Jap. J. Human Genet. **23**, 139–143, 1978

RED CELL GLYOXALASE I POLYMORPHISM IN JAPANESE: CONFIRMATION OF A LOW *GLO*^{1} FREQUENCY[1]

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Summary Haemolysate samples from 414 unrelated Japanese living in Tokyo were examined for GLO phenotypes by starch gel electrophoresis using a tris-citrate-borate-LiOH buffer system, pH 7.4. Three phenotypes were found which were classified as GLO 1 (2; 0.48%) GLO 2-1 (40; 9.66%) and GLO 2 (372; 89.86%). The corresponding gene frequency for *GLO*^{1} was 0.0531 and that for *GLO*^{2} was 0.9469. It was confirmed that the *GLO*^{1} frequency of Japanese is much lower than the values previously reported for Caucasians and Africans.

INTRODUCTION

Red cell glyoxalase I [EC 4.4.1.5] has recently been found to show a genetic polymorphism (Kömpf *et al.*, 1975). Three common phenotypes, designated GLO 1, GLO 2-1, GLO 2, are under the genetic control of two autosomal, codominant alleles, *GLO*^{1} and *GLO*^{2}. The gene frequencies in a population sample from South-Western Germany were 0.427 for *GLO*^{1} and 0.573 for *GLO*^{2} (Kömpf and Bissbort, 1975).

Kömpf and his colleagues determined the GLO phenotypes by starch gel electrophoresis using a 0.2 M Tris-histidine buffer system (pH 7.8) at 7.5 V/cm for 14 hours .Since this buffer system was relatively expensive and our preliminary results of GLO typing using this buffer system seemed not satisfactory, attempts were made to find another buffer and electrophoretic condition suitable for the GLO typing. This paper reports the result of GLO typing of 414 unrelated Japanese (Tokyo) using a tris-citrate-borate-LiOH buffer system at pH 7.4, which proved to be satisfactory.

Received February 6, 1978

[1] This study was partially aided by the grant of the Ministry of Education, and the grant of Japan Society for the Promotion of Science.

MATERIALS AND METHODS

Blood specimens from 414 unrelated Japanese living in Tokyo were kindly supplied by the Division of Biochemistry, Toranomon Hospital, Tokyo. Furthermore, samples from 20 pairs of monozygotic twins (9 male and 11 female pairs) and their parents were examined. Red cells were washed three times in 0.9% NaCl, centrifuging for five minutes at 3,000 rpm. Optimal results were obtained when hemolysates were prepared from red cells frozen with an equal volume of 3% glycerol saline solution. Horizontal starch gel electrophoresis using 11.5% Connaught Starch was carried out at 12.5 V/cm for 2.5 hours using metal cooling plates in which water at 4°C was circulating. The buffer system used (Harada and Misawa, 1976) was as follows:

Bridge buffer: 0.1 M Tris, 0.034 M citric acid, 0.039 M boric acid, 0.025 M LiOH, pH 7.4; gel buffer: a 1 : 10 dilution of the bridge buffer.

Sample insertion was made using the 5×7 mm piece of filter paper, Toyo No. 1.

Staining of GLO isozymes was performed at the initial stage of this study by the method of Kömpf *et al.* (1975) with slight modifications. Later, however, the method of Parr *et al.* (1977) was preferred and adopted in this study in the following manner.

1st step. The reaction mixture containing 0.25 ml of 40% methylglyoxal and 20 mg reduced glutathione (Sigma) in 5 ml 0.2 M phosphate buffer, pH 6.8, was applied onto the cut surface of the gel as filter paper overlay. Incubation time at 37°C was 30 min.

2nd step. After the filter paper was removed, the gel was blotted free from excess reagent. The gel was then soaked in the developing mixture containing 20 mg iodine and 40 mg KI in 50 ml distilled water. After a minute, developing mixture was removed and the gel was washed by distilled water. The gel was left at room temperature for 5–10 min. The GLO isozyme appeared as the blue bands against colorless background.

RESULTS AND DISCUSSION

Three GLO phenotypes corresponding to those described by Kömpf and his colleagues (1975) were clearly demonstrable in the present material of Japanese (Fig. 1) using the buffer system mentioned above. The most common type was the fast migrating type (GLO 2), while the slow migrating type (GLO 1) was uncommon and encountered only twice in 414 (0.48%). The apparent heterozygote (GLO 2-1) had a triple banded pattern suggesting the dimeric subunit structure of GLO-I in agreement with the original description (Kömpf *et al.*, 1975).

The results of the GLO typing in the present Japanese population are given

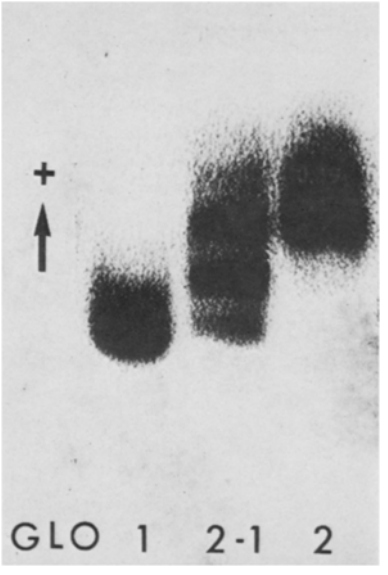

Fig. 1. Photograph of a starch gel showing three GLO phenotypes.

Table 1. Phenotype and gene frequency of GLO-I.

Total		GLO phenotypes			Gene frequencies
		1	2-1	2	
414	Obs. No.	2	40	372	$GLO^1=0.0531$
	%	0.48	9.66	89.86	$GLO^2=0.9469$
	Exp. No.	1.17	41.63	371.17	S.E.=0.0078

$\chi^2_{(1)}=0.7336$ $0.3< <0.5$

in Table 1. The allele frequencies estimated for GLO^1 and GLO^2 were 0.0531 and 0.9469, respectively. The observed numbers of phenotypes were in good agreement with the number expected on the basis of Hardy-Weinberg's law. No unusual phenotype was observed.

The gene frequency for GLO of Japanese has recently been reported: Harada and Misawa (1976) found a GLO^1 frequency of 0.0883 in a sample from blood donors in Tokyo (N=572), while Kuwata and Ishimoto (1976) reported a slightly lower frequency, 0.079, in a sample of Mie Prefecture (N=346), Western Central Japan. A similar GLO^1 frequency was reported in a sample from Yokohama (Yoshida *et al.*, 1977). The present study, therefore, not only confirms the low GLO^1 frequency in Japanese population, but also indicates a frequency of GLO^1 which is even lower than the values previously reported in Japan. The difference in allele frequency is statistically significant, the $\chi_{(1)}^2$ value being 4.07 ($P<0.05$) for the comparison between the present data and that of Kuwata and Ishimoto (1976).

Table 2. Gene frequency of GLO^1 reported in various populations.

Population	Number tested	GLO^1 frequency	Reference
Dutch (Leiden)	757	0.454	Meera Khan *et al.* (1976)
Norwegians	216	0.442	Olaisen *et al.* (1976)
English	296	0.44	Parr *et al.* (1977)
Southwestern German	655	0.427	Kömpf & Bissbort (1975)
Southern German	1,025	0.424	Berg *et al.* (1977)
American Whites	101	0.42	Weitkamp & Guttormsen (1975)
Lapps	184	0.304	Olaisen *et al.* (1976)
American Blacks	107	0.28	Weitkamp & Guttormsen (1975)
Jali (Ganbia)	506	0.28	Parr *et al.* (1977)
South African Negroid	843	0.259	Bender *et al.* (1977)
Philippine Negrito	128	0.244	Omoto *et al.* (1978)
Japanese (Tokyo)	572	0.088	Harada & Misawa (1976)
Japanese (Mie)	346	0.079	Kuwata & Ishimoto (1976)
Japanese (Yokohama)	493	0.068	Yoshida *et al.* (1977)
Japanese (Tokyo)	414	0.053	This study

The gene frequency for GLO^1 reported thus far ranges 0.30–0.45 among European populations while it is slightly lower, 0.26–0.28, in African populations (Table 2). A relatively high GLO^1 value (0.244) was recently observed in a Negrito sample of the Philippines (Omoto *et al.*, 1978). Compared with these values it is apparent that the Japanese values are much lower. It is probable that the GLO^1 frequency is relatively low in Mongoloid populations.

Among twin samples only those from one pair of male and two pairs of female monozygotic twins showed GLO 2-1 type, while the others were GLO 2 type. The finding together with the GLO types of the parents of the twins with the type 2-1 was consistent with the hypothesis that the GLO phenotypes were controlled by the autosomal, codominant alleles.

The buffer system and the electrophoretic condition used in the present study are quite useful. The buffer is relatively inexpensive compared with the original 0.2 M Tris-histidine buffer, and it enables a quick separation of GLO isozymes. Also, it is adequate to use the other half of the sliced gel for a variety of enzyme typing, for example, ESD, PGD and ADA.

Acknowledgements The authors are deeply indebted to Dr. Motoshi Kitamura, Toranomon Hospital, Tokyo, for providing them with blood samples. They are also grateful to Prof. Eiji Inouye, the University of Tokyo, and the members of the Committee for the Twins in the Junior High School Attached to The University of Tokyo, for the blood samples and the diagnosity data of the twins.

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