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Gc SUBTYPES DEMONSTRATED BY ISOELECTRIC FOCUSING: FURTHER DATA AND DESCRIPTION OF NEW VARIANTS AMONG AN AFRICAN SAMPLE (FULA) FROM SENEGAL

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Summary The polymorphism of the Group Specific Component (Gc) was studied in serum samples from a Fula population of Senegal (Western Africa) using isoelectric focusing in slab polyacrylamide gel followed by immunofixation. The isoelectric focusing patterns of various Gc phenotypes were compared to the patterns obtained by polyacrylamide disc electrophoresis. Besides the two subtypes, Gc^{1F} and Gc^{1S} of Gc^1 and the Gc^{Ab} gene, a Gc^1 variant and a Gc^2 variant were discovered and named Gc^{1b} and Gc^{2b} respectively. The results were compared to the data obtained in a Pygmy population. They confirmed the high degree of polymorphism of the Gc protein in African populations.

INTRODUCTION

Investigations on population genetics very often include the determination of the Group Specific Component polymorphism. Various methods have already been described and successive improvements have made them more easily reproducible and more sensitive. Recently new techniques have been adapted to the study of the Gc protein. One of them is based on the fact that this protein is capable of fixing radioactive vitamin D. After a migration on agarose gel the electrophoretic pattern is developped on a photographic plate (Daiger *et al.*, 1975). Another improvement is represented by the immunofixation electrophoresis on agarose (Alper and Johnson, 1969).

More recently, isoelectric focusing on slab polyacrylamide gel followed by a specific immunofixation was applied to the study of the Gc polymorphism (Constans and Viau, 1977). This method possesses a resolving power superior to all others and offers the advantage of an immunospecificity.

Using this technique the presence of a microheterogeneity of Gc^1 has been demonstrated and two subtypes Gc^{1F} (F=fast) and Gc^{1S} (S=slow) were described

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(Constans and Viau, 1977). Using an immunological method, Ruoslahti (1965) reported the possible existence of such a heterogeneity. He proposed the theory of two codominant alleles: Gc^{1a} (gene frequency 0.096) and $Gc^{1\ non\ a}$ (gene frequency 0.675). The data we gathered in a Caucasoid population enabled us to notice that the Gc^{1F} and Gc^{1S} alleles showed similar frequencies. Unfortunately however, it has not been possible to carry out camparison between the electrophoretic mobilities of the phenotypes described by Ruoslahti.

In this work, we describe the results we obtained in the study of the Gc polymorphism in a sample of Fula population of Senegal, West Central Africa, using isoelectric focusing in slab polyacrylamide gel followed by immunofixation.

MATERIAL AND METHODS

To determine the Gc polymorphism each serum sample was studied simultaneously by two techniques.

The first one is the polyacrylamide disc electrophoresis according to Kitchin (1965). This method makes it possible to classify the samples according to the three phenotypes: Gc 1–1, Gc 2–1 and Gc 2–2.

The second method used is an isoelectric focusing electrophoresis carried out on slab polyacrylamide gel containing a solution of LKB ampholine pH range 4–6 polymerized in presence of riboflavin (Karlsson *et al.*, 1973). After a four hour migration period an immunofixation is carried out on a cellulose acetate strip soaked with a dilution of a monospecific anti Gc serum (Behring) according to Viau *et al.* (to be published). The cellulose acetate strip is then removed from the gel, washed in saline solution and stained with a Coomassie Brilliant Blue solution (R 250).

This method enables us to separate the two Gc^1 subtypes (Gc^{1S} and Gc^{1F}) and also new variants in 357 sera collected in a group of Peuhl (Fula) living in Senegal. The transmissions of the two subtypes and of the new variants are confirmed by family pedigrees.

RESULT AND DISCUSSION

The Gc^1 gene subtypes

The electrophoretic patterns obtained in this study (Fig. 1) confirmed the heterogeneity of the Gc^1 zone as we have described it formerly (Constans and Viau, 1977). Two Gc^1 subtypes are present. According to the electrophoretic mobilities Gc^{1F} is represented by two protein bands in anodal position while Gc^{1S} is characterized by two protein bands in a more cathodal position.

By measuring the pH at the gel surface under the conditions of the migration (12°C) the Gc^1 protein bands are located in a pH zone between 4.8 to 5.2. In this study the three phenotypes corresponding to the Gc^{1F} and Gc^{1S} subtypes are observed: Gc 1F-1F, Gc 1F-1S and Gc 1S-1S (Fig. 1).

The polyacrylamide gel disc electrophoresis cannot reach such a resolution.

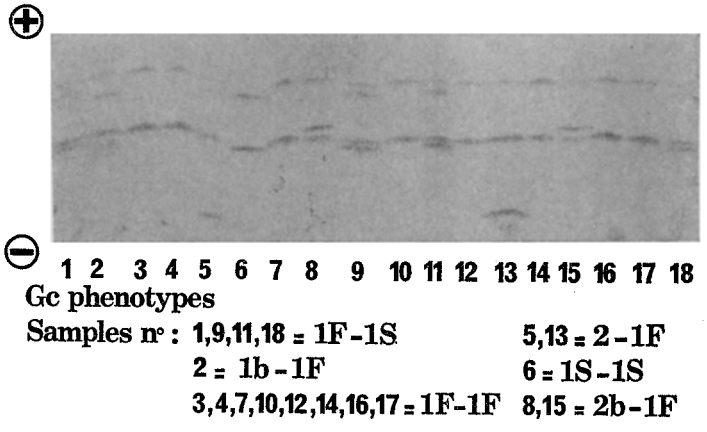

Fig. 1. Immunofixation pattern obtained after isoelectric focusing electrophoresis of different samples in this study. The Gc^{2b} variant (sample 8) is clearly distinct in this figure from the Gc^{1} bands. In the sample 2 a four band electrophoretic pattern is obtained with the presence of the Gc^{1b} variant.

The three subtype phenotypes represent the usual electrophoretic pattern of the Gc 1-1 phenotype.

Description of two new variants: Gc^{1b} and Gc^{2b}

In polyacrylamide gel disc electrophoresis the presence of these two variants is quite impossible to disclose. As it appears from Fig. 2 (A) only two large bands strongly stained (phenotype Gc 1b-1) or as disequilibrium in the intensity of the protein bands (phenotype Gc 2b-1) point out the possible existence of a variant at the Gc^{1} and Gc^{2} gene level. In this case the isoelectric focusing electrophoresis gives a four band pattern, two of them corresponding to the product of Gc^{1F} or Gc^{1S} subtypes whereas the other two bands are moving in a more cathodal position than the Gc^{1S} gene bands as it is possible to see in the Fig. 2 (B). Their presence is explained by the existence of a Gc^{1} new variant that we shall call Gc^{1b}. The family study (Fig. 3) corroborated the transmission of that variant as a codominant character.

Besides, we were able to notice that on polyacrylamide disc electrophoresis some sera possessed an electrophoretic pattern very similar to that of the Gc 1-1 phenotype, the only difference being the presence of a stronger intensity on the anodal band, as it appears in the Fig. 2 (A).

These samples studied by isoelectric focusing presented a three band pattern as in the case of the heterozygote Gc 2-1F or Gc 2-1S phenotypes. Besides the two bands corresponding to the Gc^{1F} or Gc^{1S} genes, another band can be seen. It is located between the bands of the Gc^{1F} gene, close to the cathodal one (Fig. 1, 2B). The mobility of this band is almost symmetrical to the one which characterizes the

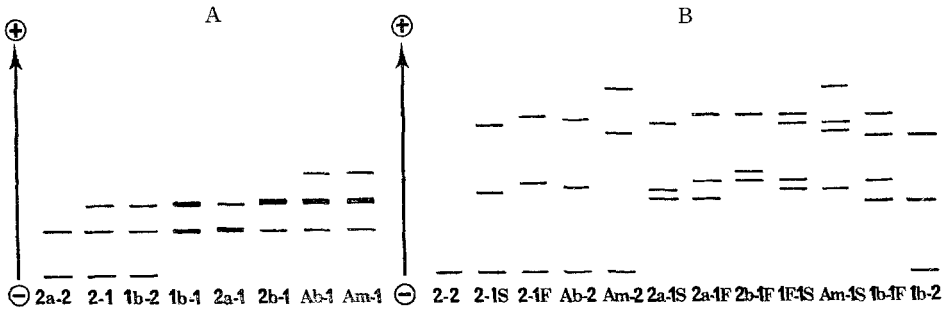

Fig. 2. Scheme of electrophoretic pattern obtained using polyacryamide gel disc electrophoresis (A) and isoelectric focusing electrophoresis (B) in the study of an African serum sample. The Gc^{Am} and Gc^{2a} described previously in an Amerindian and Pygmy sample are presented in comparison to the new variants Gc^{1b} and Gc^{2b} described.

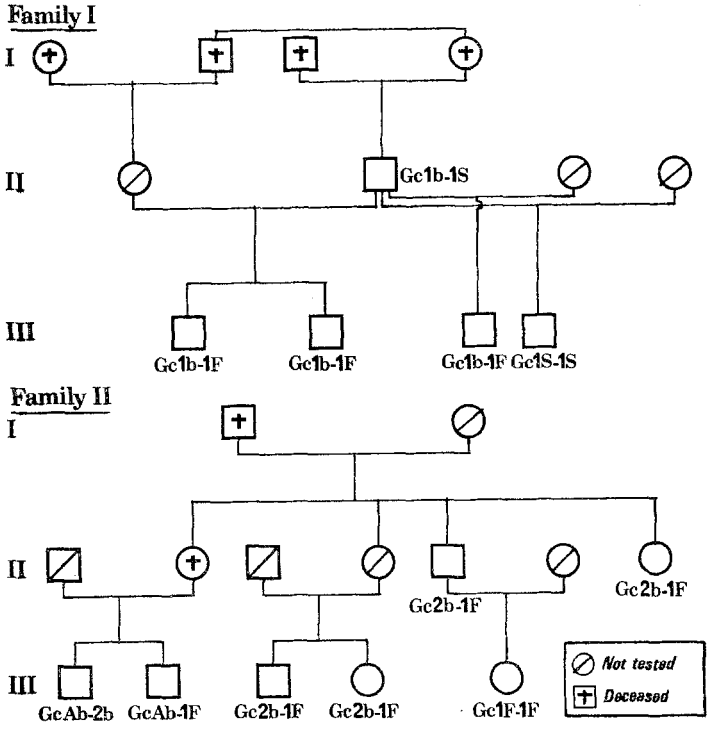

Fig. 3. Two pedigrees of families illustrating the transmission of the Gc^1 gene subtypes and the new variants Gc^{1b} (family I) and Gc^{2b} (family II).

Table 1. Phenotype and gene distribution obtained: 6 genes and variants are present and correspond to 21 expected phenotypes.

Gc phenotypes		1F-1F	1F-1S	1S-1S	Ab-1F	Ab-1S	Ab-Ab	2-Ab	2-1F	2-1S	2b-2	Ab-2b	2b-1S	2b-1F	2b-2b	2-2	1b-1F	1b-1S	Ab-1b	1b-1b	1b-2	1b-2b	
COUNTRY	Population																						Total
SENEGAL	Peuhls (Fula)																						
	obs.	227	48	9	14	3	0	0	29	9	0	2	2	7	0	0	5	2	0	0	0	0	357
	exp.	217,3	64	4,7	14,8	2,2	0,3	1	29,6	4,4	0,6	0,3	1,3	8,6	0,1	1	5,5	0,8	0,2	0	0,4	0,1	

GENE FREQUENCIES

Gc^{1F} = 0.780
Gc^{1S} = 0.115
Gc^{1b} = 0.010
Gc^{Ab} = 0.027
Gc^{2} = 0.053
Gc^{2b} = 0.015

Gc^{2a} variant that we described in a Pygmy population (Constans *et al.*, 1978). A study of family II (Fig. 3) allows us to ascertain the fact that this new variant of the Gc^{2} gene is transmitted in a simple Mendelian manner: we shall call it Gc^{2b}.

Phenotypes and allele distribution in the Peuhl-Fula population

In the course of the study, samples from 357 individuals were examined. For the first time 12 electrophoretic phenotypes were revealed in the group specific component polymorphism of one population. They correspond to the presence of two subtypes Gc^{1F} and Gc^{1S} and their association with the Gc^{1b} and Gc^{2b} variants we have described or again with the Gc^{Ab} and Gc^{2} genes.

The data gathered from the electrophoretic patterns and from the family studies allow us to consider the possible existence of 6 codominant alleles with 21 possible corresponding phenotypes (Table 1).

The presence of Gc 1F-1F individuals (over 60% in our sample) explains the high frequency of the Gc^{1F} gene (0.780). There is however an important deficiency of heterozygous Gc 1F-1S individuals (48 observed and 64 expected) together with a slight excess of Gc 1S-1S individuals. The discrepancy between the number of the observed electrophoretic phenotypes and the ones expected is also to be found between the number of heterozygous Gc 1b-1F, Gc 2b-1F and Gc Ab-1F individuals (26) and the number of heterozygous Gc 1b-1S, Gc 2b-1S and Gc Ab-1S (7). Considering the frequencies of Gc^{1F} and Gc^{1S} genes in the population the different variants Gc^{1b}, Gc^{2b} and Gc^{Ab} proportionately combine much more with the Gc^{1S} gene than with the Gc^{1F} gene. But the numbers observed in each phenotype are not large enough and the χ^2 (0.07) obtained in the comparison of the variants distribution in relationship with the presence of the Gc^{1F} or Gc^{1S} gene makes the difference non

Table 2. The results obtained using the isoelectric focusing method in the study of the Gc polymorphism in four different populations.

Country	Population	Gc^{1F}	Gc^{1S}	Gc^{Ab}	Gc^{Am}	Gc^{1b}	Gc^{2}	Gc^{2a}	Gc^{2b}	χ^2	Total	*
Senegal	Peuhls (Fula)	0.780	0.115	0.027	—	0.010	0.053	—	0.015	4.81	357	(1)
Central Africa Empire	Pygmees	0.584	0.191	0.054	—	—	0.064	0.107	—	2.35	267	(2)
Bolivia	Amerindian	0.231	0.636	—	0.009	—	0.122	—	—	1.05	253	(2)
France	Pyrenees (vallée de l'Ouzom)	0.077	0.512	—	—	—	0.410	—	—	0.66	290	(2)

* : (1) This study. (2) Constans *et al.*, 1978.

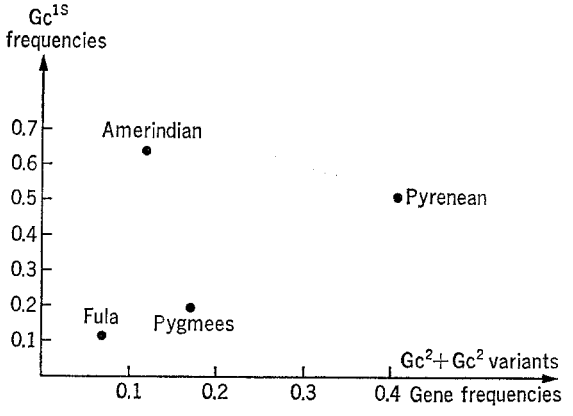

Fig. 4. The repartition of the four population samples according to the Gc^{1s} and Gc^{2} gene frequencies revealed their anthropological and geographical differences.

significant. More data are needed. These results may reflect one important feature of the sample studied:

1) The blood samples came from related individuals.

2) There exists an important heterogeneity within the population and its effects are emphasized when related subjects are studied.

The higher frequency of the Gc^{1F} gene in relation to the one of the Gc^{1S} gene leads to a result similar to the one we had already noticed in the Pygmy population (Constans *et al.*, 1978) and would make up a characteristic of the African populations (Table 2). The same remark goes for Fig. 4 in which the Fula group comes very significantly near the Pygmy group. These cases appear to give further confirmation to the relation in the various human population between the Gc^{1S} gene frequency on one hand and of the Gc^{2} gene or its variants on the other.

Walter and Steegmuller (1969) had already observed a similar phenomenon

when considering the Gc^1 and Gc^2 gene variations in populations.

What is particular in the Peuhl population sample we studied is the presence of the two new variants: Gc^{1b} and Gc^{2b} with frequencies close to one another being respectively 1 and 1.5%. Their presence in this group together with the absence of the Gc^{2a} variant we observed in the Pygmy population is an example of the new anthropological interest of the study of the group specific component polymorphism. The frequency of the Gc^2 gene in our sample is very much the same as the one found in the literature dealing with populations living in the same region (Mourant *et al.*, 1976). Together with it, the presence of the Gc^{Ab} variant in the Fula group shows that the latter were in contact with populations coming from the south, among which the Gc^{Ab} gene seems to be more frequent.

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