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Detection of the Rare PGM_1^3 Allele. Further Biochemical and Genetic Characterization of the PGM_1^3 Isozymes

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ABSTRACT: A family possessing the rare PGM_1^3 allele has been found in North Carolina, and criteria for the electrophoretic separation and accurate typing of the PGM_1^3 isozymes are outlined. The PGM_1^3 isozymes detected proved to be useful in helping to determine parentage in an incest investigation. The pattern of segregation of the PGM_1^3 allele in four generations of this family and thermostability studies on the PGM_1^3 isozymes are presented.

KEYWORDS: pathology and biology, genetic typing, phosphoglucomutase

Phosphoglucomutase (PGM) (E.C. 2.7.5.1.) is an important metabolic enzyme that functions to bring storage polysaccharides into the glycolytic cycle. PGM polymorphism in man was first described by Spencer et al [*1*], who described two alleles, PGM_1^1 and PGM_1^2, which code for the three common phenotypes PGM_1 1-1, PGM_1 2-1, and PGM_1 2-2. Subsequent work by Hopkinson and Harris [*2,3*] identified the additional rare alleles PGM_1^3, PGM_1^4, PGM_1^5, PGM_1^6, PGM_1^7, and PGM_1^8, which occur at the same structural locus as PGM_1^1 and PGM_1^2.

The PGM_1^3 allele, which is the focus of this report, was first described in a single English family by Hopkinson and Harris [*2*]. Blake and Omoto [*4*], in a review of PGM types in the Asian-Pacific area, reported the detection of the PGM_1^3 allele in Japanese, Malaysian, and Indian populations, but not in polymorphic frequencies. However, the PGM_1^3 allele has been reported by Blake and Omoto [*4*] to reach polymorphic frequencies in certain areas of New Guinea and the Western Caroline Islands.

This report deals with the detection of the PGM_1 3-1 and PGM_1 3-2 phenotypes in a U.S. family, the usefulness of the phenotypes in helping to solve an incest case, the pattern of segregation of the PGM_1^3 allele in the family under study, and a look at the relative thermostability of the PGM_1 1-1, PGM_1 3-1, and PGM_1 2-2 phenotypes.

Experimental Procedures

Preparation of Samples

Blood samples were collected with the voluntary consent of all individuals tested by either venipuncture or finger puncture. Blood taken by venipuncture was drawn into tubes that

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contained no preservatives or anticoagulants. After the removal of the plasma and the completion of serological groupings, a portion of the packed red cells was transferred to a piece of clean white cotton sheeting. Because of the age and health of some of the participants and their unwillingness to leave their homes, some samples were taken by finger puncture onto clean white cotton sheeting. Each stain prepared on the sheeting was allowed to air-dry thoroughly and then was placed in an individual envelope and frozen at −20°C until analyzed.

Electrophoretic Separation and Enzyme Assay

PGM phenotyping was accomplished with a 1-mm horizontal starch gel that was allowed to run overnight in the refrigerator at 7 V/cm. The buffer system was a tris(hydroxymethyl)-aminomethane (Tris)/maleic acid/ethylenediaminetetraacetic acid (EDTA) buffer (pH 7.4), and the enzyme assay was a mixture of glucose-1-phosphate containing glucose-1,6-diphosphate, nicotinamide adenine dinucleotide phosphate (NADP), phenazine methosulfate (PMS), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) (all from Sigma), and glucose-6-phosphate dehydrogenase (Boehringer Mannheim) in a Tris hydrochloride buffer (pH 8.0). This technique was similar to that described by Wraxall and Culliford [5] except that the gel buffer was a 1:15 dilution of the electrode buffer and there were several other minor modifications.

Thermostability Studies

Thermostability studies were carried out with an apparatus similar to the one described by McAlphine et al [6]. Three sets of the PGM phenotypes PGM_1 1-1, PGM_1 3-1, and PGM_1 2-2 were run on a single gel plate as previously described, and at the completion of the run this gel was divided into three separate strips. Since these strips were thin and had a tendency to crack, they were transferred to a piece of thin plastic sheeting to facilitate handling. One strip was left as a control and the other two strips were placed on heating plates for varying times. When heated at 60°C the gel strips had a tendency to dry out and become brittle, and therefore it was necessary to keep these strips slightly damp at all times. After the gel strips had been heat-treated for the appropriate times, the strips were reassembled next to the control gel strip and all were simultaneously assayed for PGM activity.

Results and Discussion

A zymogram illustrating the PGM_1 3-1 and PGM_1 3-2 phenotypes is shown in Fig. 1. The $PGM_1{}^3$ allele codes for two isozyme bands: the major band, referred to in this report as the 3 band, is slightly cathodal to the d band, and the minor band, referred to in this report as the 3′ band, is slightly anodal to the e band. These isozyme patterns agree with those published by Hopkinson and Harris [2] and Blake and Omoto [4] and have been further confirmed by comparison with a known PGM_1 3-1 stain provided by Brian Parkin of the Metropolitan Police Forensic Laboratory in London, England. The blood samples have also been tested by Dr. Harvey Mohrenweiser of the University of Michigan for phosphopentomutase reactivity of the 3 and 3′ isozyme bands and no reactivity was found. The PGM_1 locus isozymes, unlike the PGM_2 locus isozymes, have no phosphopentomutase activity [7]; hence, the lack of phosphopentomutase activity by the 3 and 3′ bands further confirms that the variant described in this report must be a PGM_1 locus variant.

In a PGM_1 3-2 sample the overlapping d and 3 bands stain more intensely than the b band, whereas in a fresh PGM_1 2-2 sample the b band stains more intensely than the d band. However, it is unwise to rely on this distinction alone since most of the blood samples

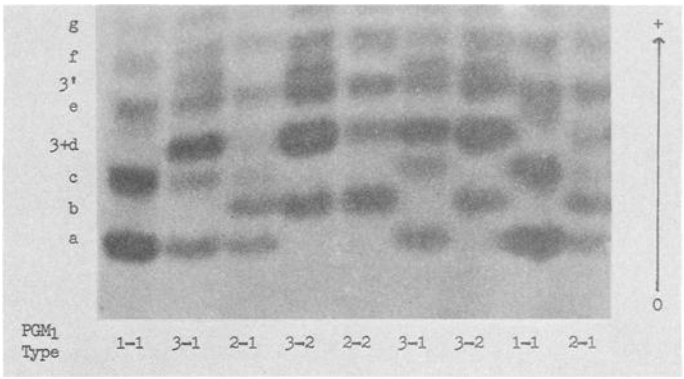

FIG. 1—*Photograph of a starch gel comparing the rare PGM phenotypes PGM_1 3-1 and PGM_1 3-2 with the more common phenotypes PGM_1 1-1, PGM_1 2-1, and PGM_1 2-2.*

received in a forensic science laboratory are not fresh and since occasionally in our laboratory a PGM_1 2-2 sample has been seen with equal staining intensities of the b and d bands, and rarely with a greater intensity of the d band. The PGM_1 3-1 sample poses less of a problem to type since the intensely staining 3 band is clearly separated from the c band. To avoid the problems posed by the overlapping of the d and 3 bands and to accurately type a PGM_1 3-1 and especially a PGM_1 3-2 sample, it is essential to achieve clear separation of the 3′ from the e band. Blake and Omoto [*4*] report that using a 1:10 dilution of the PGM electrode buffer in the gel will not give clear separation of the 3′ from the e band, but that using a 1:15 dilution will. Satoh et al [*8*] have speculated that improper separation of the PGM isozyme bands may have led to confusion in typing PGM_1^5 and PGM_1^3 phenotypes in Japanese populations. It is therefore suggested that maximum caution be used when typing an unknown stain as being PGM_1 3-1, or especially PGM_1 3-2, unless the 3′ band is clearly separated from the e band.

The PGM_1 3-1 phenotypes obtained were detected in the course of an incest investigation. Blood samples were brought into the laboratory from the individuals involved and the results of grouping tests obtained are shown in Table 1. The victim had an illegitimate child and had implicated her father (Suspect 1) and her uncle (Suspect 2) as the only possible fathers of her child. The individual numbers in Table 1 correspond to that individual's location in the family pedigree shown in Fig. 2, which will be discussed later.

Examination of the blood types shown in Table 1 clearly eliminates Suspect 1 as being the father of the child on the basis of the ABO and erythrocyte acid phosphatase (EAP) systems. Table 1 also shows that Suspect 2 could be the father of the illegitimate child in question to the extent of the blood groups tested. The fact that both the child and his suspected father possess the PGM_1^3 allele and that this allele is extremely rare in European populations greatly supports the victim's allegations that the father of her child was a member of her immediate family.

Of the original blood samples submitted to the laboratory, only PGM_1 3-1 and PGM_1 1-1 phenotypes were obtained. It was decided to test other family members in hopes of finding a PGM_1 3-2 phenotype and to study further the segregation of the PGM_1^3 allele in this family.

The family pedigree is shown in Fig. 2 and spans four generations. The oldest individual of this family is 85 years of age and the youngest is 2 years of age. The suspected father of Individual IV.1 is shown with a dotted line as being Individual II.5. In all, 21 individuals were tested and of these, seven were typed as being PGM_1 3-1 and four were typed as being PGM_1 3-2. The pattern of inheritance noted here further confirms the work of Hopkinson and Har-

TABLE 1—*Blood grouping data on individuals involved in an incest case.*

	Individual	ABO	Lewis		Rh					Erythrocyte Acid Phosphatase	Phosphoglucomutase	Haptoglobin	Esterase D	Glyoxalase	Adenylate Kinase	Adenosine Deaminase
			a	b	D	C	E	c	e							
Suspect 1	II.2	O	+	−	−	−	−	+	+	BB	3-1	2-1	1-1	2-1	1-1	1-1
Suspect 2	II.5	A	+	−	−	−	−	+	+	BA	3-1	2-1	1-1	2-1	1-1	1-1
Victim	III.5	O	+	−	+	−	+	+	+	BB	1-1	2-1	1-1	2-1	1-1	1-1
Child	IV.1	A	+	−	+	−	+	+	+	BA	3-1	2-2	1-1	2-1	1-1	1-1

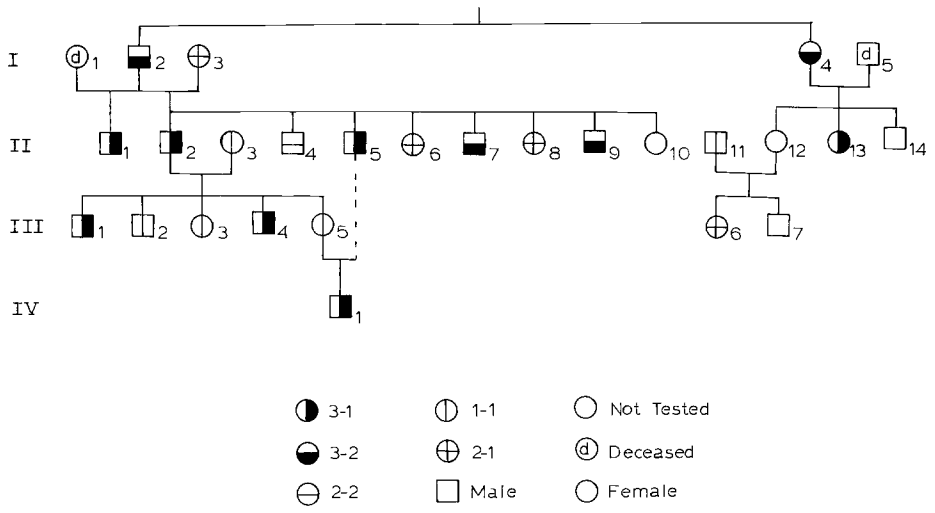

FIG. 2—*Pedigree of the family under study in this report showing the segregation of the* PGM_1^3 *allele through four generations.*

ris [2] and shows the PGM_1^3 allele to be codominant and autosomal in its mode of inheritance, which is the same as for all the other PGM_1 alleles.

The PGM_1^3 isozymes were also tested for their thermostability relative to the PGM_1^1 and PGM_1^2 isozymes. The method employed allows a visual comparison of the relative thermostabilities of the PGM isozymes without purification and *in situ* after electrophoresis. According to McAlphine et al [6], this method is comparable with the more traditional methods for studying the thermostabilities of isozymes.

Figure 3 is a photograph of a thermal inactivation test performed on PGM_1 1-1, PGM_1 3-1, and PGM_1 2-2 phenotypes at 60°C. After 5 min of heating, very little effect is noted in the isozyme patterns with the exception of a slight loss of staining intensity. After 10 min of heating, the minor isozyme bands of the phenotypes represented (the c band of PGM_1 1-1, the c and 3′ bands of PGM_1 3-1, and the d band of PGM_1 2-2) are seen to be decreasing rapidly in intensity. After 15 min of heating, most of the activity of the phenotypes has been eliminated and the only isozyme bands easily distinguishable are the a band of PGM_1 1-1, the a and 3 bands of PGM_1 3-1, the b band of PGM_1 2-2, and the e and f bands of all three phenotypes. After 30 min of heating, all the isozymes coded by PGM_1 have disappeared, leaving only the faintly staining e and f isozyme bands coded by PGM_2.

The PGM_1^3 isozymes have a relative rate of thermal inactivation similar to that of the PGM_1^1 and PGM_1^2 isozymes. McAlphine et al [6] reported that the PGM_1^1 and PGM_1^2 isozymes have a similar rate of thermal inactivation, but that the PGM_1^4 isozymes were less thermostable than the PGM_1^1 or PGM_1^2 isozymes. They also reported that the PGM_2 isozymes are more thermostable than the PGM_1 isozymes, and that fact is also shown in this study.

Thermostability studies may prove useful as a preliminary test in distinguishing PGM_1 locus variants from PGM_2 locus variants, especially for laboratories without access to substrates specific for the phosphopentomutase activity of the PGM_2 isozymes. However, this assumes that all PGM_1 locus variants are more thermolabile than PGM_2 locus variants, and that assumption has not yet been confirmed. Further experimentation into the usefulness of thermostability studies in PGM typing is continuing.

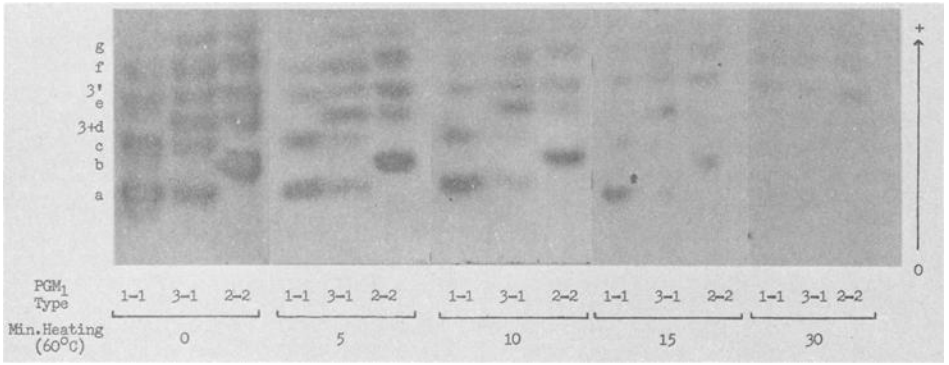

FIG. 3—*Photograph of a starch gel showing the relative thermostabilities of the PGM phenotypes PGM_1 1-1, PGM_1 3-1, and PGM_1 2-2 heated in situ at 60°C for 0, 5, 10, 15, and 30 min.*

Summary

1. Several individuals possessing the rare PGM phenotypes PGM_1 3-1 and PGM_1 3-2 have been detected, and electrophoretic techniques have allowed separation of the 3′ and e bands seen in these phenotypes. Criteria for accurate typing of PGM_1 3-1 and PGM_1 3-2 samples have been discussed.

2. The use of blood grouping tests to determine parentage in an incest case was aided by the detection of a PGM_1 3-1 phenotype in both the child and his suspected father.

3. A study of the segregation patterns of the PGM_1^3 allele in 21 members of the family in question has shown genetic control to be due to a codominant, autosomal allele at the PGM_1 locus.

4. Studies have shown that the PGM_1^3 isozymes have a relative rate of thermal inactivation similar to the PGM_1^1 and PGM_1^2 isozymes. Thermostability studies further support the contention that the blood samples identified as PGM_1 3-1 and PGM_1 3-2 in this report are a PGM_1 locus variant.

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