INCOMPATIBLE MOTHER-CHILD PAIRS FOUND IN THE PGM_1 SYSTEM.

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Introduction

Since Spencer et al. (1964) demonstrated polymorphism within human red cell phosphoglucomutase incompatible mother-child pairs have been reported. When Bark et al. (1976) succeeded in subtyping PGM_1 this deviation from the rules of inheritance also appeared within the subgroups revealing incompatible homozygosity (Prokop et al. 1981 and Polesky et al. 1983) as well as incompatible heterozygosity (Martin, 1981).

The present paper calls in question the use of PGM_1^a as a single excluding marker in paternity cases.

Material and Methods

Hemolysates

Venous blood samples from 8686 mother-child pairs involved in cases of disputed paternity in Sweden during the years 1980-1984 were collected without additive. The blood cells were washed three times with isotonic saline and lysed by the addition of 0.2 % mercaptoethanol followed by freezing and thawing to achieve complete hemolysis. The lysates were tested within a week after venepuncture. All samples were examined twice and the mother-child exclusions were confirmed by repeated sampling.

Isoelectric focusing (IEF)

Apparatus: LKB 2117 Multiphor; LKB 2103 Power Supply. Gel: LKB ampholine PAGplates pH 5-6,5 and 0.5 mm gels (T = 5.5 %; C = 3.3 %) containing 4 % LKB ampholine pH 5-7. Electrode solutions: 0.01 M sodium hydroxide (cathode) and 1 % acetic acid (anode). Temperature: + 5°C. Application: The hemolysates were applied directly onto the gel surface with 25 ul microcaps 1.5 cm from the anode. Focusing procedure: Prefocusing was performed during 30 min at 2000 V, 20 mA and 25 W followed by 2.5 h of focusing at the same adjustment. Identification: The phenotypes were visualized by an agar overlay method (Sutton and Burgess, 1978).

Agarose gel electrophoresis (AGE)

All samples have also been investigated by thin layer agarose (0.8 % Litex HSC) gel electrophoresis. This was performed with a 0.025 M tris-maleic buffer as bridge buffer and 1/5 dilution as gel buffer during 2 h and at 12 V/cm.

Nomenclature

The nomenclature proposed by Kühnl et al. (1977) was used.

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Table I. Mother-Child exclusions within PGM_1 during 1980-1984 in Sweden (8686 combinations tested).

	Child					
Mother	a1	a2	a3	a4	a3-a2	Total
a1		1	1	1		3
a2	2					2
a3	3					3
a3-a2	1					1
a4-a1					1	1
Total	6	1	1	1	1	10

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

Phenotypes PGM_1^a	Observed n	Expected n	X^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. = 3 $p < 0.05$
The rare phenotypes are not included.

Results and Discussion

Out of 8686 investigated mother-child combinations ten incompatible pairs were found, which gives a frequency of 0.0012 (table I).

Eight of these exclusions show contrary homozygosity. No extra bands have been observed by seven of them, pointing towards some rare allele, consequently the probable explanation is the occurence of a silent allele. Neither family studies nor quantitative enzymatic investigations have been performed. The eighth mother-child exclusion a1/a3 show a discrepancy against the AGE typings where both mother and child were typed as 2-1. A closer investigation revealed a very weak blur in the a2 position on the IEF-gel. This problem is earlier discussed (Svensson, Wetterling, 1982) and if the explanation is some rare allele, unsensitivity in the method or both I will leave unsaid.

Furthermore, two pairs show contrary heterozygosity (a4a1/a3a2 and a3a2/a1). A larger list of a Swedish PGM_1^a material exhibits an excess of the type a3a2 and a corresponding deficit of a2a1 (table II). This would imply that certain a2a1 were mistyped as a3a2. But there is no such risk due to similarities between the phenotype patterns. Experimentally by repeated freezing and thawing of the blood samples the PGM_1^{a1} allele shows a clear trend to go over to the PGM_1^{a3} allele after about three weeks. This indicates that the PGM_1^{a1} allele in some way is labile. However, no discrepancies of the type a3a2 against a2a1 have been observed since the subgrouping of PGM started and the child a3a2 with mother a4a1 have been clearly typed on fresh samples by three occasions. Heterozygote mother-child exclusions have also been reported by Martin (1981) a3a2/a4a1 and by Vivian Johnsson, Helsinki, a3a2/a1 (personal communication).

Conclusion

Due to problems with the relatively high frequency of silent and rare alleles and even supposed incorrect typings within PGM_1^a, it is recommended not to base any exclusions in paternity cases on this system solely.

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