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Electrophoretic and Kinetic Studies of Glucosephosphate Isomerase (GPI) in Two Different Japanese Families with GPI Deficiency

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In recent years, several types of inborn red cell enzyme deficiencies have been reported among the etiologies of nonspherocytic hemolytic anemia. Glucosephosphate isomerase (GPI; EC 5.3.1.9) catalyzes the reversible interconversion between glucose-6-phosphate and fructose-6-phosphate in the Embden-Meyerhof pathway. Although the pentose-phosphate shunt provides an alternative route for metabolism of glucose-6-phosphate and GPI is not a rate-limiting enzyme for the Embden-Meyerhof pathway, deficiency of this enzyme still is a cause of hereditary nonspherocytic hemolytic anemia. Thirteen cases from 10 different families with GPI deficiency have been observed and documented [1–7].

Recently two additional cases of nonspherocytic hemolytic anemia with GPI deficiency in different families were investigated in our laboratory. This paper describes the electrophoretic and kinetic characteristics of enzymes and genetic studies of these families. The clinical features, levels of glycolytic intermediates and adenine nucleotides, and hemolytic studies are to be reported elsewhere [8, 9].

MATERIALS AND METHODS

Routine hematological studies were performed by standard laboratory methods. Red cells were obtained from heparin or ACD samples. Red cell enzymes were assayed by a method [10] which is similar to that described by Beutler [11]. All the substrates, adenine nucleotides, and enzymes employed were purchased from Boehringer-Mannheim and Sigma. Starch-gel electrophoresis was carried out with 9% hydrolyzed starch (Connaught Medical Research Laboratories, Toronto) at 4° C for 17 hr at 8 v/cm using the technique described by Detter and co-workers [12], modified by using a gel 0.3 cm in thickness and an agar-free staining mixture. A thermostability test was performed by the method of Blume et al. [7]. The *p*H curves were obtained with our GPI assay system in a triethanolamine-HCl buffer at varying *p*H. The *p*H value was measured in each enzyme assay mixture immediately after GPI estimation.

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RESULTS

The propositus (K.S.), a Japanese boy 2 years and 2 months old, was born at term by a normal delivery, followed by moderate jaundice which lasted for a month. Anemia was observed at 8 months. He had been hospitalized because of marked anemia and jaundice at 1 year, but the etiology was not elucidated. He was readmitted for fever, anemia, and jaundice and was referred to us. The second case, T.H., was a 25-year-old Japanese woman admitted to the Shinshu University Hospital for evaluation of jaundice, anemia, and left-breast mass, which was diagnosed as a lipoma. In her neonatal period, jaundice was moderate to marked, and during childhood and teens she had mild, fluctuating jaundice. No neurological or muscular impairments were seen in either propositi. Hematological findings of the two cases are shown in table 1, and family pedigrees are given in figure 1, showing erythrocyte GPI activities of the family members.

TABLE 1

HEMATOLOGICAL DATA IN TWO PROPOSITI

	Propositus K.S.	Proposita T.H.
Hemoglobin (g/100 ml)	8.0	8.6
Hematocrit (%)	25.0	27.5
Reticulocyte (%)	23.0	6.5
Serum bilirubin (mg/100 ml):		
Total	2.0	7.1
Indirect	1.4	6.4
Red cell life span:		
^{51}Cr half life (days)	5.0	10.5

In family S, all family members with decreased GPI activity presented an abnormal electrophoretic red cell pattern with four isozyme bands; normal individuals showed only three bands (figs. 2, 3). The cathodally fastest band migrated more rapidly than the normal main band. In propositus K.S. (VI-5), the isozyme pattern was markedly different from that of either parent (V-4, V-12) or from normal individuals. The same abnormal isozyme pattern was found in spleen, liver, and muscle extracts from biopsy specimens obtained at splenectomy (fig. 4). Zymograms of leukocyte and plasma GPI from each family member showed the same pattern as those obtained from their hemolysates.

Electrophoretic studies of proposita T.H. and her parents revealed the same isozyme bands as found in normal individuals (fig. 5). However, GPI activities of the parents and one of the sisters were approximately one-half of the normal level (figs. 1, 5); these individuals were asymptomatic. Leukocytes and plasma of all family members examined showed the same normal isozyme pattern and decreased activity for GPI. In the thermostability test, proposita T.H. had markedly labile GPI, while both parents had only a moderately unstable enzyme, as also seen in propositus K.S. (fig. 6). The *p*H activity curve of proposita T.H. was

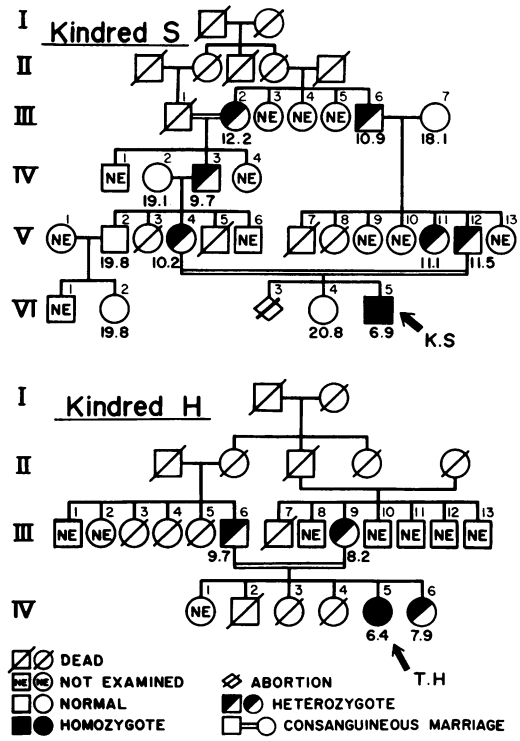

FIG. 1.—Pedigrees of kindreds S and H. The erythrocyte GPI values (in units per 10^{10} red cells) are shown directly beneath the GPI phenotype. Normal range of erythrocyte GPI values is 12.0–22.0 units per 10^{10} red cells. In kindred S, the maternal great-grandmother (III-2) is a sister of the paternal grandfather (III-6), and they have lower GPI activities. The maternal great-grandmother (III-2) and the maternal great-grandfather (III-1) are first cousins. The mother experienced a spontaneous abortion at the time of the first pregnancy. In kindred H, the parents are first cousins. All living members except for propositi in both kindreds have had no episodes of anemia and jaundice.

shifted to the acidic side (fig. 7) but that of propositus K.S. appeared normal. These results were obtained in a duplicate experiment.

Enzyme activities were checked throughout these experiments as a precaution against possible loss of activity. The Michaelis constant (K_m) for F-6-P of the hemolysate in the two propositi and their family members was not different from that of normal individuals.

DISCUSSION

Detter and co-workers [12] intensively investigated GPI variants. Electrophoretic studies of GPI in family S, in the propositus K.S., and in family members possessing lower GPI activity revealed different electrophoretic patterns than those previously reported [2, 3, 12, 13]. At first glance the parents' GPI pattern appears similar to PHI (GPI) 7-1 described by Detter et al. [12]. However, they differ in two respects. The cathodally fastest band of the parents migrated slightly

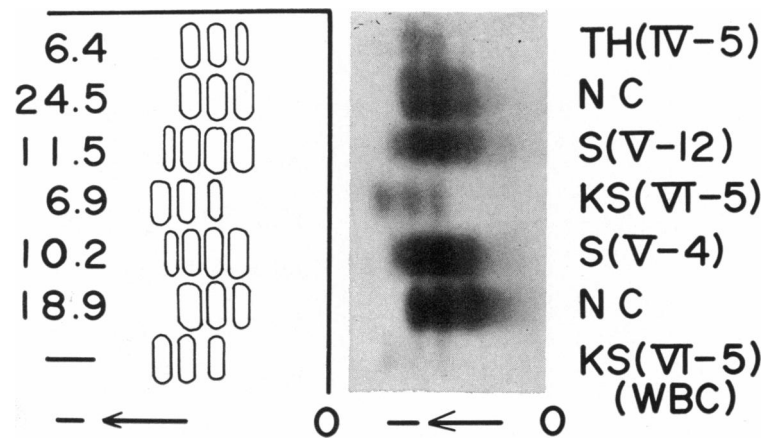

FIG. 2.—Photograph (right) and diagram (left) of starch gel electrophoresis of GPI of hemolysates (bottom sample is from leukocytes). At left are GPI activities in units per 10^{10} red cells (normal 12.0–22.0). To demonstrate the minor bands more clearly (see fig. 3 for comparison), the sliced gel was rinsed with water and dried before enzyme reaction. See pedigree for identification of subjects. NC = normal control; 0 = origin. Propositus K.S. has low GPI and abnormal isozyme pattern with three bands; proposita T.H. has low GPI and normal isozyme pattern.

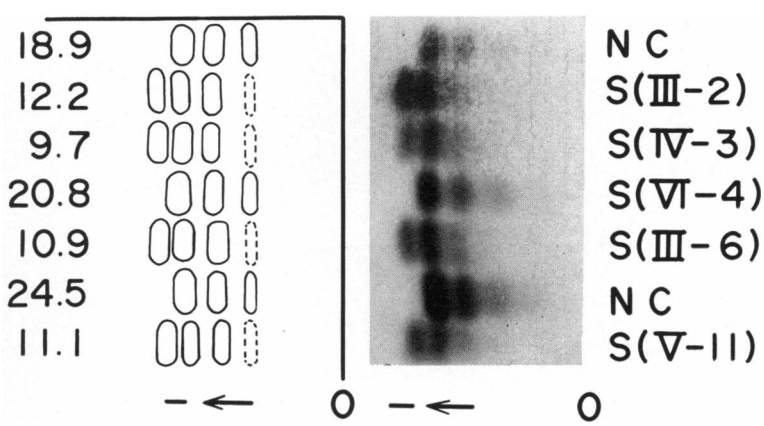

FIG. 3.—Photograph (right) and diagram (left) of starch gel electrophoresis of erythrocyte GPI in kindred S. The GPI values (in units per 10^{10} red cells) are shown on the left. Electrophoresis was carried out at higher voltage (10 v/cm) for 17 hr to separate the fastest band in heterozygotes from the normal main bands. NC = normal control; 0 = origin. All family members with decreased GPI activity presented an abnormal isozyme pattern with four isozyme bands, whereas normal control and sister have normal isozyme pattern with normal GPI activity.

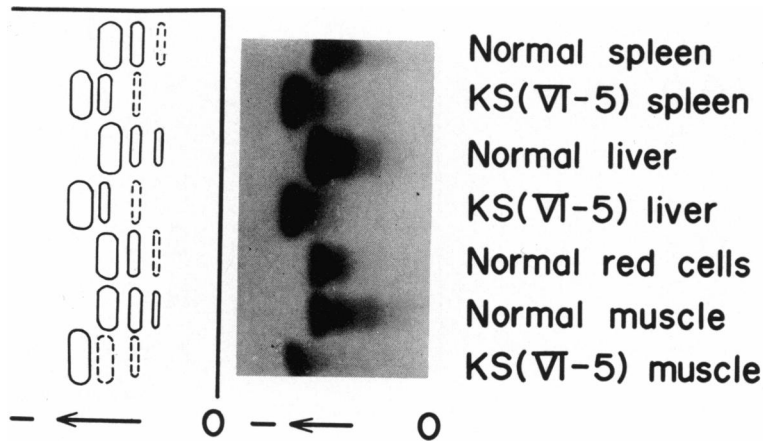

FIG. 4.—Photograph (right) and diagram (left) of GPI zymogram of tissue extracts. Propositus's samples were obtained from biopsy specimens at splenectomy; normal samples were obtained from an autopsy case. Gel was stained by standard procedure. All samples from propositus K.S. show abnormal migrations.

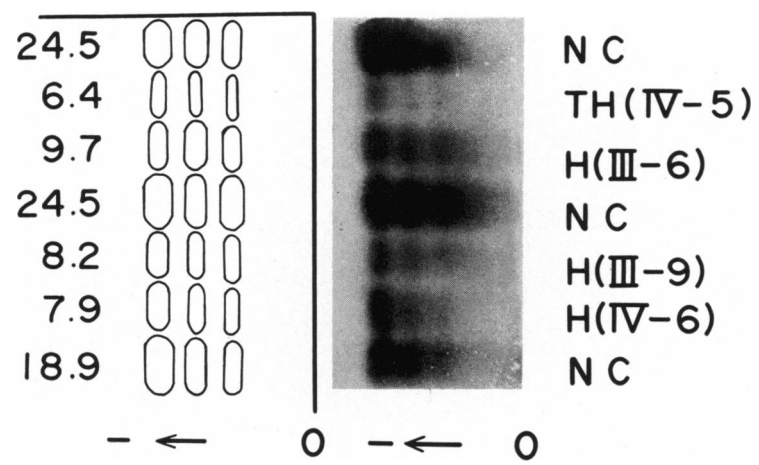

FIG. 5.—Photograph (right) and diagram (left) of erythrocyte GPI zymogram in kindred H. The GPI values (in units per 10^{10} red cells) are shown at left. All samples show the same electrophoretic migration. The decrease in the band density of the three isozymes was proportional to the total GPI activities as compared with normal GPI. NC = normal control; 0 = origin.

more slowly than that of Detter's PHI 7-1, and the total activities of the parents' GPI was reduced to approximately half the normal value. Detter et al. neither mentioned total GPI activity nor discussed whether their case had hemolytic anemia. In addition, the pattern of propositus K.S. was clearly different from PHI 7-1 because three isozyme bands were present whereas four were demonstrated in

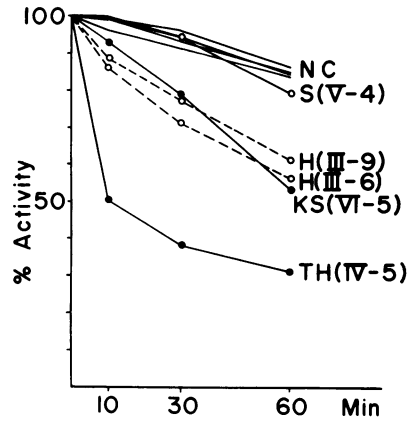

FIG. 6.—Thermostability test of GPI. NC = normal control

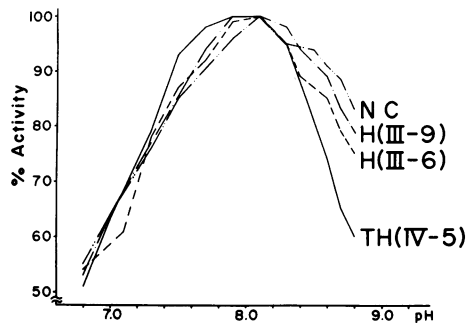

FIG. 7.—*p*H-activity curve of GPI. NC = normal control

PHI 7-1. In the cases of a deficient GPI variant reported by Baughan et al. [2] and of GPI–Los Angeles discovered by Blume et al. [7], isozyme patterns indicated that each parent had a different variant gene with patterns different from our first case.

Since it is not feasible to determine the amino acid sequence of GPI isozyme molecules, differentiation of GPI variants is best achieved by electrophoretic comparison of each sample on the same plate and by kinetic characterizations. In spite of the difficulties of this identification process, it seems reasonable to conclude that propositus K.S. has a new variant, GPI "Narita." The consanguineous marriages within this family and the electrophoretic patterns of GPI from the family members indicate that both mutant alleles of propositus K.S. (VI-5) are derived from a common ancestor. Thus the propositus is thought to be homozygous for the GPI "Narita" variant gene. Since GPI has been proved to be a dimer [12, 14], one subunit in heterozygotes and both subunits in homozygotes should be affected, resulting in different isozyme patterns.

In family H the parents are first cousins, and both parents had GPI activities almost one-half of the normal level with normal electrophoretic mobility. Proposita

T.H. and her parents had the same migration pattern. This evidence strongly suggests that both GPI alleles of proposita T.H. had the same origin. Moreover, she possessed a markedly thermolabile enzyme while her parents had only moderately labile GPI; and her GPI had a slightly acidic optimal *p*H curve. The heat instability found in the parents' GPI can be interpreted to mean that one-half of their enzyme is contributed by the subactive labile enzyme. Because of the normal electrophoretic migration, it is also possible that a control gene might have been affected without any abnormality arising in the GPI structural genes. However, the results of the thermostability test do not support this possibility. By analogy, there is a group of unstable abnormal hemoglobins, such as Hb Hammersmith [15], which does not separate from Hb A by electrophoresis but is unstable and thermolabile. In red cell enzyme observations examples also exist (such as G6PD Mediterranean [16]) which are indistinguishable from the normal by electrophoresis but are thermolabile.

Thus, family H probably possesses a mutant structural gene which produces an unstable GPI with normal electric charge. Since there has been no previous report of a GPI variant with deficient activity but with the same electrophoretic mobility in the homozygous and heterozygous cases as the normal, the GPI variant of family H should be regarded as new, GPI "Matsumoto." Blume et al. [7] suggested that most of the so-called homozygotes for GPI deficiency are heterozygous for two different deficient alleles, that is, double heterozygotes. However, the results mentioned above from consanguineous families indicate that the first case is homozygous for a mutant structural allele, GPI "Narita," while the second case is homozygous for another mutant allele, GPI "Matsumoto."

SUMMARY

Electrophoretic, kinetic, and pedigree studies of two new patients with nonspherocytic hemolytic anemia due to glucosephosphate isomerase deficiency were described. In the first case, abnormal isozymes were found in the propositus and among family members; in the second case normal electrophoretic mobility of a thermolabile enzyme was observed and the optimal *p*H curve was shifted toward the acidic side. Consanguineous marriages were noted in both families, and pedigree studies revealed that both propositi were homozygous for variant genes rather than heterozygous for two different variant genes. These findings indicated that both cases possess enzyme variants different from those previously reported in the literature. The first case was designated GPI "Narita" and the second, GPI "Matsumoto."

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REFERENCES

1. BAUGHAN MA, VALENTINE WN, PAGLIA DE, WAYS PO, SIMONS ER, DEMARSH QB: Hereditary hemolytic anemia associated with glucosephosphate isomerase (GPI) deficiency, a new enzyme defect of human erythrocytes. *Blood* 32:236–249, 1968

2. PAGLIA DE, HOLLAND P, BAUGHAN MA, VALENTINE WN: Occurrence of defective hexosephosphate isomerization in human erythrocytes and leukocytes. *New Eng J Med* 280:66–71, 1969
3. SHRÖTER W, BRITTINGER G, ZIMMERSCHMITT E, KÖNIG E: A new hemolytic syndrome with glucosephosphate isomerase (GPI) and glucose-6-phosphate dehydrogenase (G6PD) deficiency of the erythrocytes: biochemical studies. *Europ J Clin Invest* 1:145, 1970
4. ARNOLD H, BLUME KG, BUSCH D, LENKELT U, LÖHR GW, LÜBS E: Klinische und biochemische Untersuchungen zur Glucosephosphatisomerase normaler menschlicher Erythrocyten und bei Glucosephosphatisomerase-Mangel. *Klin Wschr* 48:1299–1308, 1970
5. OSKI F, FULLER E: Glucose-phosphate isomerase (GPI) deficiency associated with abnormal osmotic fragility and spherocytes. *Clin Res* 19:427, 1971
6. SCHRÖTER W, BRITTINGER G, ZIMMERSCHMITT E, KÖNIG E, SCHRADER D: Combined glucosephosphate isomerase and glucose-6-phosphate dehydrogenase deficiency of the erythrocytes: a new haemolytic syndrome. *Brit J Haemat* 20:249–261, 1971
7. BLUME KG, HRYNIUK W, POWARS D, TRINIDAD F, WEST C, BEUTLER E: Characterization of two new variants of glucose-phosphate-isomerase deficiency with hereditary nonspherocytic hemolytic anemia. *J Lab Clin Med* 79:942–949, 1972
8. MIWA S, NAKASHIMA K, ODA S, ODA E, MATSUMOTO N, OGAWA H, FUKUMOTO Y: Glucosephosphate isomerase (GPI) deficiency hereditary nonspherocytic hemolytic anemia: report of the first case found in Japanese. *Acta Haemat Jap.* In press, 1973
9. MIWA S, NAKASHIMA K, ODA S, MATSUMOTO N, OGAWA H, KOBAYASHI R, KOTANI M, HARATA A, ONAYA T, YAMADA T: Glucosephosphate isomerase (GPI) deficiency hereditary nonspherocytic hemolytic anemia: report of the second case found in Japanese. *Acta Haemat Jap.* In press, 1973
10. MIWA S, NISHINA T, KAKEHASHI Y: Blood cell enzymes. *Sogorinsho* 18:1743–1753, 1969
11. BEUTLER E: *Red Cell Metabolism: A Manual of Biochemical Methods.* New York, Grune & Stratton, 1971
12. DETTER JC, WAYS PO, GIBLETT ER, BAUGHAN MA, HOPKINSON DA, POVEY S, HARRIS H: Inherited variations in human phosphohexose isomerase. *Ann Hum Genet* 31:329–338, 1968
13. GIBLETT ER: *Genetic Markers in Human Blood.* Oxford, Blackwell, 1969
14. CARTER ND, YOSHIDA A: Purification and characterization of human phosphoglucose isomerase. *Biochim Biophys Acta* 181:12–19, 1969
15. DACIE JV, SHINTON NK, GAFFNEY PJ, CARRELL RW, LEHMANN H: Haemoglobin Hammersmith (β42(CDI)Phe→Ser). *Nature* 216:663–665, 1967
16. WHO SCIENTIFIC GROUP: Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. *WHO Techn Rep Ser* no. 366, 1967