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Characterization of a series of electrophoretic and enzyme activity variants of human glucose-phosphate isomerase

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Summary. A total of 3438 cord blood samples were screened for variants of erythrocyte glucose-phosphate isomerase. The five different electrophoretic, three activity/deficiency, and one thermostability variants distributed among 27 unrelated Caucasian families of that population, plus two electrophoretic variants previously described from three Amerindian tribes were subsequently examined for cryptic variation using activity and thermostability criteria. Although thermostability differences were observed between electrophoretic variants, no microheterogeneity within any one class of variant was detected.

Introduction

Glucose-phosphate isomerase (GPI, E.C. 5.3.1.9) is a dimeric enzyme that catalyzes the interconversion of glucose-6-phosphate and fructose-6-phosphate. In animals, the enzyme is the product of a single structural locus (Womack 1982) with a subunit molecular weight of 63,000 daltons (Gracy 1982).

Numerous mobility differences exist in the standard GPI from different species (Scopes 1968). In addition, many electrophoretic variants of GPI occur as intraspecific polymorphisms within animal species (Richkind and Richkind 1978; Sandberg 1973; VanderBerg and Stone 1978). In contrast to this, however, among humans, GPI tends to be largely monomorphic. The electrophoretic variants which have been reported tend to be rare with frequencies of less than 0.1%, although variants with frequencies approaching 1% have been reported for some populations (Harris and Hopkinson 1976). The variant alleles usually occur in heterozygous combination with the normal allele. In addition to electrophoretic variants, a number of activity and thermostability variants of GPI have also been reported (Mohrenweiser 1981; Mohrenweiser and Neel 1981). On the clinical level, the absence of GPI results in a metabolically significant genetic disease. GPI deficiency is the third most prevalent cause of hemolytic anemia in humans (Arnold 1979; Kahn et al. 1979).

Although the variants of GPI are usually differentiated by their electrophoretic mobility, electrophoretic variants within a mobility class may often be further subdivided on the basis of activity and thermostability characteristics. Such microheterogeneity within an electrophoretic class was demonstrated for GPI variants from Japan (Satoh and Mohrenweiser 1979). Also both activity and thermostability differences for GPI could be demonstrated for isozymes with similar electrophoretic mobilities but from different species, and an electrophoretically "silent" variant was detected by thermostability techniques among a series of chimpanzee blood samples (Naidu et al. 1984).

The present paper reports on the electrophoretic and thermostability characteristics of nine different electrophoretic or activity/thermostability variants of GPI from 27 unrelated families which were observed in an unselected sample of newborns from Ann Arbor, MI. Two variants observed among three different Amerindian tribes (Neel et al. 1977; Tanis et al. 1973) were also characterized.

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Materials and methods

The Ann Arbor study population consisted of all infants born at the University of Michigan Women's Hospital between January 1977 and January 1985, whose parents agreed to donate blood samples. Samples were collected as described by Mohrenweiser (1981). Enzyme assays were performed on the miniature centrifugal fast analyzer as described by Fielek and Mohrenweiser (1979). Thermostability studies were conducted as described by Mohrenweiser and Neel (1981), Naidu et al. (1984), and Satoh and Mohrenweiser (1979). Electrophoresis on 7 or 8% polyacrylamide gels was performed as described by Neel et al. (1980), and on starch gels using a variety of buffer systems as described by Harris and Hopkinson (1976). Km determinations were conducted as described by Mohrenweiser and Novotny (1982).

The electrophoretically identifiable variant alleles were assigned designations using nomenclature similar to the system described by Wurzinger and Mohrenweiser (1982). Thus, variants with mobilities less cathodal than the normal enzyme were considered slow or type 2 variants and variants with mobilities more cathodal than the normal isozyme were classed as fast or type 3 variants. The AA in the variant designations stands for Ann Arbor.

Results

A total of nine different (five electrophoretic, three activity, one thermostability) variant classes of GPI, distributed among 27 unrelated individuals from the Ann Arbor study population, as well as two different electrophoretic variants which

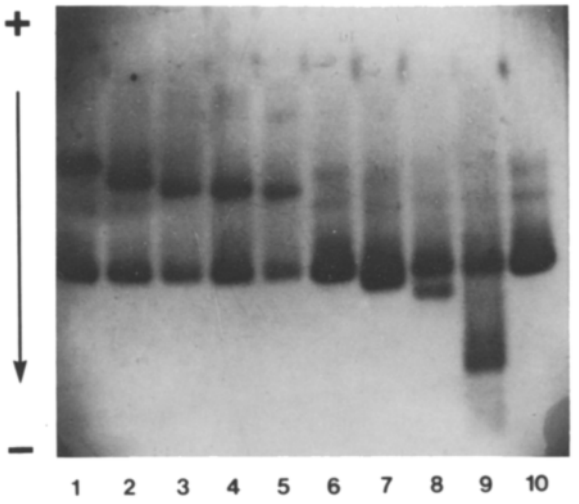

Fig. 1. Polyacrylamide gel electrophoretic pattern of human erythrocyte GPI variants. The phenotypes of the individuals are as follows: *lane 1,* 1-2AA-1; *lane 2,* 1-2AA-2; *lane 3,* 1-2WAP-1; *lane 4,* 1-2AA-3; *lane 5,* 1-2CAY-1; *lane 6,* normal (1-1); *lane 7,* 1-3AA-1; *lane 8,* 1-3AA-2; *lane 9,* 1-3NG-1; *lane 10,* normal (1-1). The origin is at the top of the gel and the direction of migration is indicated

occur in the Cayapo, Kraho, and Wapishana Amerindian tribes were examined for cryptic variation utilizing the thermostability methods previously described. All variant individuals examined were heterozygous, having the normal plus a variant allele. In all cases the variant allele was inherited, being found both in a child and in one of its parents.

The polyacrylamide gel electrophoretic pattern of the electrophoretic variants is presented in Fig. 1. Human GPI is a cathodally migrating enzyme with an electrophoretic pattern that consists of a primary isozyme and usually one or two minor, less cathodally migrating bands of activity. The variants typically display the three-banded pattern characteristic of dimeric enzymes. This three-banded pattern, which is usually seen most clearly in fresh samples, is sometimes altered in aged samples in which the variant homodimer is no longer readily observed, suggesting that many of the variant isozymes tend to be less stable than the normal enzyme.

Nineteen variant individuals displaying electrophoretic mobility variation were identified during the screening of 3438 newborn infants. The following variants were differentiated.

Variant 2AA-1 had normal levels of activity and thermostability. It was the least cathodally migrating of the variants with a relative mobility of 46. The variant was observed in two male children from two apparently unrelated Caucasian families. The variant was inherited from the father in one family and in the second family from the mother.

Variant 2AA-2 was observed in a male child who had inherited the variant from his father. The variant had a relative mobility of 54 and possessed normal levels of activity and thermostability.

Variant 2AA-3 was observed in a male child to whom it was transmitted by the mother. The variant had normal levels of activity with a thermolabile thermostability profile. The GPI activity in the hemolysate from the affected individuals, after 30 min of heating at 50.5°C, was 49% and 73% of the GPI activity in the unaffected parent whose thermostability profile was in the normal range for control samples. The variant had a relative electrophoretic mobility of 59.

Variant 3AA-1 was seen in 14 individuals belonging to both sexes. In all instances, the variant was inherited. The individuals who carried the variant all possessed normal levels of activity. The thermostability profile was normal thus no microheterogeneity was detected. On polyacrylamide gels, variant 3AA-1 appeared as a broad, intensely staining band of activity, especially on gels on which equal amounts of GPI activity had been loaded. The relative mobility of variant 3AA-1 was 103, and although clearly recognizable on 8% polyacrylamide gels, this variant was not able to be differentiated from the normal enzyme on starch gels.

Variant 3AA-2 was seen in a female child of Asian Indian ancestry. The variant was inherited from the mother. It had normal levels of activity and thermostability (in the original sample) and a relative mobility of 110. The reduced intensity of the heterodimer (variant plus normal subunit) reflects the loss of activity of the variant isozyme associated with several freeze/thaw cycles. The bands were of more equal intensity on the original gel (not shown).

In addition to the variants observed in the Ann Arbor study populations, we were able to examine several electrophoretic mobility variants from other populations.

Variant 2WAP-1 occurred in the Wapishana Indians of South America. Two individuals in the same family were heterozygous for this variant (Neel et al. 1977). In the original report this variant was called 3-WAP-1. This variant had normal levels of activity and a thermolabile thermostability profile. The two individuals had 65% and 66% of the activity of the normal Indian control values after heating at 48.5°C for 30 min. The variant allele had a relative electrophoretic mobility of 58.

Variant 2CAY-1 occurred in the Cayapo and Kraho Indians of South America (Tanis et al. 1973; Neel 1978). This variant had normal levels of activity and thermostability. It had a mobility of 60 relative to the normal enzyme.

In addition to these variants we were also able to perform an electrophoretic comparison of a GPI variant (3NG-1) found among the Gainj tribe of Papua/New Guinea (Long et al. 1986). This variant had a mobility of 156 relative to the normal enzyme. Activity and thermostability studies could not be performed on this variant.

In addition to the screen for electrophoretic variants in this unselected group of newborns, a subset of 2084 newborns were screened for the presence of enzyme activity variants and another subset of 100 of these newborns were screened for variants which were detected by altered thermostability profile (Mohrenweiser 1981; Mohrenweiser and Neel 1981). The activity variants were classified as enzyme deficient if the activity was less than 65% of normal and more than 3 SD below the mean, hypoactive if the activity was between 65 and 75% of normal and more than 2.5 SD below the mean, and hyperactive if the activity was greater than 140% of normal and more than 4 SD above the population mean.

A hyperactivity variant was detected in a male child who had inherited the variant from his father. The levels of activity were 168% (>7SD) and 145% (>4SD) above the appropriate population means for child and parent respectively (Mohrenweiser 1981). This variant had a normal thermostability profile.

Table 1. Characteristics of GPI variants. TS, thermostability; N, normal; L, labile; RM, relative mobility; 1°, null

Allele designation	No. of individuals	Activity level	TS	RM
1	–	N	N	100
2AA-1	2	N	N	46
2AA-2	1	N	N	54
2WAP-1	2	N	L	58
2AA-3	1	N	L	59
2CAY-1	2	N	N	60
3AA-1	14	N	N	103
3AA-2	1	N	N	110
Hyperactivity	1	Hyper-	L	100
Hypoactivity	2	Hypo-	N	100
1°	4	Deficient	–	100
Labile	1	N	L	100

The GPI deficiency variants were identified in four unrelated families, one of which was previously reported by Mohrenweiser (1981). These individuals had activity levels of approximately 50% of the appropriate population mean for GPI and normal activity for 11 other erythrocyte enzymes. This level of GPI activity is more than four SD below the population mean and is consistent with the presence of a null allele at the GPI locus. The thermostability profile of the deficiency (null) variants was normal. This is as expected unless the gene product of the null allele was destabilizing the functional enzyme. Two other child-parent diads had activity levels of 65%–75% of normal, a level 2.5–3.5 SD below the mean, consistent with the existence of an allele associated with an unstable gene product rather than a null allele. The thermostability profile of each of these hypoactivity variants was normal.

The thermostability variant was previously described in detail by Mohrenweiser and Neel (1981). This variant had normal levels of activity and a thermolabile thermostability profile. It was transmitted to the affected male child by his father.

All of the activity and thermostability variants possessed the normal electrophoretic pattern for GPI. In addition, all of the variants examined, both electrophoretic and activity, possessed Km values that were well within the normal range established for the control samples. The results of this study on the variants of GPI are summarized in Table 1.

Discussion

A total of 3438 cord blood samples were screened for electrophoretic variants of GPI. The ethnic distribution within the Ann Arbor study population was 88% Caucasian, 10% Black, 1% Oriental, and 1% unidentified or other. No electrophoretic variants of GPI were detected in the non-Caucasian segment of the population. A total of five different electrophoretic variants distributed among 19 Caucasians out of a total of 3015 unrelated Caucasian newborn individuals were resolved. The overall GPI electrophoretic variant frequency in Caucasians in the Ann Arbor area is thus 0.3%, two thirds of this being contributed by a single variant (3AA-1). This frequency

Table 2. Comparative frequency of rare variants at the GPI locus as determined by three criteria

Variant class	No. of determinations	No. of variants	Frequency no./1000
Electrophoresis	3438	19	5.5
Thermostability	100	1	10.0
Deficiency/activity	2084	7	3.3
Total			18.8

is essentially in agreement with other studies on Caucasian populations (Harris and Hopkinson 1976).

Although thermostability differences were detected between different variant classes, no microheterogeneity within any one electrophoretic type of variant was detected using heat-denaturation methods. This is in contrast to the results of Satoh and Mohrenweiser (1979) who were able to discern several thermostability differences within two electrophoretic classes of GPI variants identified in Japan.

The variants identified in this study were resolved on 8% polyacrylamide gels, thus we are hesitant to compare them to the GPI variants previously described, all of which were resolved on starch gels. Should such a comparison be desirable, one could hazard a guess and suggest that variant 2AA-1, 2AA-2, 3AA-1, and 3AA-2 are similar to the variant alleles designated 2, 3, 9, and 7 respectively by Detter et al. (1968). The variants 2WAP-1, 2AA-3, and 2CAY-1 are similar to the 4 allele of Detter et al. (1968). These three variants could not be differentiated from each other on starch or 7% acrylamide gels, but were resolved and differentiated on 8% polyacrylamide gels.

This report summarizes the frequency of rare variants at a single structural locus (GPI) generated by three different independent approaches, viz; electrophoresis, activity measurements, and thermostability. Thus it may be used to calculate an overall variant (electrophoretic and silent or non-electrophoretic) frequency (Table 2), a figure which is not available for most loci. The difference between the total variant frequency and the electrophoretic variant frequency indicates again that one must consider multiple approaches when asking questions concerning genetic variation or mutation rates.

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