On the significance of isolated exclusions in the PGM1 subtype system

J. Bertrams, W. Weber*, P.G. Höher**

Prof. Dr. J. Bertrams, Abteilung für Laboratoriumsmedizin, Elisabeth Krankenhaus, Moltkestr. 61, 4300 Essen 1/FRG
*Institut für Blutgruppenforschung, Olpenerstr. 110, 5000 Köln 91/FRG
**Hygienisch-Bakteriologisches Institut, Klinikum Barmen, Heusnerstr. 40, 5600 Wuppertal 2/FRG

PGM1 subtyping by thin layer polyacrylamide gel isoelectric focusing (PAGIF) has become a commonly used part of paternity testing, since the method is simple, highly reproducible and relatively cheap. According to advantageous frequencies of the four common alleles PGM1*1A or a1(0.6356), PGM1*1B or a3(0.1194), PGM1*2A or a2(0.1910), and PGM1*2B or a4(0.0525) the exclusion probability increases from about 15% with conventional PGM1 typing to over 30% with isoelectric focusing techniques (IEF). The practicability of PGM1 subtyping for paternity testing is, however, restricted not only by a relatively high frequency of silent alleles, of partially deficient alleles, and of a large number of variants, but especially by the occurrence of incompatible heterozygous mother-child pairs and isolated exclusions.

Martin was the first to describe such a mother-child exclusion in 1981 (Table 1). Wetterling (1986) found two more cases of incompatibility among 8686 mother-child combinations and reported an additional case, communicated to her by V. Johnsson/Helsinki. All pairs were typed several times and confirmed by at least two experts. The mysterious inheritance of PGM1 subtypes, as given by IEF, prompted the Scandinavian colleges, not to base any paternity exclusion on results obtained only by PGM1 subtyping (Wetterling 1986).

Table 1 Isolated mother-child incompatibilities in the PGM1 subtype system

	Child	Mother
Martin 1981	PGM1 1A,2B (a1-a4)	1B,2A (a3-a2)
Wetterling 1986	PGM1 1A (a1)	1B,2A (a3-a2)
	PGM1 1B,2A (a3-a2)	1A,2B (a1-a4)
Johnsson 1985	PGM1 1A (a1)	1B,2A (a3-a2)

Our findings of three cases with an isolated exclusion of the putative father according to the results of PGM1 subtyping (Table 2) causes us to point again at the unsolved problem of formal genetics of PGM1 subtypes.

Advances in Forensic Haemogenetics 2
Edited by W.R. Mayr

Table 2 Isolated father-child incompatibilities in the PGM1 subtype system

	Child	Mother	Putative Father
Weber 1985	PGM1 1A,2A (a1-a2)	1A,2B (a1-a4)	1A (a1)
Weber 1985	PGM1 1A,1B (a1-a3)	1A (a1)	1A,2B (a1-a4)
Höher 1986	PGM1 2A,2B (a2-a4)	1B,2A (a3-a2)	1A,2A (a1-a2)

In the first case the child has inherited the allele PGM1*1A (a1) from its mother and accordingly the allele PGM1*2A (a2) from its father. The accused man was homozygous for PGM1*1A (a1) and could not be excluded by any other investigated genetic polymorphism. The probability of paternity according to Essen-Möller was 99,999999%. Altogether 30 genetic systems have been tested including the HLA antigens. The isolated PGM1 exclusion could be confirmed by repeated blood collections and different investigators. Also the use of additional IEF techniques, like LKB Immobiline Dry Plates, pH 5.6-6.6 (Application note 473) showed the same results.

The second case was also reported by Dr. Weber in 1985. In this case the mother was homozygous for the PGM1*1A (a1) allele and accordingly the child has inherited this allele from its mother and the PGM1*1B (a3) allele from its father. The accused man, however, showed the phenotype PGM1 1A,2B (a1-a4), lacking the allotype 1B (a3). Again a very high probability for paternity was calculated (W=99.9998%). Like in the first case the HLA system was also included.

In the third case, found by Dr. Höher, the child inherited the allele PGM1*2A (a2) from the mother and the PGM1*2B (a4) allele from its father. The disputed father, however, was heterozygous for 1A and 2A (a2-a1). This situation was faced by a probability for paternity of W=99.99%, which resulted from testing altogether 27 polymorphisms including HLA.

In all three cases PGM1 subtyping was performed by PAGIF according to the LKB instruction No 1804-121 with only minor modifications. The characteristics were as follows:

Gel: LKB ampholine PAG plates, pH 5.0-6.5 (T=5.5%; C=3.3%).
Electrode solutions: 0.01 M sodium hydroxide (cathode), 1% acetic acid (anode).
Temperature: 5°C.
Application of hemolysates:~3µl by sample applications pieces about 2cm from the anode.
Focusing data: Prefocusing for 30min. at 2000V, 15mA and 20W, followed by 150min. focusing time at the same conditions(80min. with and 70min. without application pieces).
Identification of phenotypes by functional agarose overlay according to Sutton and Burgess (1978).

As mentioned above the usefulness of PGM1 subtyping by IEF for paternity testing is complicated by the existence of silent alleles with a relatively high frequency, of rare alleles with reduced activity and of a large number of rare variants.

Silent alleles have been reported by Fiedler and Pettenkofer (1968,1969), Kaplan et al. 1970, Wendt et al (1971), Brinkmann et al. (1973), Horai (1974), Ueno et al. (1976), Schon and Thalhammer (1977), Gahr and Schroter (1981), Herzog and Libich (1982), and Ferrell et al. 1984. The frequency of PGM1*QO probably lies in the order of 0.001.

Partially deficient alleles of the PGM1 system were described by Brinkmann et al. (1972) and by Bertrams et al. (1986). The variant PGM1*W31 observed by Bertrams et al. had a reduced enzyme activity of only 25% of the normal heterozygote PGM1 A1 protein. According to this deficiency the variant could not be detected by conventional electrophoresis on cellulose acetate membranes or agarose gel, even after prolonged staining and overloading of the samples. A similar variant with a reduction to 5% of normal heterozygote activity, described by Brinkmann et al. (1972), could not be identified in conventional starch gel electrophoresis and resulted in an apparent mother-child exclusion in the second order.

It is well known that the PGM1 1B(a3) band in most cases is less intense than the 1A(a1) band. Accordingly PGM1 2-1 heterozygotes often show a characteristic pattern in starch gel or agarose gel electrophoresis with more or less reduced activity of the PGM1 bands ("PGM1 2-1red."). One can expect that "PGM1 2-1red."-types include a high percentage of PGM1 1B subtypes according to the decreased activity of this allotype. In an analysis of altogether 2151 samples with the PGM1 2-1 phenotype Weber found a highly significant association between PGM1 1B(a3) containing phenotypes and samples, which were classified as "PGM1 2-1red." according to faint PGM1 1 bands: PGM1 1B,2A (a3-a2) occurred in 49.71% among 175 "PGM1 2-1red." samples, compared to only 9.92% among 1976 PGM1 2-1 types with normal activity (Figure 2).

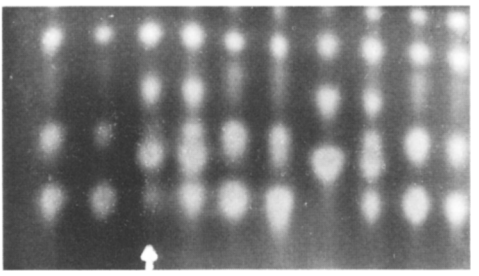

Figure 1. "PGM1 2-1red." phenotypes in starch gel electrophoresis.

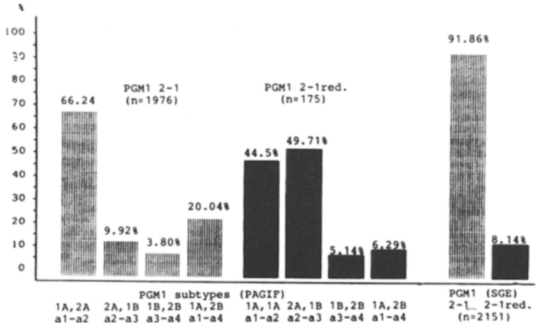

Figure 2. Distribution of PGM1 subtypes among 1976 PGM1 2-1 samples with equal activity of PGM1 1 and 2 bands compared to 175 samples with a reduced PGM1 1 activity ("PGM1 2-1red.").

Rare alleles of the PGM1 system could be located between as well as anodal or cathodal to the common PGM1 subtypes, partially overlapping the normally seen major and minor bands of classical subtypes and of PGM2 bands as well. At least 30 different rare variants were found and characterized up to date by conventional electrophoresis and IEF techniques (Dykes et al. 1985). It is necessary to use a minimum of two techniques to completely differentiate rare variants. All currently known variants can be detected by a combination of thin layer agarose gel electrophoresis (AGE), pH 7.4 (as described by Martin 1978) and PAGIF, pH 5-7. Additional IEF in agarose gel (AGIF) at a pH gradient of 4-8 (Dykes 1982) could be useful for identification of rare anodal variants, and eventually PAGIF, pH 6-8, for the identification of rare cathodal variants.

The above mentioned isolated mother-child and child-man exclusions, however, could not be explained - as far as heterozygotes are concerned - by rare alleles or alleles with reduced activity. Mistypings could be excluded according to repeated testing of freshly drawn blood samples and by different experts. Accordingly in some rare cases a clear discrepancy between gene and gene product does exist. It is striking that the PGM1 phenotype 1B,2A(a3-a2), normally occurring in only about 5%, was found in five of the seven cases reported here (Tables 1 and 2). In this connection it may be important, that in a larger Swedish material (n=6800) a significant excess of PGM1 1B,2A(a3-a2) heterozygotes and a corresponding deficit of PGM1 1A,2A(a1-a2) was found (Wetterling 1985). As shown in table 3 Martin (1981) and Kühnl et al. (1977) also observed more 1B,2A(a3-a2) phenotypes than expected, the difference, however, was not significant. In our own material from the Essen area observed and expected numbers of PGM1 1B,2A heterozygotes were exactly identical.

Table 3. Observed and expected numbers of PGM1 1B,2A heterozygotes in different population samples.

	Phenotype PGM1 1B,2A(a3-a2)		
	observed(%)	expected(%)	χ^2
Wetterling 1986	349/6800(5.13%)	306.1(4.50%)	6.01
Martin 1981	89/1580(5.63%)	75.6(4.79%)	2.37
Kühnl et al.1977	76/1506(5.05%)	72.89(4.84%)	0.14
Driesel et al.1982	22/496 (4.44%)	24.25(4.89%)	0.22
Bertrams 1987	100/2181(4.56%)	100 (4.56%)	0.00

A corresponding deficit of the phenotype PGM1 1A,2A(a1-a2) existed only in Wetterling's material (χ^2=2.52), but was absent in the data obtained by Martin (1981), Kühnl et al. (1977) and Driesel et al. (1982) and in our own analysed material. Thus a deviation from the Hardy-Weinberg equilibrium apparently does not exist.

In conclusion PGM1 subtyping by IEF is complicated by the existence of silent alleles, by at least 30 rare variants, by a number of hypo-synthetical variants and last but not least by a number of mysterious, inexplicable isolated mother-child and child-man exclusions. Accordingly we recommend again (Wetterling 1985), not to base an exclusion of paternity on isolated incompatibilities of PGM1 subtypes.

References

Bertrams J, Hintzen U, Barberan F (1986) A new partially deficient variant in the phosphoglucomutase 1 system, PGM*W31. Hum. Genet. 72: 177-178

Brinkmann B, Koops E, Klopp O, Heindl K (1972) Inherited partial deficiency of the PGM_1^1 gene: biochemical and densitometric studies. Ann Hum Genet Lond 35: 363-366

Brinkmann B, Riemann U, Heide KG, Sachs W, Walcher A, Heindl K, Hoppe H (1973) Demonstration of the silent allele PGM_1^0 in three families and description of a new variant. Z. Rechtsmedizin 73: 207-217

Driesel AJ, Basler M, Henke J (1982) PGM_1^a subtyping in Western Germany (Düsseldorf region). Z Rechtsmed 88: 313-316

Dykes DD, Copouls BA, Polesky HF (1982) Routine phenotyping of phosphoglucomutase (PGM1) by thinlayer focusing: Isoelectric points of 14 different variants. Electrophoresis 3: 165-168

Dykes DD, Kühnl P, Martin W (1985) PGM1 system. Report on the International Workshop, Munich 1983. Am J Hum Genet 37: 1225-31

Ferrell RE, Escallon M, Aguilar L, Bertin T (1984) Erythrocyte phosphoglucomutase: A family study of PGM1 deficient allele. Hum Genet 67: 306-308

Fiedler H, Pettenkofer H (1968) Ein "neuer" Phänotyp im Isoenzymsystem der Phosphoglukomutasen des Menschen (PGM1). 1. Mitteilung. Blut 18: 33-34

Fiedler H, Pettenkofer H (1969) Ein "neuer" Phänotyp im Isoenzymsystem der Phosphoglukomutasen des Menschen (PGM1). 2. Mitteilung. Blut 18: 358-362

Gahr M, Schroter W (1981) Red cell phosphoglucomutase (PGM) deficiency: hereditary defect of the PGM1 locus. Eur J Pediatr 136: 63-65

Herzog P, Libich M (1982) A new case of a silent allele in the PGM1 system. Hum Hered 32: 293-295

Horai S (1974) A new case of partial deficiency in red cell PGM isoenzyme. Jap J Legal Med 28: 259

Johnsson (1985), personal communication to Wetterling (1985)

Kaplan JC, Alexandre Y, Dreyfuß JC (1970) Deficit selectif dun des loci genetiques de la phosphoglucomutase dans les globules rouges. CR Seances Acad Sci (III) 270: 1061-1063

Kühnl P, Schmidtmann U, Spielmann W (1977) Untersuchungen zum Polymorphismus der Phosphoglucomutase (PGM-E.C.: 2.7.5.1) mittels isoelektrischer Fokussierung auf Polyacrylamidgel (PAGIF). Lectures 7th International Congress of the Society for Forensic Haemogenetics, Hamburg p 243

Martin W, Ziegler CH (1978) Die Darstellung der Erythrozyten-Isoenzymsysteme ADA (E.C.3.5.4.4) AK (E.C.2.7.4.3) und PGM1 (E.C.2.7.5.1) in der Agarosegel-Dünnschichtelektrophorese. Ärztl Lab 24: 125-129

Martin W (1981) Red cell enzyme groups in paternity testing. Lectures 9th International Congress of the Society for Forensic Haemogenetics, Bern, p 221

Schon R, Thalhammer O (1977) False positive galactosaemia screening. Lancet I: 43

Sutton JG, Burgess R (1978) Genetic evidence for four common alleles at the phosphoglucomutase locus (PGM1) detecable by isoelectric focusing. Vox Sang 34: 97-103

Ueno S, Yoshida H, Kiribayaski K, Omoto K (1976) The demonstration of the silent PGM1 allele in a Japanese family. Jap J Human Genet 21: 79-84

Wendt GG, Kirchberg G, Rube M, Ritter H (1971) Problematischer Mutter-Kind-Ausschluss mit PGM1. Humangenetik 11: 171-174
Wetterling G (1986) Incompatible mother-child pairs found in the PGM1 system. In: Brinkmann B, Henningsen K (eds) Advances in Forensic Haemogenetics Vol I. Springer Verlag Berlin, p 186