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THE DISTRIBUTION OF THE GROUP-SPECIFIC COMPONENT (Gc) SUBTYPES IN JAPANESE

Keiichi OMOTO and Kazuhiko MIYAKE*

Department of Anthropology, Faculty of Science, The University of Tokyo, Tokyo
**First Department of Internal Medicine, Faculty of Medicine, The University of Tokyo, Tokyo*

Summary. The distribution of Gc subtypes in a sample from 310 unrelated healthy Japanese living in Tokyo was examined by a slab polyacrylamide gel isoelectrofocusing followed by immunofixation. At least 9 phenotypes were distinguished which can be ascribed to four alleles: Gc^{1F}, Gc^{1S}, Gc^{2} and Gc^{J}. The allele frequencies were 0.4656, 0.2590, 0.2574 and 0.0180, respectively. It was confirmed that Gc^{J} has a polymorphic frequency in Japanese. Furthermore, two unusual phenotypes were encountered, one with a variant Gc 1 and the other with a variant Gc 2 protein, indicating the occurrence in Japanese of two further Gc alleles.

INTRODUCTION

Recently, stimulating new methods have been developed to detect genetic variation of the group-specific component (Gc) of human serum. These are (1) agarose gel electrophoresis followed by immunofixation (Alper and Johnson, 1969; Johnson *et al.*, 1975), (2) autoradiography of the ^{14}C-vitamin D-labeled Gc after electrophoresis in polyacrylamide gels (Daiger *et al.*, 1975; Daiger and Cavalli-Sforza, 1977), and (3) isoelectrofocusing in thin layer polyacrylamide gel followed by immunofixation (Constans and Viau, 1977; Constans *et al.*, 1978a). In population genetic studies, in particular, the last mentioned method seems to be superior to others in both resolving power and convenience. Thus, it was shown by this method that samples of the classical Gc 1-1 phenotype can be divided into three subtypes, Gc 1F-1F, Gc 1F-1S and Gc 1S-1S, which are considered to be determined by a pair of alleles, Gc^{1S} and Gc^{1F}. Also, two subtypes, Gc 2-1F and Gc 2-1S, are confirmed in the classical Gc 2-1 phenotype (Constans and Viau, 1977). Thus far, samples from only four populations have been examined by this method, namely, a Caucasian, two African and an American Indian populations, and the universal occurrence of the Gc subtypes mentioned was shown together with the discovery of a number of relatively uncommon Gc variants (Constans *et al.*, 1978 a, b).

In this report, we present the result of the Gc subtyping of a Japanese sample.

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As a by-product of the survey, it was shown that Gc^{J} (Gc^{Japan}) which was regarded as rare variant allele (Omoto *et al.*, 1972) has a "polymorphic" frequency in Japanese.

MATERIALS AND METHODS

A total of 310 sera were obtained from unrelated healthy adults living in Tokyo and stored at −20°C for about 5 months until tested. They were essentially without hemolysis, although a few samples showed a slight sign of hemolysis.

The slab gel (16 × 10 × 0.1 cm) was prepared by mixing 4 ml acrylamide solution (29.1 g/100 ml), 4 ml N,N'-methylenbisacrylamide solution (0.9 g/100 ml), 8 ml sucrose solution (20 g/100 ml), 1.6 ml Ampholine pH 4–6 (LKB Produktor, Bromma, Sweden), 16 mg ammonium persulfate. The gel was polymerized within 20 min at room temperature and was precooled for 10 min at 5°C. Paper strips saturated with 0.5% ethylene-diamine and 0.5% phosphoric acid were used as cathode and anode, respectively. 5 × 5 or 5 × 3 mm pieces of filter paper (Whatman 3 MM) saturated with *ca.* 10 μl of serum (diluted 1 : 3 or 1 : 4 with saline) were applied at the cathodic side of the gel.

Isoelectrofocusing was carried out using the apparatus manufactured by Joko Sangyo, Tokyo, in which cooling water at approximately 4°C was circulating. The voltage was increased gradually in 60 min from the initial 300 V to 1,000 V, and then this final voltage was maintained for 5 hours.

For immunofixation of Gc proteins the ingenious method developed by Dr. J. Constans and his colleagues (Constans *et al.*, 1978b; Viau *et al.*, 1978) was used. The strip of cellulose acetate membrane (Separax, Joko Sangyo, Tokyo) was soaked with the anti-Gc serum diluted 1 : 3 or 1 : 4 with saline and was dried. The monospecific anti-Gc serum (goat) was obtained from Atlantic Antibodies, Westbrook, Maine, USA. The membrane was placed onto the gel after isoelectrofocusing, left for a few minutes and then soaked overnight in saline. Staining was performed in the usual manner using Amido Black 10B or Coomassie Brilliant Blue.

The immunofixation electrophoresis using agarose gels was carried out as described (Alper and Johnson, 1969; Johnson *et al.*, 1975).

RESULTS AND DISCUSSION

Photograph of the acetate membrane showing the isoelectrofocusing-immunofixation patterns of various Gc types is shown in Fig. 1 (a–d). It was shown that sera of the classical Gc type 1-1 can clearly be divided into three different phenotypes. The comparison with a control of the Caucasian origin confirmed that they correspond to the Gc subtypes described by Constans and Viau (1977), namely, 1F-1F, 1F-1S and 1S-1S. Among these, Gc 1F-1F and Gc 1S-1S have a two-banded pattern and are considered to be homozygotes, while Gc 1F-1S with a four-banded pattern is considered to be a heterozygote. Further, there are the three-banded

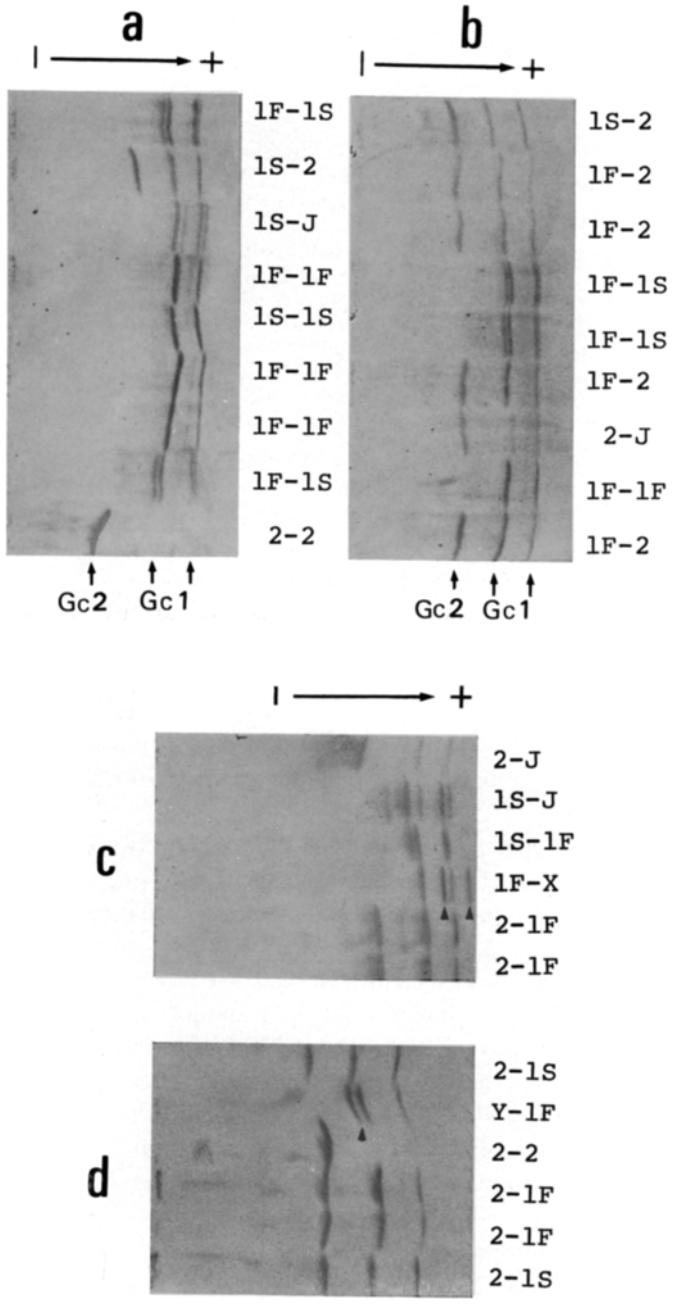

Fig. 1. Photographs of the cellulose acetate membranes showing isoelectrofocusing-immunofixation patterns of various Gc phenotypes found in Japanese. a, b: Common phenotypes including Gc 1-J and Gc 2-J. c: The unusual type with the "X" band marked by arrows. d: The unusual phenotype with the "Y" band marked by an arrow, probably a variant of Gc 2 protein.

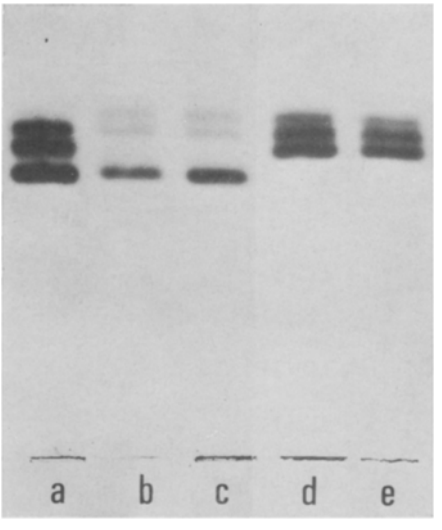

Fig. 2. Photograph of the agarose gel showing immunofixation electrophoretic patterns of samples of Gc J phenotypes. a: Gc 2-1, b: Gc 2-J (this survey), c: Gc 2-J (control), d and e: two different samples of Gc 1-J (this survey).

phenotypes, Gc 2-1F and Gc 2-1S. Gc 2-2 is shown as a single-banded phenotype. By far the most samples examined could be typed as one of the six phenotypes mentioned above.

Besides these phenotypes, however, eleven samples had two Gc bands migrating more anodally than the Gc 1F components, together with one of the common Gc components, Gc 1F, Gc 1S and Gc 2. These samples were directly compared with the control sample of Gc J-2, the variant which has been discovered in Japanese (Omoto *et al.*, 1972), and it was confirmed that the variant Gc component was indistinguishable from Gc J(Gc Japan). Also, it should be noted in Fig. 1 (a, b) that the anodal, variant bands are stained weakly, the feature known to be peculiar to Gc J phenotypes. A comparison run of agarose gel electrophoresis followed by immunofixation (Johnson *et al.*, 1975) also confirmed that the variant protein is Gc J (Fig. 2), although the two subtypes of Gc 1 protein are not distinguishable by this method. The combined frequency for Gc J heterozygotes was estimated at approximately 3.5%.

Furthermore, five samples had deviating patterns clearly distinguishable from all of the above mentioned phenotypes. One of them, which was encountered four times, had in addition to the Gc 1F components two Gc components migrating more anodally than Gc J ("X" in Fig. 1c). Although it could be another genetic variant, these samples were excluded from the gene frequency calculation, since two samples among four showing this phenotype had a slight indication of hemolysis.

Table 1. Distribution of Gc phenotypes as the results of isoelectrofocusing / immunofixation of 305 sera from healthy Japanese adults.

Phenotypes	Number observed	Number expected	χ^2
1F-1F	66	66.12	.0002
1F-1S	69	73.56	.2827
1S-1S	17	20.46	.5851
2-1F	76	73.11	.1142
2-1S	53	40.67	3.7381
2-2	13	20.20	2.5722
J-1F	7	5.11	.6990
J-1S	2	2.84	.2484
J-2	2	2.84	.2484
J-J	0	0.10	.1000
Total	305	305.00	8.5883

(6 df, .20>P>.10)

Allele frequencies: Gc^{1F} 0.4656±0.0202, Gc^{1S} 0.2590±0.0177, Gc^{2} 0.2574±0.0177, Gc^{J} 0.0180±0.0054

The other deviating phenotype had a three-banded pattern with usual Gc 1F comnents and a Gc component migrating slightly cathodal to the main band of the Gc 1S component ("Y" in Fig. 1d). The variant component differs clearly in the isoelectric position from the similar kind of variant discovered in an African population, Gc 2b (Constans *et al.*, 1978b). It is likely that this variant is a heterozygote for Gc^{1F} and a new, rare Gc allele which derive from a Gc^{2} allele. However, since it was found only once, it was similarly excluded from the calculation of allele frequencies.

Among 305 serum samples excluding those with deviating phenotypes mentioned above, nine phenotypes were clearly distinguishable. The distribution of observed and expected numbers is shown in Table 1, together with the frequencies estimated for four alleles. The deviation from the state of panmixia as measured by the chi-square test for the whole sample was statistically non-significant (χ^2= 8.5883, 6 df, .20>P>.10). However, it is obvious from Table 1 that in the present sample, the observed numbers for only two phenotypes, Gc 2-1S and Gc 2-2, tend to deviate markedly from the corresponding expected numbers. The reason for this is unknown, though it may be either accidental or due to the presence of further microheterogeneity in the Gc system.

The allele frequency for Gc^{2} (0.257) falls within the range of frequencies reported for Japanese population, namely, 0.22–0.27, (Omoto, 1975). The frequency of Gc^{1F} and Gc^{1S} combined is 0.7246. On the other hand, the frequency for Gc^{J} (0.018) that was estimated for the first time in the present study is considerably high and regarded as "polymorphic." Thus far, only a few examples of this variant were reported in Japan (Omoto *et al.*, 1972; Nakajima *et al.*, 1976; Ishimoto *et al.*, 1977). It has also been discovered recently among Chinese (Johnson *et al.*, 1975).

Recently, Daiger and Cavalli-Sforza (1977) reported the existence of a polymorphic Gc variant in Japanese and Chinese samples using the autoradiography of the ^{14}C-vitamin D-labeled Gc protein. Though they failed to distinguish this variant from that found among Africans (Gc Ab), it is very likely that this variant is Gc J. It now appears to be true, that the Gc J phenotypes have been overlooked in population studies. This is obvious, since in the conventional immunoelectrophoretic pattern they were not distinguishable from Gc 1-1 or Gc 2-1 phenotypes, unless observation was made with a special care.

The data of Gc subtypes in other population groups are still meagre. According to Constans and his co-workers (1978 a, b), the frequency for Gc^{1F} is high (0.58–0.78) in two African samples (Pygmy and Fula), lower (0.23) in an American Indian sample, and markedly low (0.08) in a French Pyrenean sample. Rather contrary to this trend, the frequency of Gc^{1S} is high in the American Indian (0.64) and French (0.51) samples, while it is low (0.11–0.19) in the African populations. In the present Japanese sample, the frequency for Gc^{1F} was relatively high (0.47), while that for Gc^{1S} was relatively low (0.26). Further data of other asiatic populations are clearly needed to confirm whether the distribution of Gc subtypes found in the present study along with the occurrence of Gc^{J} is characteristic of Mongoloid groups of Asia. In any case, it is clear that the Gc subtype system detected by the isoelectrofocusing-immunofixation method is a valuable addition of genetic markers in man suitable for population genetic studies.

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