Discrepancy between Gene and Protein Products within the PGM_1 System Shown by Improved Resolution on Immobiline Gels

G. Wetterling

State Institute for Blood Group Serology
SRL, Regionsjukhuset
S-581 85 Linköping, Sweden

INTRODUCTION

Maternity exclusions according to the 1st rule of heredity have been observed within the PGM_1 system (Martin 1981; Wetterling 1986). In order to further investigate this deviation from the genetic rules, four such cases have been retested on LKB immobiline Dry Plates, which so far give the best separation of the PGM_1 isozymes.

MATERIAL and METHODS

Hemolysates

Blood samples from four incompatible mother-child pairs were obtained. Venous blood was collected without additive. Washed and packed red cells were lysed by addition of two volumes redestilled water containing 0.2 % mercaptoethanol as antioxidant. Complete hemolysis was achieved by freezing and thawing.

Isoelectric Focusing

Apparatus: LKB 2217 Multiphor II electrofocusing unit, LKB 2297 Macrodrive 5 power supply, LKB 2219 Multitemp II cooling bath and LKB 2117-915 reswelling cassette for dry gels.
Gel: LKB 1824-560 Immobiline^R Dry Plate pH range 5.6-6.6. Before use the gel is rehydrated in 0.5 % ampholine pH 5-7 over night.
Electrode solutions: 10 mM glutamic acid for the anode and 10 mM sodiumhydroxide for the cathode.
Temperature: +4^{o}C.
Application: 15 µl of the hemolysate were applied directly on the gel surface 1 cm from the anode (square 12) after prefocusing.
Running conditions: Prefocusing was performed during 30 minutes at 5000 V, 4.0 mA and 12 W followed by 3 hours of focusing at the same adjustment.

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Identification

The PGM phenotypes were visualized by an agar overlay method by Sutton and Burgess (1978), except that 0.08 mM Meldola blue was used instead of phenazine methosulphate.

RESULTS and DISCUSSION

During the years 1980-1986 12 682 mother-child pairs were typed for PGM_1. Fifteen incompatible pairs were found corresponding to a frequency of 0.0012 (Table 1). Four of these were incompatible according to the 1st rule of heredity (opposite heterozygosity) and eleven according to the 2nd rule of heredity (opposite homozygosity). These eleven can consequently be explained by the existence of a silent or an undetected rare allele and are not further discussed here.

The results from the other four mother-child exclusions were verified by new sampling at at least three different occasions with identical and clear results (Fig. 1). The blood grouping results of the mother, the child and the putative father in the four cases are shown in Table 3. Probability values of maternity and paternity were calculated and these values show strong relationship between mother and child. In two families the maternal grandmothers have been investigated and in one family a half-sister without finding any abnormal PGM_1 pattern.

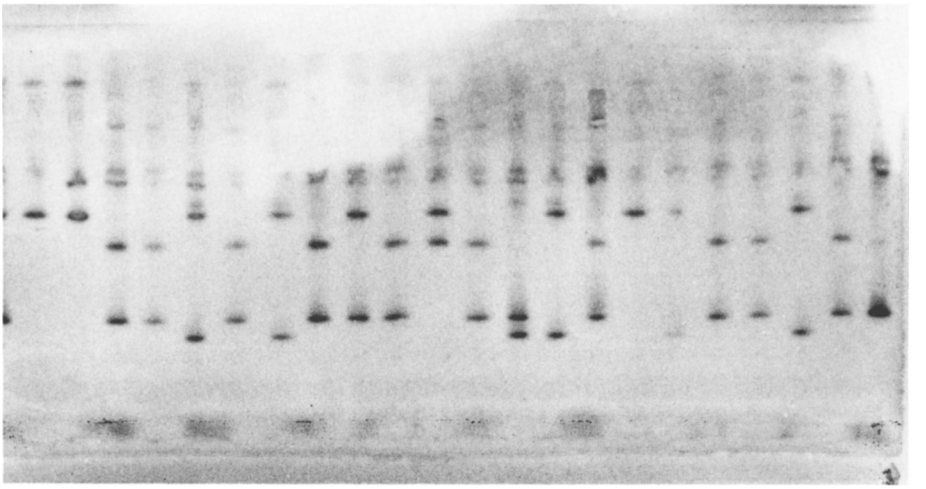

Fig. 1. PGM_1 phenotypes from left to right: a2a1, a2, a2, a4a1, a4a1(GM), a3a2, a4a1(M), a3a2(C), a4a1, a2a1, a4a1(GM), a4a2, a4a1(M), a3a1, a3a2(C), a4a1, a2, a3a2, a4a1(GM), a4a1(M), a3a2(C), a4a1 and a1. GM = grandmother, M = mother and C = child, all from case 4.

Documented mother-child exchange or similar exclusion has not been described in any other blood group system in the Swedish paternity test material.

In some cases there was an incompatibility within the PGM_1 system between father and child, although these fathers had very high indexes (e.g. 976) and accordingly were not excluded as possible fathers.

In other population studies similar maternity exclusions have been observed within the PGM_1 system namely an a3a2-a4a1 exclusion by Martin (1981) and an a3a2-a1 exclusion by Vivian Johnson, Helsinki (personal communication).

It is remarkable that the phenotype a3a2 is present in all these cases. Furthermore, in a large Swedish material (Table 2) a significant excess of the phenotype a3a2 and a deficit of the phenotype a2a1 are observed. When tested for Hardy-Weinberg equilibrium the whole material is very close to the 5 % significance level, mostly due to these deviations.

One explanation for these findings might be that the PGM-locus is relatively sensitive for mutations, which also is indicated by the many described rare alleles.

At the present it is preferable not to base any paternity exclusions on the PGM_1 system only.

REFERENCES

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Table 1. Mother-Child exclusions within PGM_1 during 1980-1986 in Sweden (12 682 pairs tested).

	Child					
Mother	a1	a2	a3	a4	a3a2	Total
a1		1	1	2		4
a2	2					2
a3	5					5
a3a2	2					2
a4a1					2	2
Total	9	1	1	2	2	15

Table 2. Distribution of PGM_1^a phenotypes in a sample of 6800 unrelated adult Swedish persons.

Phenotypes PGM_1^a	Observed n	Expected n	χ^2
a1	2804	2751.9	0.9864
a2a1	1186	1241.9	2.5162
a3a1	1313	1356.6	1.3630
a4a1	546	547.6	0.0047
a2	146	140.1	0.2485
a3a2	349	306.1	6.0124
a4a2	127	123.6	0.0935
a3	169	167.2	0.0194
a4a3	132	135.0	0.0667
a4	28	27.2	0.0235
Total	6800		11.3343

For d.f. = 6 $0.10<p<0.05$*
The rare phenotypes are not included.

Table 3. Blood grouping results of the excluded child (C), the mother (M) and the putative father (PF) from the four cases with PGM_1^a exclusion according to the 1st rule of heredity

Blood Group	Case 1			Case 2			Case 3			Case 4		
	M	C	PF	M	C	PF	M	C	PF	M	C	PF
ABO	A_1	A_1	A_1	A_1	A_1	A_2	A	O	A	O	O	O
MNSs	N	N	N	NS	MNSs	MSs	MNSs	MNs	MNs	Ns	MNs	MSs
Rh	cde	cDEe	cDE	CcDEe	cDEe	cDEe	CcDe	cde	CcDe	cDEe	cDEe	cDEe
Kk				k	k					k	k	k
Fy_a				a	a					ab	ab	a
Jk_a				a+	a+							
Xg_a				a+	a+							
Gm				1,-2	1,-2							
P_1				+	+							
Hp	2	2,1	1	1	2,1	2	2	2	2	2	2	2,1
Gc	2,1S	2,1S	2,1S	2,1S	1S	1S	1S	1S	1S	2,1S	2,1S	2,1S
C3	2	2	2	2	2	2						
Tf	2,1	1		2	2	3,2						
Bf				F	F,S							
Pi				2	2,1							
F13B				2,1	1							
PGM	**a3a2**	**a1**	**a3a1**	**a4a1**	**a3a2**	**a2a1**	**a3a2**	**a1**	**a3a1**	**a4a1**	**a3a2**	**a3a1**
EAP	B	BA	BA	BA	BA	B	CB	CB	B	B	BA	BA
GLO	2	2	2	2,1	1		2	2,1	2,1	1	1	2,1
EsD	2,1	2,1	1	1	1							
AK				1	1					1	1	1
ADA				2,1	2,1					1	1	1
HLA										A2; B12,40	A2,10; B18,40	A9,10; B18,35
Probability of maternity %	95.3			99.9			85.5			98.4		
Probability of paternity %			99.0			89.9			77.7			99.6