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GENETIC POLYMORPHISM OF WHITE BLOOD CELL GLUCOSE DEHYDROGENASE IN JAPANESE

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Summary Genetic polymorphism of the glucose dehydrogenase in white cells extracts from random adult Japanese was investigated using polyacrylamide gel isoelectric focusing or agarose gel isoelectric focusing, followed by a specific zymogram technique. Three common phenotypes, which might correspond to GDH 1, GDH 2 and GDH 2-1 reported by King and Cook (1981), were observed at the p*I*s between pH 6.56–6.76 on the gel. No phenotypes with GDH 3 component were detected so far. The allele frequency of GDH^3 may be very low among Japanese. The results of family study suggest that these phenotypes are inherited in the autosomal codominant trait. The allele frequencies were $GDH^1=0.522$ and $GDH^2=0.478$.

INTRODUCTION

Glucose dehydrogenase [GDH, EC 1.1.1.47], a microsomal enzyme, is identical with hexose-6-phosphate dehydrogenase (H6PD). It oxidize glucose, glucose-6-phosphate, galactose-6-phosphate and 2-deoxyglucose-6-phosphate utilizing as a coenzyme either nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP). In mammalian tissues GDH is distinguishable from glucose-6-phosphate dehydrogenase [G6PD, EC 1.1.1.49] in several properties as subcellular localization, genetic trait, molecular weight, electrophoretic mobility, substrate specificity and immunochemical precipitation or inactivation (Metzger *et al.*, 1964, 1965; Shaw, 1966; Ohno *et al.*, 1966; Beutler and Morrison, 1967; Kimura and Yamashita, 1972; Srivastava *et al.*, 1972; Blume *et al.*, 1975).

King and Cook (1981) have demonstrated genetic polymorphism of GDH using various tissue extract samples in a population of unrelated White British people. Six phenotypes determined by three alleles (GDH^1, GDH^2 and GDH^3) at an autosomal locus were detected by a polyacrylamide gel isoelectric focusing (PAGIEF) followed by a zymogram technique for the developing the enzyme activity on the gel. They have suggested that GDH is also polymorphic in Indians, Chinese, Black Africans, Finns, Greeks and Burmese. Recently Abe *et al.* (1982)

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examined post-morten liver extract samples in Japanese, and described that no phenotypes with GDH 3 component were observed. In order to make it easy to investigate this polymorphism on a large scale in various population groups, an utilization of samples prepared from white blood cells seems to be necessary and useful. We reported here GDH polymorphism in the Japanese population using white blood cells extracts as the enzyme sources. Both methods of PAGIEF and agarose gel isoelectric focusing (AGIEF) were used in this study.

MATERIALS AND METHODS

Sample preparation

Whole blood samples from 296 unrelated healthy Japanese people and 13 matings with 21 children were collected using 5 mM Na_3-EDTA or 13 mM trisodium citrate as anticoagulants. The buffy coat from 20 ml of whole blood was collected and transferred into a 10 ml plastic test tube. This sample contained a small amount of red blood cells. To remove this red blood cells, 3 ml of 0.2% NaCl was added to the tube and mixed well. After 30 sec, 3 ml of 1.6% NaCl was added to the mixture, followed by a centrifugation. These procedures were repeated four times. One hundred μl of cold distilled water (or 0.02 M phosphate buffer containing 1% Triton X-100, pH 7.5) was added to a final pellet of white blood cells, placing the tube at -80°C, rapidly freezing and thawing three times, and centrifuging. The supernatant was used as the enzyme source.

Isoelectric focusing

Twenty-five μl of white blood cells extract was applied onto the gel surface with a sample applicator of paper (5×10 mm). The isoelectric points in the gel were determined using the Isoelectric Focusing Calibration Kit pH 5–10.5 (Pharmacia). Isoelectric focusings were performed using two different gel plates and conditions. (1) Polyacrylamide gel plate was prepared according to Hobart (1979) but 1 ml of Ampholine pH 3.5–10 (LKB), 0.3 ml of Ampholine pH 5–8 and 0.3 ml of Ampholine pH 7–9 were used. Isoelectric focusing was carried out for 3.5 hr at a constant power of 10 W (1,200 V maximum) with a coolant temperature of 4°C. Sample applicators were removed after 90 min. Electrode solutions were 1 M H_3PO_4 (anode) and 1 M NaOH (cathode). (2) Agarose gel plate ($123 \times 136 \times 1$ mm) consisted of 0.18 g of Agarose IEF (Pharmacia), 2.16 g of D-sorbitol, 1.2 ml of Pharmalyte pH 5–8 (Pharmacia) and 16.2 ml of distilled water. Isoelectric focusing was carried out for 2 h at a constant power of 7.5 W (1,500 V maximum) at 8°C. Sample applicators were removed after 1 hr. Electrode solutions were 0.05 M H_2SO_4 (anode) and 1 M NaOH (cathode).

Zymogram technique

Each of GDH or G6PD activities on the gel was developed according to Harris and Hopkinson (1976) with minor modifications.

Neuraminidase treatment

One U of neuraminidase, lyophilized powder purified from *Arthrobactor ureafaciens* (Nakarai Chemicals Ltd., Japan) was added to 100 μl of extract sample, followed by an incubation at 8°C overnight for 17–18 hr.

RESULTS AND DISCUSSION

In the case of using white blood cells extracts from healthy individuals, three GDH phenotypes were observed by AGIEF (Fig. 1a). The isoelectric points (p*I*s) of a basic, an acidic and an intermediate types were calibrated to be respectively pH 6.66–6.76, pH 6.56–6.66 and pH 6.56–6.76. Each of the basic and the acidic types consisted of 1 major and 2 minor isozyme bands. The intermediate type had 4 or 5 bands. The difference of p*I*s of among the isoprotein bands in each types was calibrated to be 0.03–0.04 pH unit. The same samples were also subjected to PAGIEF and the similar banding profiles of GDH activity were observed (Fig. 1b). In the sample extraction, the addition of Triton X-100 to the white cells pellet did not affect isozyme pattern and it gave slightly higher intensity of isozyme profile in a comparison to the addition of distilled water. Samples, which were stored for a long term and repeatedly used, caused the original patterns to smear and developed strong anodal repeats. The difference of p*I*s between the original pattern

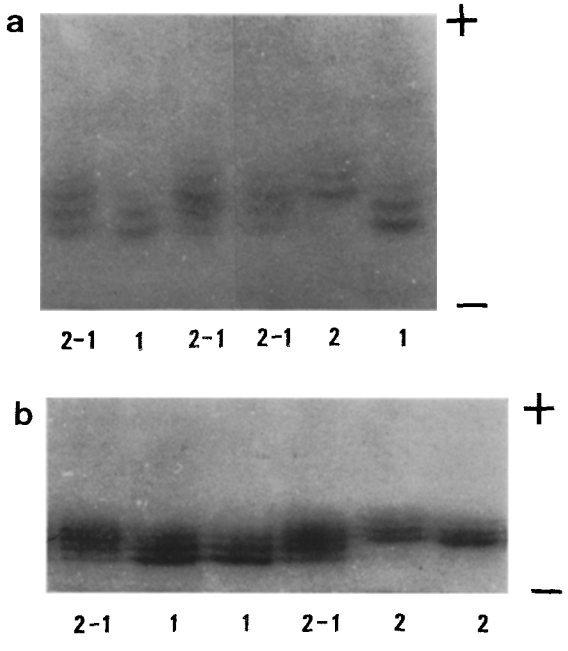

Fig. 1. Photographs of three GDH phenotypes run by AGIEF (a) and PAGIEF (b).

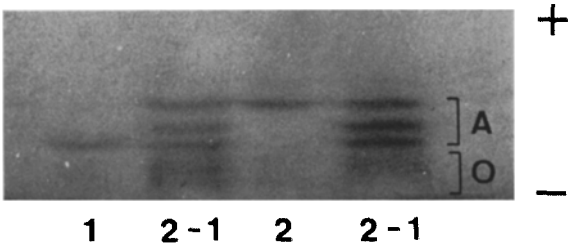

Fig. 2. Photograph showing the anodal repeats (A) and the original patterns (O) of three GDH phenotypes run by PAGIEF.

and the anodal repeat was 0.3–0.4 pH unit in each type. In the patterns of anodal repeats, each of the basic and the acidic types consisted of a single major isozyme bands and the intermediate type had three bands. These pattern seemed to be characteristic of a dimeric protein with two alleles (Fig. 2). Preliminarily, red blood cells hemolysates were used as an enzyme source. No GDH isozyme was detected on the gel by AGIEF or PAGIEF. After AGIEF or PAGIEF, the technique of G6PD staining was also applied to the gel (not shown). The staining profiles suggested that red cells hemolysate showed only G6PD and white cells extract had both GDH and G6PD, and that p*I*s of G6PD was lower than those of GDH. These results were coincident with the previous studies (Shaw, 1966; Ohno *et al.*, 1966). Therefore, these GDH patterns were not contaminated by G6PD.

In the previous investigation (King and Cook, 1981), using liver or placental extracts, GDH 1, GDH 2 and GDH 3 were homozygotes and each of which was a single major isozyme. GDH 2-1, GDH 3-1 and GDH 3-2 each had three isozyme bands in heterozygous form. GDH 1+1− consisted of 2 bands was observed in white cells or placental extracts, and it was not a constant characteristic of placental samples since different extracts from the same placenta could be typed as GDH 1 or GDH 1+1− (King and Cook, 1981). On the other hand, in the present study, each of the basic and acidic types which seemed to be homozygote by the family study had a microheterogeneous form. The microheterogeneous forms seemed to be constant characteristics. For, samples repeatedly obtained from a same individual in the different days always gave an identical isozyme pattern in each types. The microheterogeneity might exist only in white cells extract. As these microheterogeneity did not reduce or change even after the treatment with neuraminidase, these were not due to unequal contents of sialic acid in isoproteins (not shown).

If symbols of the alleles or phenotypes designated by King and Cook (1981) were used, the basic, the acidic and the intermediate types might correspond to GDH 1, GDH 2 and GDH 2-1, respectively. A family study was carried out to ascertain genetic basis (Table 1). The results were not contradictory to the hypothesis that these phenotypes were inherited in the autosomal codominant trait. The

Table 1. Distribution of GDH phenotypes in 13 matings with 21 children.

Parental types	No. of matings	No. of children types					
		1		2-1		2	
		♂	♀	♂	♀	♂	♀
1×1	1	1	1	0	0	0	0
1×2-1	1	0	0	1	0	0	0
2-1×1	2	1	0	1	0	0	0
2-1×2-1	4	1	0	3	3	0	0
2-1×2	1	0	0	0	0	1	0
2×2-1	3	0	0	2	2	2	0
2×2	1	0	0	0	0	2	0

Table 2. Distribution of GDH phenotypes and allele frequencies in the Japanese population.

	Phenotypes			Total
	1	2-1	2	
Observed No.	88	160	74	322
%	27.3	49.7	23.0	100.0
Expected No.	87.7	160.7	73.6	322.0

Allele frequencies. GDH^1=0.522, GDH^2=0.478. χ^2=0.006, d.f.=1, $0.90<p<0.95$.

Table 3. Allele frequencies of GDH in various population.

Population	Samples	n	Common	Second common[a]	Third common[c]	Authors
White British	Li[d], Pl[e], WBC[f]	373	0.723	0.194	0.083	King and Cook (1981)
Caucasians	Sa[g]	190	0.755	0.245	—	Tan and Ashton (1976)
Chinese	Sa	34	0.706	0.294	—	Tan and Ashton (1976)
Japanese	Sa	104	0.659	0.341	—	Tan and Ashton (1976)
Japanese	Li	125	0.520	0.480	—	Abe *et al.* (1982)
Japanese	WBC	322	0.522	0.478	—	Present study

[a] GDH^1 or Sgd^1. [b] GDH^2 or Sgd^2. [c] GDH^3. [d] Li=livers. [e] Pl=placentas. [f] WBC=white blood cells. [g] Sa=saliva.

distribution of GDH phenotypes and allele frequencies in the Japanese population were given in Table 2. The population materials consisted of 296 unrelated healthy Japanese people and parents (N=26) from family materials. The observed number fit to those expected on Hardy-Weinberg equilibrium. Allele frequencies of GDH^1 and GDH^2 were estimated at 0.522 and 0.478, respectively. These values of allele

frequencies are nearly equal to another report on the Japanese population in Tokyo Metropolis, using post-morten liver extracts (Abe *et al.*, 1982). In the present study, only two alleles were detected and no phenotypes with GDH 3 component was observed so far. These results are also coincident with the another report on Japanese (Abe *et al.*, 1982). The allele frequency of GDH^3 might be very low among Japanese people.

The allele frequency of GDH^1 in the Japanese population was lower than that in the White British (King and Cook, 1981) shown in Table 3. Tan and Ashton (1976) reported an autosomal H6PD (GDH) polymorphism in human saliva by using polyacrylamide gel electrophoresis. In which, the allele frequencies among Japanese people in Hawaii were different from those in the present study. These differences may be due to racial or regional, methodological differences.

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