Phosphoglycerate Kinase: An X-Linked Polymorphism in Man

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Phosphoglycerate kinase (PGK: E.C.2.7.2.3) catalyzes the glycolytic pathway reaction in which 1,3-diphosphoglycerate (1,3-DPGA) is reversibly converted to 3-phosphoglycerate (3-PGA). In the forward reaction, the energy generated as ATP is used to maintain normal red cell viability. Thus PGK deficiency, described in two reports [1, 2], is associated with hemolytic anemia. In one large Chinese kindred [2], the history and biochemical studies indicated that the enzyme deficiency was probably X-linked. In an attempt to prove X-linkage by demonstrating inherited structural variation in PGK, Beutler [3] developed a method for staining the enzyme after starch gel electrophoresis. However, a uniform isozyme pattern was observed in all 345 specimens he tested.

This report describes a PGK variant found in three of 161 blood specimens obtained from two districts of New Guinea. A family study showed that the structural locus for this enzyme is X-linked.

MATERIALS AND METHODS

Blood specimens from random healthy natives of the Butibum and Lumi districts of New Guinea were collected in ACD solution and shipped to Seattle by air. To prepare the hemolysates, washed red cells were lysed with one volume of distilled water and one-half volume of toluene, and stroma was removed by centrifugation for 20 minutes at 17,400 *g*. Vertical starch gel electrophoresis [3] was performed at 4°C for 18 hours at 8 v per centimeter and *p*H 7.5 (electrode buffer: 0.1 M tris, 0.028 M citric acid; gel buffer: one-tenth dilution of electrode buffer).

The sliced gel was stained for PGK activity by incubating at 37°C for approximately one hour in a mixture of 0.1 M tris-HCl buffer (*p*H 8.1), 4 mM 3-PGA, 4 mM Na_2ATP, 3 mM $MgCl_2$, 1.4 mM Na_2EDTA, and 1 unit per milliliter glyceraldehyde 3-phosphate dehydrogenase. The sites of enzyme activity were revealed by the defluorescent zones against a fluorescent background under a long wave, ultraviolet light. Photography was performed at the same wavelength, using a yellow filter and Polaroid camera.

Activity of PGK was determined by a modification of the method described by Scopes

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[4], measuring the rate of absorbence change at 340 mμ at 25°C. The reaction mixture contained 10 mM 3-PGA, 4 mM ATP, 6 mM $MgCl_2$, 0.2 mM EDTA, 0.1 M glycylglycine buffer (*p*H 8.0), 0.2 mM NADH, and approximately 1 unit of glyceraldehyde 3-phosphate dehydrogenase per milliliter. One unit of PGK produces 1 μmole of 1,3-diphosphoglycerate per minute. Hemoglobin was estimated by the cyanmethemoglobin method. Activity of glucose-6-phosphate dehydrogenase (G6PD) was measured by a standard method [5].

RESULTS

The usual PGK zymogram (designated PGK 1) consists of three anodally migrating components, as shown in figure 1. The band with strongest staining intensity has the slowest mobility, migrating about 1 cm from the origin. The fastest band is frequently very faint. This PGK 1 pattern was uniformly observed in the hemolysates

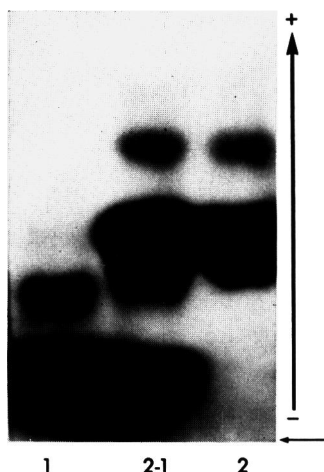

FIG. 1.—Starch gel electrophoresis patterns of three PGK phenotypes—PGK 1, PGK 2-1, and PGK 2. The arrow points to the origin.

of normal blood donors in Seattle, consisting of 67 Caucasians (48 men and 19 women), 134 Afro-American men, and 34 Oriental men, mainly of Japanese origin. It was also found in hemolysates of 118 Filipinos (men and women, sex unspecified) and 96 male Chinese whose blood had been obtained during previous studies. The stability of the enzyme was demonstrated by the fact that in these hemolysates, frozen for as long as five years, the isozyme pattern showed no apparent change when compared with fresh lysates.

Among 161 specimens from New Guinea (146 men and 15 women), all but three had the common PGK 1 phenotype. Of these three, two were males, and their zymogram (labeled PGK 2 in fig. 1) resembled PGK 1, except that all of its components had considerably faster mobility. A second exceptional pattern (labeled PGK 2-1 in fig. 1) contained isozymes with the same electrophoretic mobility as those of PGK 1 and PGK 2. It was found in a female.

Figure 2 shows the results of studying the family of one of the men with PGK 2. The same phenotype was found in his brother (II-2) and in the son (III-8) of one of their sisters. That sister (II-7), as well as the propositus's daughter (III-3) and

mother (I-1), had PGK 2-1 (i.e., both the common and variant isozymes were present). Thus, the grandmother (I-1) transmitted the variant allele to her two sons and to one of her daughters. The propositus (II-4) gave the allele to his daughter, but not to his son. In his brother's family, neither of the sons inherited the allele, but his sister (II-7) apparently gave the gene to one of her two sons.

The red cell PGK activity of three subjects with PGK 2 and four with PGK 2-1 was compared with that of 24 other New Guinea natives (nine men and 15 women) with the PGK 1 phenotype. In all instances, the value obtained fell within the range of 102.1 ± 12.4 units per gram of hemoglobin.

In studies of G6PD activity among the family members (fig. 2), the grandmother

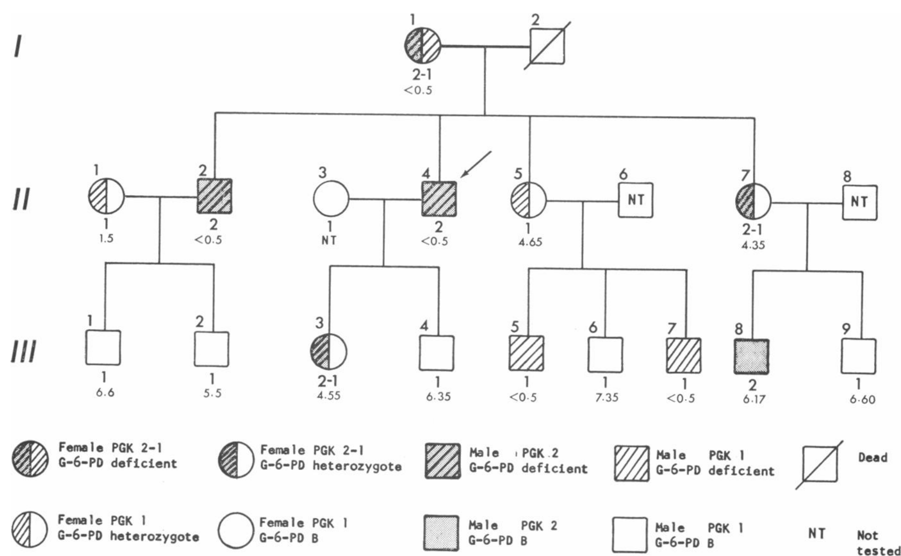

FIG. 2.—The pedigree of the New Guinea family showing the X-linked inheritance of PGK, and indicating both PGK and Gd phenotypes. The G6PD values (in units per gram of hemoglobin) are shown directly beneath the PGK phenotypes. The arrow points to the propositus.

(I-1) was found to have less than 0.5 units per gram of hemoglobin, a value consistent with homozygosity for G6PD deficiency. Her two sons were also deficient, and her daughters appeared to be heterozygotes. Some data suggesting that the loci for PGK and G6PD are not closely linked were also obtained. Thus, the propositus's sister (II-7), who was heterozygous at both loci, had two sons with normal G6PD activity, but one had PGK 1, while the other had PGK 2. Assuming X-linkage of both loci, this pattern of inheritance cannot be explained without involving crossover between them.

No useful linkage data on the Xg and PGK loci could be obtained, since the family members were all Xg(a+).

DISCUSSION

The evidence presented here, together with the data from the family with PGK deficiency described by Valentine et al. [2], strongly support X-linkage of the PGK

locus in man. First, figure 2 shows that there was no male-to-male transmission of the variant enzyme, in spite of three opportunities (II-2 to III-1 and III-2; II-4 to III-4). Second, in each instance of the PGK 2 phenotype, the variant enzyme apparently was transmitted from mother to son (I-1 to II-2 and II-4; II-7 to III-8). Third, the heterozygous phenotype, PGK 2-1, was not found in any male tested, while it occurred in three females of the tested family and in one other unrelated woman. Finally, although the appearance of the PGK 2 phenotype suggests homozygosity for the variant allele, the inheritance study indicates that men with this electrophoretic pattern are almost certainly hemizygous for a structural gene with an X-chromosomal locus. Unfortunately, no blood could be obtained from I-2 and II-8, the men whose sons had PGK 2. However, it is highly unlikely that both of these untested fathers were carriers of an allele with such a low frequency (about .014 in this population). Furthermore, the fact that the propositus and his brother (both PGK 2) had children with the PGK 1 type shows that neither could be homozygous for a variant allele on an autosomal locus.

In accordance with the nomenclature recommended for G6PD, another X-linked enzyme [6], we are designating the locus defined by these studies as Pgk. The alleles coding for the usual (slow) and variant (fast) forms of the enzyme are thus Pgk^1 and Pgk^2. Genotypes in males are Pgk^1 or Pgk^2, while in females they are Pgk^1/Pgk^1, Pgk^2/Pgk^1, or Pgk^2/Pgk^2. The latter must be extremely rare.

Very close linkage between the Pgk and Gd loci seems unlikely, since there was an instance of recombination in the family described here; however, further linkage data with Gd and with other loci on the X chromosome are now required. In addition, it should be possible to determine the likelihood of random inactivation at the X-linked Pgk locus in females—as it was for the Gd locus [7]—by studying cultured clones of skin fibroblasts from heterozygotes, since the PGK in various tissues is presumably under the control of the same structural locus [3]. The similarity of PGK enzyme activity in the two sexes, indicative of probable female dosage compensation, is consistent with random inactivation. Also, if the enzyme (as G6PD) is composed of two or more subunits capable of undergoing hybridization, failure to observe evidence of molecular hybrids in the heterozygous PGK 2-1 electrophoretic pattern could be taken as further evidence for random inactivation of the Pgk locus.

The frequency of the Pgk^2 gene (.014) in the New Guinea population sample places this enzyme variability within the range generally accepted for genetic polymorphism. Recently, we received six blood specimens from male natives of Samoa, and found that one of the six had a variant electrophoretically indistinguishable from the PGK 2 encountered in New Guinea. It is therefore conceivable that the Pgk^2 allele has its origin among the peoples of Oceania, and that some characteristic of the environment is responsible for its maintenance in that region.

SUMMARY

An electrophoretic variant of phosphoglycerate kinase (PGK) was detected in a New Guinea population, the variant gene having a frequency of about .014. A family study showed that the structural locus for PGK is on the X chromosome, supporting the previously reported X-linked inheritance of PGK deficiency. The red cell PGK

activity in subjects with the electrophoretic variant fell within the normal range. Preliminary evidence suggests that the loci for PGK and G6PD enzymes are not closely linked.

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Participants who wish to submit a paper for presentation should send the name(s) of author(s), title, and an abstract (200 words maximum) to the general secretary, Dr. Jean de Grouchy, Clinique de Génétique Médicale Hôpital des Enfants Malades, 149, Rue de Sèvres, 75-Paris 15. Further information on the Congress can be obtained from Dr. de Grouchy, and a preliminary program will be published in the March *Journal.* Deadline for abstracts is March 31, 1971.

An American Society of Human Genetics travel committee has been formed to provide travel information and assistance for North American residents who plan to attend the Paris Congress. A newsletter regarding possible modes of travel and application forms for travel grants are available from Prof. T. E. Reed, ASHG Travel Committee, Department of Zoology, University of Toronto, Toronto 5, Ontario, Canada. Nonmembers will be considered for grants, but, because funds are expected to be very limited, other sources of travel funds should be looked for.

The membership of the American Society of Human Genetics has voted not to hold their 1971 meeting to avoid conflicts with the Paris Congress.