ja} (Gc^{1F} 0.466, Gc^{1S} 0.259, Gc^2 0.257 and Gc^J 0.018). In addition, they observed 4 cases of the Gc 1F-X, which are probably identical to the present Gc Jc-1F.

Family studies

The family data of the Gc variants are summarized in Table 3. Although the numbers analysed are still not large, it is apparent that the 6 variant alleles determined in this study are all transmitted from parents to offspring in a simple Mendelian manner.

DISCUSSION

This study further confirms that the immunofixation isoelectric focusing possesses a resolving power much superior to the ordinary immunoelectrophoresis once employed frequently to examine Gc polymorphism. Indeed, this method revealed an unexpected degree of Gc variation in Japanese population, and we could identify a total of 24 phenotypes determined by 9 alleles in more than 1,000 sera. The present study was conducted independently in 2 laboratories, some samples of variants being exchanged, and got to identical results, although the differences to be disclosed are rather subtle on the immunofixation pattern.

The nomenclature for Gc variants seems to be confusing, since names of the locality where a mutant was first discovered or of the ethnic group characteristic for a variant have been widely accepted before, while numbers with a subscript like 1a or 2b are also used in recent literatures. Therefore, the notations given in the above section are tentative and only valid for explanation in this paper.

Among the 6 variants described, judging from the electrophoretic patterns and frequencies in the population, we have no doubt that the present Gc Ja is identical with the original Gc J reported by Omoto *et al.* (1972). Furthermore, the Gc Jc is very likely to be the same as Gc Am (Amerindian) which was observed in South American Indians from Bolivia (Constans *et al.*, 1978a), judging from their peculiar patterns in immunofixation isoelectric focusing and in crossed electrophoresis. Recently, Cleve *et al.* (1978) reported the presence of 3 Gc variants in European populations. From the figures in their paper, the present Gc Jd and Gc Ok seem to correspond to their Gc V^2 and V^1 , respectively, both observed from Germans in Munich.

In the field of forensic medicine, the Gc system has been utilized as a genetic marker for paternity tests. The exclusion chance of a falsely alleged father was thus far calculated to be 16.2% by the three-allele model (Gc^1 , Gc^2 and Gc^J). Now, when the subtyping is carried out, the exclusion rate goes over 38.2% based on the 4-allele

model (Gc^{1F} , Gc^{1S} , Gc^2 and Gc^{var}) in Japanese. It is clear that the Gc subtyping is a valuable aid in the examination of cases of disputed paternity.

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