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GROUP-SPECIFIC COMPONENT (Gc) SUBTYPES IN TWO CHINESE POPULATIONS

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Summary The genetic polymorphism of serum group-specific component (Gc) was studied in two Chinese (Han) populations using isoelectric focusing followed by immunofixation. Six common and seven rare variant phenotypes were observed among 155 samples from Beijing and 256 samples from Guangzhou. The frequencies for the common alleles, *Gc*1F*, *Gc*1S*, and *Gc*2*, were 0.4774, 0.2000, and 0.3065, respectively, for Beijing, and 0.4316, 0.2891, and 0.2734, respectively, for Guangzhou. Only the frequency of *Gc*1S* showed a statistically significant difference between the two localities. The rare Gc variants observed were: Gc 1A3, Gc 1A8, Gc 1C18 and Gc 2A4. Furthermore, two new rare Gc variants were detected and named Gc 1C50 and Gc 2A19.

INTRODUCTION

The isoelectric focusing in thin layer polyacrylamide gel followed by immunofixation has been used since 1977 for subtyping of the group-specific component (Gc), a vitamin D binding protein in human serum (Constans and Viau, 1977; Constans *et al.*, 1978). Using this technique, approximately 50,000 individuals from more than 160 different populations have been tested for the Gc polymorphism, making this system one of the most intensively studied among human blood genetic markers (Kamboh and Ferrell, 1986). Besides the universally common alleles, *Gc*1F*, *Gc*1S* and *Gc*2*, the number of variant alleles at the Gc locus now exceeds 85 (Constans *et al.*, 1983; Nakasono *et al.*, 1985).

In east Asia, the Japanese may be the most intensively studied population for Gc subtypes (Omoto and Miyake, 1978; Ishimoto *et al.*, 1979; Shibata, 1983; Yuasa *et al.*, 1984; Omoto, 1986). As to the Chinese, only a few data concerning the populations outside the mainland China have been available in the literature (Kim *et al.*, 1981; Tan *et al.*, 1981; Matsumoto *et al.*, 1980; Kamboh *et al.*, 1984). In

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this study, a pilot investigation of Gc subtype distribution was carried out in two geographically distant populations of mainland Chinese (Han).

MATERIALS AND METHODS

Blood samples were obtained from healthy, unrelated Chinese of Han nationality living in Beijing (N=155) and Guangzhou (N=256) and stored at −30°C prior to examination.

Gc subtyping was carried out by isoelectric focusing in a thin-layer slab polyacrylamide gel followed by print immunofixation (Constans *et al.*, 1978; Omoto and Miyake, 1978). For preparation of a 0.5 mm thick gel, 3 ml of 29.1% acrylamide solution, 3 ml of 0.9% *N,N'*-methylen bisacrylamide solution, 2 ml glycerol, 1.0 ml Ampholine pH 4–6 (LKB), 20 μl TEMED (*N,N,N',N'*-tetramethyl ethylene diamine), 0.5 ml riboflavin solution (0.004%) and 10.0 ml distilled water were mixed. After the photopolymerization at room temperature for about 1.5 hr, the gel was cooled prior to electric focusing. 5×5 mm pieces of filter paper (Whatman No. 1) were soaked with the plasma samples diluted 1 : 3 or 1 : 4 with saline, and placed on the gel surface at the cathodic side. Isoelectric focusing was carried out using LKB Multiphor apparatus at 10°C with an initial voltage of 800 V. After 1 hr, the sample-soaked filter paper was removed and the electric focusing was continued for 5 hr, while the voltage increased gradually from 900 to 1,200 V. The cellulose acetate sheet (Separax, Joko Sangyo, Tokyo) was soaked with the anti-Gc antiserum (Dako-patts a/s, Glostrup, Denmark) diluted 1 : 3 with saline and then dried. It was placed on the gel after isoelectric focusing for about 2 min and then soaked in saline overnight. For staining a 1% solution of Amino Black 10B was used.

RESULTS

The photograph of the cellulose acetate membrane showing the Gc subtypes is shown in Fig. 1. The diagram of Gc subtypes observed in present study is shown in Fig. 2. Six phenotypes ascribed to three common alleles, *Gc*1F*, *Gc*1S* and *Gc*2*, together with several rare variant phenotypes were observed both in Beijing and in Guangzhou population samples. The distribution of Gc phenotypes and allele frequencies of the present study are shown in Table 1 (Beijing) and Table 2 (Guangzhou). The observed phenotypic distribution were in good agreement with the Hardy-Weinberg expectation in both population samples.

In the Beijing sample, four unusual phenotypes were observed which were considered to be heterozygous for one of at least three rare variant Gc alleles. Two of these variants were identified as *Gc*1A3* and *Gc*1A8*, while the other was considered to be a new Gc 1C variant. By the courtesy of Drs. H. Cleve and J. Constans, it was compared with the reference samples of Gc variants and confirmed to be the new variant which should be called as Gc 1C50.

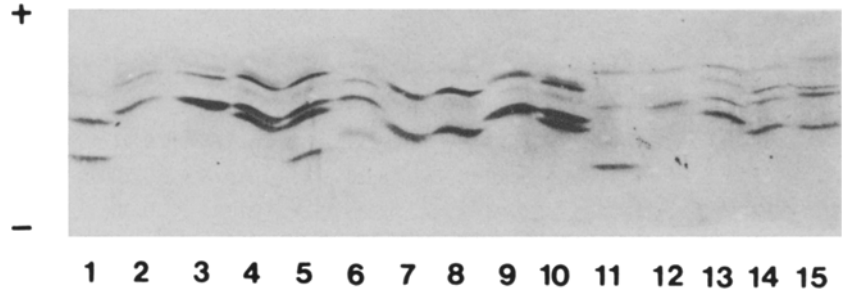

Fig. 1. Photograph showing Gc phenotypes demonstrated using isoelectric focusing followed by immunofixation technique. (1) 2-2A4, (2) 1F-2A19, (3) 1F-2A19, (4) 1F-1S, (5) 1S-1C50, (6) 1F-1C18, (7) 1S-1C2 (control), (8) 1S, (9) 1F, (10) 1F-1S, (11) 2-1A2 (control), (12) 1A2 (control), (13) 1F-1A3, (14) 1S-N (control: a variant found in the Philippine Negrito), (15) 1S-1A8.

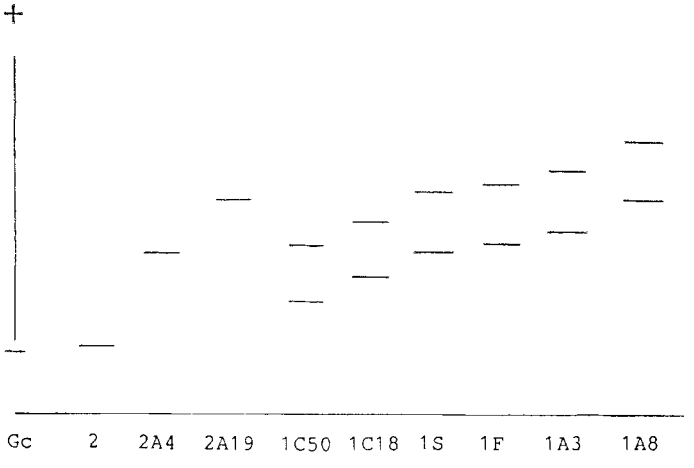

Fig. 2. Diagram of Gc subtypes identified in the present study.

In the Guangzhou sample, four kinds of variant Gc bands were observed, among which only one (Gc 1A3) was identical to that found in the Beijing sample. Drs. Cleve and Constans identified the other variants as Gc 1C18, Gc 2A4 and a new Gc 2A variant, which was named Gc 2A19.

The frequencies of the common Gc alleles in the present study were compared with those reported previously for Chinese populations (Table 3). A statistically significant difference was found only for the frequency of *Gc*1S* between Beijing and Guangzhou population samples (Table 4).

DISCUSSION

To our knowledge, there are relatively few reports on the Gc subtypes of the

Table 1. Distribution of Gc phenotypes and allele frequencies (Beijing).

Phenotypes	Obs. N.	%	Exp. N.	Allele frequencies
1F	33	21.29	35.33	
1F-1S	37	23.87	29.59	*Gc*1F*: 0.4774±0.0284
1S	2	1.29	6.20	*Gc*1S*: 0.2000±0.0227
2-1F	43	27.74	45.36	*Gc*2*: 0.3065±0.0262
2-1S	19	12.26	19.00	
2	16	10.32	14.56	
Rare variants:				Rare alleles:
1F-1A3	2	1.29	1.44	*Gc*1A3*: 0.0097±0.0056
2-1A3	1	0.65	0.92	*Gc*1A8*: 0.0032±0.0032
1S-1A8	1	0.65	0.20	*Gc*1C50*: 0.0032±0.0032
1S-1C50	1	0.65	0.20	
Total	155	100.01	152.21	$\chi^2=5.1197$, d.f.=3, $p>0.10$

Chi-square was calculated on the common phenotypes only.

Table 2. Distribution of Gc phenotypes and allele frequencies (Guangzhou).

Phenotypes	Obs. N.	%	Exp. N.	Allele frequencies
1F	40	15.62	47.69	
1F-1S	73	28.52	63.44	*Gc*1F*: 0.4316±0.0219
1S	17	6.64	21.10	*GC*1S*: 0.2871±0.0200
2-1F	65	25.39	60.42	*Gc*2*: 0.2734±0.0197
2-1S	40	15.63	40.19	
2	17	6.64	19.14	
Rare variants:				Rare alleles:
1F-1A3	1	0.39	0.44	*Gc*1A3*: 0.0020±0.0020
1F-1C18	1	0.39	0.44	*Gc*1C18*: 0.0020±0.0020
2-2A4	1	0.39	0.28	*Gc*2A4*: 0.0020±0.0020
1F-2A19	1	0.39	0.44	*Gc*2A19*: 0.0020±0.0020
Total	256	100.00	253.86	$\chi^2=4.0647$, d.f.=3, $p>0.20$

Chi-square was calculated on the common phenotypes only.

mailand Chinese populations. Considering the vast geographical areas covered by the Han group, let alone the numerous National Minority groups, it was considered to be worth investigating the Gc subtypes of northern and southern populations of

Table 3. The distributions of Gc common allele frequencies in various Chinese populations.

Population	No.	*Gc*1F*	*Gc*1S*	*Gc*2*	Authors
Beijing	155	0.4774	0.2000	0.3065	Present study
Guangzhou	256	0.4316	0.2871	0.2734	Present study
Hongkong	362	0.494	0.258	0.274	Kim *et al.*, 1981
Chinese (Taiwan)	373	0.3968	0.2708	0.3029	Matsumoto *et al.*, 1980
China	113	0.4779	0.2566	0.2611	Kamboh *et al.*, 1984

Table 4. Comparison of Gc common allele frequencies between two Chinese populations.

Population	N	*Gc*1F*	*Gc*1S*	*Gc*2*
Beijing	155	0.4774	0.2000	0.3065
Guangzhou	256	0.4316	0.2871	0.2734
		$\chi^2=1.614$	$\chi^2=8.4293$	$\chi^2=1.0956$
		d.f.=1	d.f.=1	d.f.=1
		$p>0.10$	$p<0.01$	$p>0.20$

the Han Chinese in the mainland China. For this purpose, serum samples were obtained from Beijing in northern China and from Guangzhou in southern China, two large cities separated approximately 1,900 km apart.

As shown in Tables 1, 2, and 4, the allele frequencies of the common Gc subtypes were found to be similar except for *Gc*1S* between these two localities. The *Gc*1S* frequency of this study is higher in Guangzhou than in Beijing, and the difference is statistically significant. Recently, a geographical cline of Gc subtype frequencies were noted in Japan, where the frequency of *Gc*2* tends to be higher if one goes from north-eastern to south-western part of Japan (Yuasa *et al.*, 1983; Omoto, 1986). There was also an indication for a cline for the frequency of *Gc*1F*, but not for *Gc*1S* (Omoto, 1986). Thus, the finding of this study that the frequency of *Gc*1S*, rather than that of *Gc*1F*, may show a cline in China, contrasts with the observation in Japan. It may indicate that the geographical cline of Gc subtypes in eastern Asia, if it really exists, is not due to the selective factor on the particular Gc subtype, but is explained simply by the effect of gene flow.

In this study a total of six rare variant Gc subtypes were found, two of which were newly discovered and named Gc 1C50 and Gc 2A19, respectively. The Gc 1C50 variant found in the Beijing sample is located behind Gc 1C8 and Gc 1C9 on the conventional isoelectric focusing pattern, and similar to Gc 1C10 or Gc 1C28, but the comparison run with the reference samples in the Gc reference laboratory

in Toulouse showed that it is distinct from the known variants. The Gc 2A19 variant found in the Guangzhou sample is located between Gc 2A5 and Gc 2A13 on the conventional isoelectric focusing pattern. This variant was found to be identical with the variant tentatively named Gc 2F recently reported from northern Japan (Omoto, 1986).

Among the other rare Gc variants observed in this study, only Gc 1A3 was commonly found in Beijing and Guangzhou. It has been known to be widely distributed in east Asian populations (Matsumoto *et al.*, 1980; Kamboh *et al.*, 1984; Kamboh and Ferrell, 1986). Gc 1A8 and Gc 2A4 which have been well known in Japanese (Constans and Cleve, 1979; Matsumoto *et al.*, 1980; Shibata, 1983; Omoto, 1986), were also detected in the present study. The other variant, Gc 1C18, was hitherto known also in Japanese (Constans *et al.*, 1983; Shibata, 1983).

It is worth noting that Gc 1A2, originally known as Gc J, was not found in the present Chinese samples. This variant has a polymorphic frequency throughout Japanese including the Ainu (Omoto and Miyake, 1978; Matsumoto *et al.*, 1980; Cleve *et al.*, 1981; Omoto, 1986). Also, it would be pointed out that another variant frequently observed in Japanese, Gc 1A9 (Gc TK1), was not observed in the present Chinese samples as well as in those reported by Kamboh *et al.* (1984), although these two variants were suggested to be the markers of Mongoloid populations. Future studies are needed to clarify the geographical origins of these two variants.

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