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G6PD Ube, A Glucose-6-Phosphate Dehydrogenase Variant Found in Four Unrelated Japanese Families

KOJI NAKASHIMA,[1] JUNICHIRO ONO,[1] SHIGENOBU ABE,[1] SHIRO MIWA,[1] AND AKIRA YOSHIDA[2]

Over 100 genetically determined variants of glucose-6-phosphate dehydrogenase (G6PD; E.C.1.1.1.49) have been described [1, 2]. Some of these variants are not associated with hematological disorders; their enzyme activities vary from high or normal to mild or moderately low. Another group has enzyme deficiency with hemolysis induced by agents such as drugs, infections, or fava beans. A third group has severe deficiency, abnormal enzyme kinetics, or marked enzyme instability, causing chronic hemolytic anemia even in the absence of exogenous agents.

The frequency of these variants among various populations differs according to race and geography [3]. An example is the high frequency of G6PD deficient variants among Negro and Mediterranean populations which has been explained by selection related to the presence of malaria [4, 5]. Among Mongoloid populations generalizations cannot simply be made along racial lines: only sporadic cases [7–11] of this enzyme deficiency have been reported in Japan [3, 6], while in southern China, G6PD deficiency is common [3, 12].

A systematic survey for G6PD deficiency in Japan would be useful, therefore, not only for hematological and genetical reasons but also in an effort to trace the racial origin of Japanese who have lived in a relatively isolated island country where malaria is not endemic. In Yamaguchi at the southwestern end of Honshu (the main island of Japan), 6,120 Japanese males were screened for G6PD deficiency. This paper presents a new G6PD variant which was found in four unrelated Japanese families.

MATERIALS AND METHODS

The screening for G6PD deficiency in males was performed with Beutler's fluorescent method [13] on 6,120 blood samples submitted to the Central Laboratory of Yamaguchi University Hospital and on samples donated to the Yamaguchi Red Cross Transfusion Service.

To characterize the G6PD variant, blood from the propositi, their family members, and normal

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[1] Third Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan.

[2] Department of Biochemical Genetics, City of Hope National Medical Center, Duarte, California 91010.

controls was collected in ACD solution and stored at 4°C until tested (within 5 days after harvesting). Partially purified G6PD, free of hemoglobin and 6-phosphogluconate dehydrogenase activity, was characterized by methods recommended by the WHO Scientific Group [3]. Starch gel electrophoresis used a phosphate buffer system at pH 7.0 [14], a Tris-EDTA-borate buffer system at pH 8.6 [3], and a Tris-hydrochloride buffer system at pH 8.8 [3]. Migration took 4 hr in a field of 10 V/cm with cooling. The pH optima were determined in a Tris-glycine-phosphate buffer system adjusted with HCl or NaOH [15]. The inhibition constant (Ki) for the NADPH with respect to NADP was measured as suggested by Yoshida [16], in 0.05 M Tris-hydrochloride buffer, pH 7.3, containing 0.1 KCl and 5 mM $MgCl_2$ and at 37°C.

Blood from case 1 and a normal control was sent to the City of Hope National Medical Center, Duarte, California, to compare this G6PD variant directly with G6PD A^- which is stored there as a reference standard.

All substrates, substrate analogues, and nucleotides were purchased from Boehringer-Mannheim (New York, N.Y.), except for 2-deoxy glucose-6-phosphate (2-deoxy G6P) and deamino-NADP, which were from Sigma (St. Louis, Mo.). Starch was from Connaught (Willowdale, Canada) and DEAE cellulose came from Pharmacia (Piscataway, N.J.). All other reagents were of analytical grade. The enzyme reactions were read with a Gilford 2400-S recording spectrophotometer.

CASE REPORTS

Cases 1, 3, and 4 are Japanese from Yamaguchi, located at the southwestern end of Honshu, the main island of Japan; case 2 is from Ehime on the Shikoku island.

Case 1

Case 1, a 56-year-old male, was hospitalized for leg edema. Diabetes mellitus and nephrotic syndrome were diagnosed. A blood sample, taken for a routine blood count, was found by the screening test to be G6PD deficient. He has no history of anemia, jaundice, or colored urine. Hemoglobin (Hb) was 14.3 g/100 ml; hematocrit, 42%; red blood cell count (RBC), $4.80 \times 10^6/mm^3$; reticulocyte count, 1.2%; and total serum bilirubin, 0.6 mg/100 ml.

Case 2

Case 2 is a 26-year-old male, physician. Case 2 sent a blood sample from a patient with nonspherocytic hemolytic anemia and a sample of his own blood as a normal control to our laboratory for evaluation of red cell enzyme activity. His red cells showed a low G6PD activity, while the patient's red cell enzymes were normal. He has never had anemia, jaundice, or colored urine. Hb was 16.7 g/100 ml; hematocrit, 50.4%; RBC, $5.56 \times 10^6/mm^3$; reticulocyte count 0.6%; and serum bilirubin, 1.5 mg/100 ml with 1.0 mg/ml indirect reading.

Case 3

This 57-year-old healthy male has been an occasional blood donor and has never had jaundice or anemia. One of his daughters who donated blood to the Red Cross Blood Transfusion Service was found to be G6PD deficient by the screening test. Her family was immediately evaluated. Her father (case 3) and her nephew were diagnosed as hemizygotes and she and her three sisters as heterozygotes. Case 3's blood showed: Hb, 15.2 g/100 ml; hematocrit, 46%, RBC, $5.15 \times 10^6/mm^3$; reticulocyte count, 0.8%; and serum bilirubin 0.8 mg/100 ml. The pedigree is in figure 1.

Case 4

This 34-year-old healthy male has never had anemia, jaundice, or colored urine. His blood, donated to the Red Cross Blood Transfusion Service, was found by the screening test to be G6PD deficient. His Hb was 13.5 g/100 ml; hematocrit, 39%; RBC, $3.90 \times 10^6/mm^3$; reticulocyte count, 2.7%; and serum bilirubin, 0.4 mg/100 ml with 0.2 mg/100 ml indirect reading.

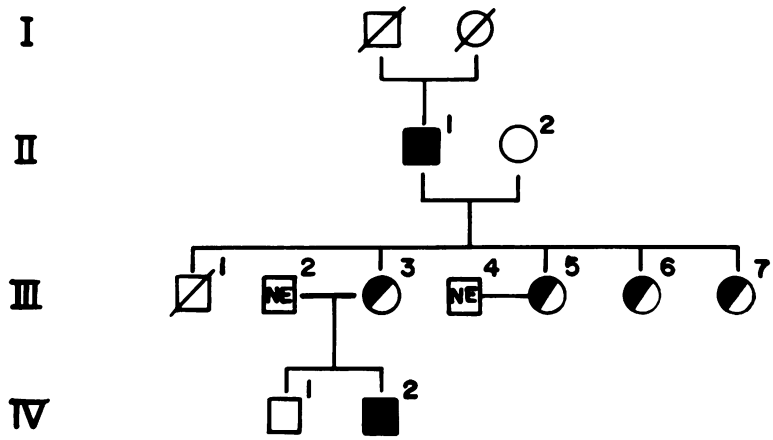

FIG. 1.—Distribution of G6PD variant in members of family T. Symbols: □ = normal male; ■ = hemizygous male; ○ = normal female; ◐ = heterozygous female; ∅ = dead; NE = not examined.

RESULTS

The G6PD characterization of four hemizygotes are given in table 1. The Km G6P, Km NADP, utilization of substrate analogues, and thermal stability were normal. The inhibition constants (Ki) by NADPH with respect to NADP were increased. The G6PD activities were moderately low. Electrophoretic mobility was fast in all propositi; the electrophoresis of kindred T is presented in figure 2. The hemizygous father (II-1) and nephew (IV-2) showed a fast G6PD variant. The heterozygous sisters (III-5 and III-6) have a broad band which is a combination of a fast variant band and a normal band; the heterozygous proposita (III-7) has a fast band with a very faint normal band. These findings are consistent with the theory of Lyon [17] for X-linked inheritance.

In the simultaneous comparison of case 1 and G6PD A^-, the electrophoretic mobilities were identical, and the activity of case 1 was higher (43% of a normal control) than that of G6PD A^- (20% of a normal control). The inhibition constants for NADPH showed a normal control, 18 μM; G6PD A^-, 24 μM; and case 1, 48 μM; other parameters were within normal ranges.

DISCUSSION

All hemizygotes presented here have moderately decreased G6PD activity, fast moving G6PD on electrophoresis, normal kinetics, stability, and pH optima, and utilization of substrate analogues, indicating that they all have the same G6PD variant. Several types of G6PD variants which have mild to moderate G6PD deficiency and fast moving G6PD on electrophoresis are classified in class 3 [1, 2]. The most common variant in this class and that which most resembles the variant described here is the Negro variant, G6PD A^-. This can be distinguished from the present variant by differences in G6PD activity and Ki NAPH. Also, the geographic areas and racial groups in which the present variant and G6PD A^- are found are quite different. For this reason, comparison with variants found in Orientals would be interesting. The present variant resembles G6PD Taipei-Hakka, but differs from it in G6PD activity, Km G6P, and heat instability.

TABLE 1

ENZYMATIC CHARACTERISTICS OF G6PD UBE

SUBJECTS	RBC ENZYME ACTIVITY (%)	ELECTROPHORESIS (%)	KM G6P (μM)	KM NADP (μM)	UTILIZATION TEST			HEAT STABILITY (% AT 20 MIN)	pH OPTIMA	KI NADPH* (μM)
					2dG6P (% of G6P)	Gal-6P (% of G6P)	Deamino-NAPD (% of NADP)			
Normal B†	100.0	100	31–71	2.6–6.6	1.6–6.4	4.3–12.7	51–69	66–99	normal	17–31
Case 1	45.2	107 (Tris) 107 (TEB) 115 (ph)	55	5.0	2.5	10.8	61	90	normal	53
Case 2	32.9	109 (Tris) 107 (TEB) 112 (ph)	50	5.5	2.5	10.4	55	90	normal	57
Case 3	33.3	107 (Tris) 107 (TEB) 112 (pH)	50	5.0	2.5	8.8	57	85	normal	48
Case 4	35.0	108 (Tris) 108 (TEB) 112 (ph)	55	6.0	3.0	8.2	53	94	normal	37

* Ki NADPH: obtained at 37°C, pH 7.3. Under the same condition, Km NADP values of the normal and variants are as follows; normal B, 12–22 μM; case 1, 17 μM; case 2, 20 μM; case 3, 22 μM; and case 4, 13μM.

† Normal B: mean ± SD, n = 10, medical and laboratory staff (male) proven to be normal.

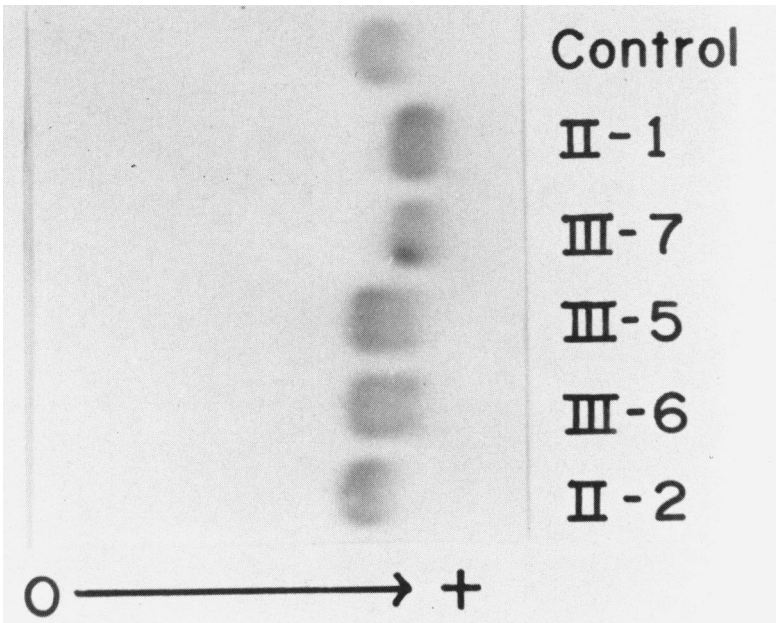

FIG. 2. — G6PD electrophoresis of members of family T in starch gel with phosphate buffer, pH 7.0: current 10 V/cm for 4 hr at 4°C. Subject number is the same as in figure 1.

G6PD Canton has lower activity, lower Km G6P, and thermal instability; other Oriental variants, as well as non-Oriental variants, can be easily distinguished from it by these biochemical parameters. The present variant was thus identified to be a new variant and has been designated G6PD Ube for the place of discovery.

Only systematic study will determine the frequency of G6PD deficiency. In the survey reported here, 5 deficient cases were discovered in 6,120 males. Cases 1 and 4 are presented here, but cases 2 and 3 were eliminated from the calculation of the frequency because they were not found by the screening test, and the other three cases (unpublished) are different from G6PD Ube. The frequency is less than 0.1%, which is quite different from that found in southern Chinese (5.5%) [12], in the Taiwan-Hakka population (5.5%) [18], and in the Philippines (6.0%) [19]. G6PD Ube does not lead to drug-induced hemolysis in spite of the deficiency; the increased Ki NADPH (i.e., the decreased inhibition of the enzyme by NADPH) favors the catalytic activity of this variant in the intra-erythrocytic condition [16]. This advantage, along with the fact that it is only a mild deficiency, probably explains the absence of hemolytic manifestations. Nonpathological variant G6PD Ube may be inherited simply without any negative selection by hemolytic disorders or any positive selective pressure of resistance to malaria, in relatively isolated islands where malaria is not endemic. This hypothesis is supported by the differences in the frequency of the G6PD variants in southern Chinese and Japanese. These differences, along with the other Japanese variants (G6PD Kyoto [9], Heian [10], Tokyo [11], and Tokushima [11]) with chronic hemolytic anemia, also

suggest the possibility that the mutation might have occurred after Japanese were isolated in the islands.

In continental Asia, immigrant G6PD variants (G6PD Taipei-Hakka and Taiwan-Hakka) are seen in the Hakka population, which moved from north China to Taiwan [18]. G6PD Canton was found in Thailand [20], and G6PD Hong Kong resembles G6PD Panay [20] found in the Philippines and G6PD West Bengal [21] found in Asiatic Indians, suggesting immigration and intermarriage. G6PD Mediterranean in the northwestern part of the Indian subcontinent suggests affinities with Mediterranean, Near East and northwest Indian populations [22]. Even if the Chinese or Philippine variant with hemolytic disorders were imported, it would have been hard for them to survive the pressure of natural selection without the positive selective pressure of malaria. The differences in the frequency and type of G6PD variants in southern Asia and Japan suggest that the Japanese may have been isolated geographically, genetically, and anthropologically long enough to have developed their own genetic traits, even though the racial origin of the Japanese is from southern and northern continental Asia.

SUMMARY

A total of 6,120 Japanese males were screened for glucose-6-phosphate dehydrogenase deficiency (G6PD). Five cases with the deficiency were discovered. Two of them and an additional two cases have the same variant, G6PD Ube, characterized by moderate enzyme deficiency, fast moving enzyme activity on electrophoresis, high Ki NADPH, utilization of substrate analogues, kinetics, pH optima, and stability. This variant was distinguished for G6PD A^- and from other Oriental variants by biochemical parameters. Differences in the frequency and type of the variants between southern Asia and Japan, suggest that the Japanese who have been isolated on islands where malaria is not endemic, may have developed their own variant traits.

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REFERENCES

1. Yoshida A, Beutler E, Motulsky AG: Human glucose-6-phosphate dehydrogenase variants. *Bull WHO* 45:243–253, 1971
2. Beutler E, Yoshida A: Human glucose-6-phosphate dehydrogenase variants: a supplementary tabulation. *Ann Hum Genet* 37:151–155, 1973
3. Report of WHO Scientific Group: Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. *WHO Tech Rep Ser* 366, 1967
4. Motulsky AG: Metabolic polymorphismus and the role of infectious diseases in human evolution. *Hum Biol* 32:28–62, 1960
5. Lazzatto L, Usanga EA, Reddy S: Glucose-6-phosphate dehydrogenase deficient red cells: resistance to infection by malarial parasites. *Science* 164:839–842, 1969
6. Miwa S, Teramura K, Irisawa K, Oyama H: Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. II. Incidence of G-6-PD deficiency in Japanese. *Acta Haematol Jpn* 28:590–592, 1965
7. Tada K: Enzymatic anomaly of erythrocytes in congenital nonspherocytic hemolytic anemia. *Tohoku J Exp Med* 75:263–268, 1961
8. Miwa S, Momma K, Komiya K, Ise T, Irisawa K, Oyama H: Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. I. Report of a case found in Japanese, with special

reference to the enzyme activation by stroma of normal erythrocytes. *Acta Haematol Jpn* 28:585–589, 1965

9. KOJIMA H: Congenital nonspherocytic hemolytic disease (CNHD) due to a G-6PD variant: G-6-PD Kyoto. *Acta Haematol Jpn* 35:32–38, 1972
10. NAKAI T, YOSHIDA A: G6PD Heian, a glucose-6-phosphate dehydrogenase variant associated with hemolytic anemia found in Japan. *Clin Chim Acta* 51:199–203, 1974
11. MIWA S, ONO J, NAKASHIMA K, ABE S, KAGEOKA T, SHINOHARA K, ISOBE J, YAMAGUCHI H: Two new glucose 6-phosphate dehydrogenase variants associated with congenital nonspherocytic hemolytic anemia found in Japan: Gd(-) Tokushima and Gd(-) Tokyo. *Am J Hematol*. In press, 1976
12. CHAN TK, TODD D, WONG CC: Erythrocyte glucose-6-phosphate dehydrogenase deficiency in Chinese. *Br Med J* 2:102, 1964
13. BEUTLER E, MITCHELL M: Special modifications of the fluorescent screening method for glucose-6-phosphate dehydrogenase deficiency. *Blood* 32:816–818, 1968
14. MATHAI CK, OHNO S, BEUTLER E: Sex-linkage of the glucose-6-phosphate dehydrogenase gene. *Equdae Nature* 210:115–117, 1966
15. BEUTLER E, MATHAI CK, SMITH JE: Biochemical variants of glucose-6-phosphate dehydrogenase giving rise to congenital nonspherocytic hemolytic disease. *Blood* 31:131–150, 1968
16. YOSHIDA A, LIN M: Regulation of glucose-6-phosphate dehydrogenase activity in red blood cells from hemolytic and nonhemolytic variant subjects. *Blood* 41:877–891, 1973
17. LYON MF: Gene action in the X-chromosome of the mouse (Mus musculus L). *Nature* 190:372–373, 1961
18. LEE TC, SHIH LY, HUANG PC, LIN CC, BLACKWELL BN, BLACKWELL RQ, HSIA DYY: Glucose-6-phosphate dehydrogenase deficiency in Taiwan. *Am J Hum Genet* 15:126–132, 1963
19. MOTULSKY AG, STRANSKY E, FRASER GR: Glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassaemia, and abnormal haemoglobins in Philippines. *J Med Genet* 1:102–106, 1964
20. MCCURDY PR, BLACKWELL RQ, TODD D, TSO SC, TUCHINDA S: Further studies on glucose-6-phosphate dehydrogenase deficiency in Chinese subjects. *J Lab Clin Med* 75:788–797, 1970
21. CHAN TK, TODD D: Characteristics and distribution of glucose-6-phosphate dehydrogenase-deficient variants in south China. *Am J Hum Genet* 24:475–484, 1972
22. AZEVEDO E, KIRKMAN HN, MORROW AG, MOTULSKY AG: Variants of red cell glucose-6-phosphate dehydrogenase among Asiatic Indians. *Ann Hum Genet* 31:373–379, 1968