"Silent" Alleles in Paternity Testing

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INTRODUCTION

"Null" alleles appear at almost all the genetic systems used in disputed parentage cases. So far, it seems that 0.001 value is a reasonable estimate for null alleles in most systems according to the reports of Gershowitz (1983) and Polesky et al. (1983).

Goya et al.(1972) mentioned a patient with a deficiency of transferrin in which only traces of transferrin are detected in his serum. His parents had a serum transferrin concentration about half the normal value. It presumably represents a case of familial congenital atransferrinemia. A transferrin "null" alleles has been observed by different authors (Weidinger et al. 1984; Giari et al.1985; Lukka et al. 1985). A alpha-1-antitrypsin (Pi) "null" allele in the heterozygotic form was also described by Weidinger and Cleve (1984) in a case involving a child-pretense father pair.

We found two cases with "silents" alleles in two polimorphic proteins — transferrin (Tf) and alpha-1-antitrypsin (Pi).

MATERIAL and METHODS

We used sera from disputed paternity cases routinely examined in our institute. Sera were separated and stored at -20 ºC.

For Tf subtype determination, the serum samples were diluted 1:5 with 0.5 % w/v ferrous ammonium sulphate and incubated overnight at 4 ºC.

Isoelectric focusing (IEF) was carried out in thin-layer polyacrylamide gels (5% T, 3 % C; 230X105X0,5 mm). Ampholyte concentration was 5,6%. For Pi typing, the pH range was established with a mixture of 0.6 ml of ampholyte pH 3.5-5 and 0.15 ml of ampholyte pH 4-6. Ampholine pH 5-7 was used for Tf typing. Staining of the gels was carried out with Coomassie Blue R 250.

In the case of Tf, immunofixation was made directly on the gel with monospecific antiserum against human transferrin at a dilution of 1:4 (Dakopatts).

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Quantitative determinations of serum transferrin and alpha-1-antitrypsin were performed by the radial immunodiffusion method on agarose plates.

RESULTS and DISCUSSION

An apparent exclusion of maternity was found in Pi system in a Caucasian trio tested for disputed paternity (Fig. 1).The phenotypes of the trio - II2; II3; III1 - would exclude the mother. However no other contradictions to the rules of inheritance were observed in the other genetic systems (red cell antigens, serum proteins and red cell enzymes). A family study was performed and a similar apparent exclusion is also present.

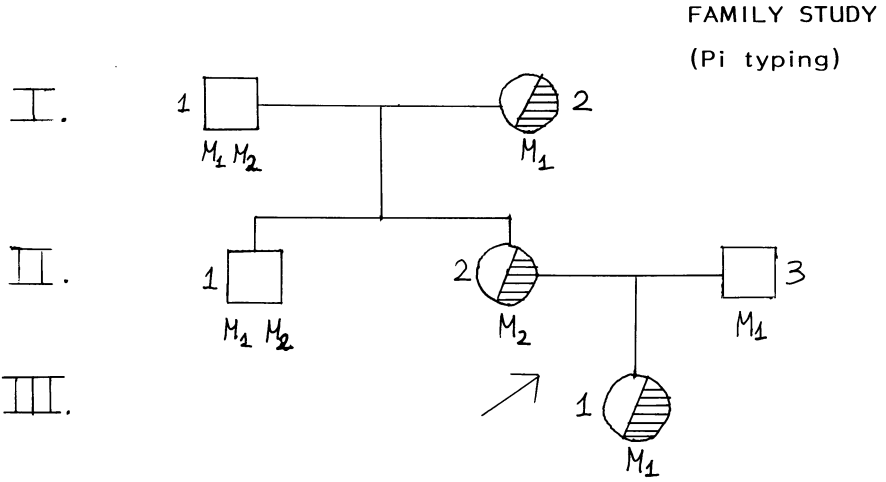

Fig. 1 - Pedigree of the family with Pi* Q0 carriers.
("null" ≡); ◪ = carriers of PiQ0

No other inconsistencies were observed in the other systems studied on this family.

The existence of a "null" allele was therefore, postulated in this case: The child was classified as PiM1Q0, the mother as PiM2Q0 and the grandmother as PiM1Q0. The results of alpha-1-antitrypsin testing are shown in Figure 2.

These finding were confirmed by quantitative studies.

The alpha-1-antitrypsin concentration in grandmother's, mother's and child's sera was reduced to approximately 50 % of normal.

In Figure 3 the results of serum α_1-AT concentrations are shown.

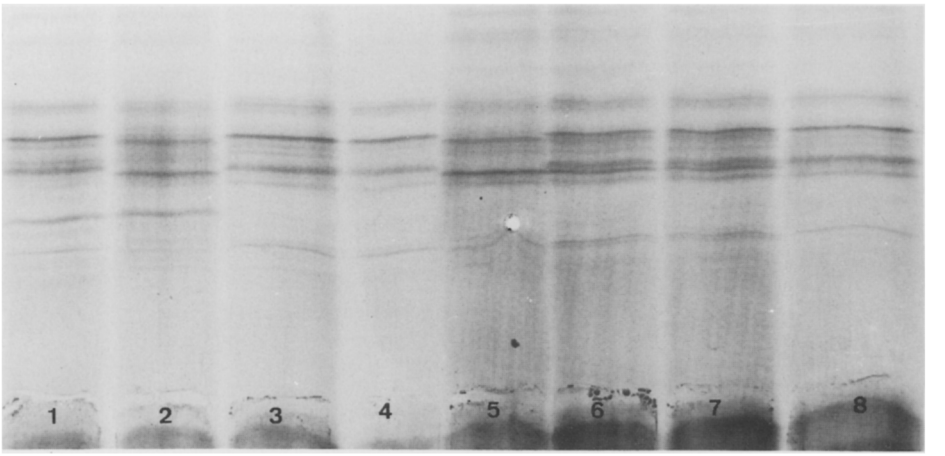

Fig. 2 - IEF of alpha-1-antitrypsin (Pi) phenotypes:
1=PiM_1S 2=PiM_2S 3=alleged father II-3, PiM_1;
4=child III-1, $PiM_1(QO)$; 5=mother II-2, $PiM_2(QO)$
6=grandfather I-1, PiM_1M_2; 7=brother II-1, PiM_1M_2
8=grandmother I-2, $PiM_1(QO)$.

		Control serum	- 275 mg/100 ml	- 100 %
Family members	II3 =	PiM_1	- 259 mg	- 94 %
	II2 =	M_2QO	- 144 mg	- 52 %
	III1 =	M_1QO	- 79,9 mg	- 29 %
	I1 =	M_1M_2	- 275 mg	- 100 %
	I2 =	M_1QO	- 156 mg	- 56 %
	II1 -	M_1M_2	- 269 mg	- 98 %

Fig. 3 - Quantitative determination of serum α_1-AT concentrations by single radial immunodiffusion.

In other case of paternity analysis, the child had a no detectable transferrin phenotype, whereas mother and pretense father had both C1 phenotypes, as can be seen in Figure 4.

The case was studied by immunofixation analysis with a monospecific anti-Tf-immunoglobulin. Neither other common variants were observed nor rare Tf variants were obtained in the child and his parents.

No other inconsistencies were observed in the other systems studied in this case, and there is no history of consanguinity.

Quantitative determinations of serum transferrin were made by the single radial immunodiffusion assay. In mother's and pretense father's serum was found less than 50 % of the normal transferrin

concentration. In child's serum was detected a very low concentration (Fig. 5).

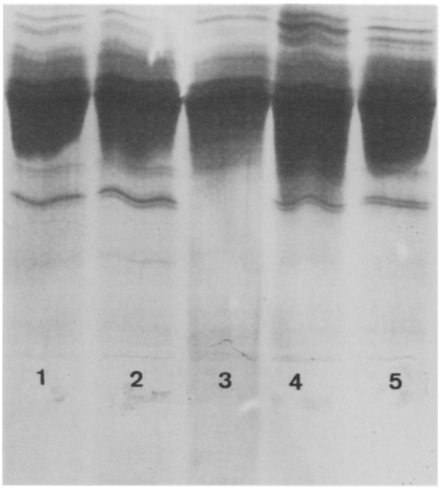

Fig. 4 - Transferrin (Tf) phenotypes: 1=C_1C_3; 2=alleged father, TfC_1QO 3=child, TfQO 4=mother, TfC_1QO 5=C_1C_2

Control serum - 280 mg/100 ml - 100 %

2. P. Father	- TfC_1QO	- 116 mg	- 41 %
4. Mother	- TfC_1QO	- 112 mg	- 40 %
3. Child	- TfQO	- 35 mg	- 12, 5 %

Fig. 5 - Quantitative determination of serum Tf levels by single radial immunodiffusion.

The presence of a "null" allele was then assumed in this trio. A Tf "null" allele was detected in both mother and pretense father in heterozygous states-Tf C1QO as well as in the homozygotic form in the child-TfQO.

When "null" alleles occurs in a polimorphic protein systems, difficulties may arise without a more accurate study. The presence of such alleles should be considered, in reaching a conclusion in disputed paternity.

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