An Evaluation of the Use of DNA Probe hMF1 in Cases of Disputed Paternity by Comparison with a Range of Well Established Blood Group Polymorphisms

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INTRODUCTION

Recently discovered polymorphisms at the DNA level appear to offer great potential in the field of parentage testing (Jeffreys 1985a, 1985b).

DNA probe hMF1 (human Molecular Fingerprinting) was discovered in the laboratory of one of us (DIH) and its applications to parentage problems has been investigated.

In this study the results from tests using some 17 well established red cell antigen, serum protein and red cell enzyme polymorphisms are compared with results obtained using probe hMF1. Conventional systems used included ABO, MNSs, Rh, Duffy, Kell, Lutheran, Hp, EAP, Glo, PGM, Gc, ESD, ADA, AK, PLG, Tf and Pi.

Probe hMF1 is a member of the Midi-Sat family of hypervariable tandem repeats and has a repeat unit of 40bp.

METHODS

The tests for the 17 conventional blood grouping systems were carried out by standard methods.

For hMF1 testing, DNA samples for analysis were routinely prepared from 100ul of whole blood largely following the method of Gill (1985). Following digestion with a suitable restriction enzyme, in most cases Taq 1, they were electrophoresed on 25cm 0.7% agarose/TAE gels at 30-50 V for 20-48 hours. The DNA was Southern blotted onto Biotrace RP nylon membrane (Gelman Sciences Ltd., Northampton, U.K.). The probe was labelled by nick translation as the isolated insert and hybridisation was carried out at 65°C overnight. Washing was generally to 0.1 x SSC at 65°C and autoradiography was for a period of 6-48 hours.

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

RESULTS

A total of 51 families were tested by both hMF1 and conventional methods. Of those, 14 gave no result by DNA testing, either as a result of insufficient DNA in one or more of the samples, or as a result of incomplete digestion of the DNA in one or more of the samples. Both of these problems have now been overcome by refinements to the method of DNA preparation.

1. Families Showing No Exclusions on Conventional Tests

Table 1. Results of families showing no exclusions on conventional tests.

	Conventional tests	hMF1 tests		
	Non-Ex	Non-Ex	A-Ex	NUR
No. of families	26	19	2[a]	5

Non-Ex : Non-excluded men
A-Ex : Apparently excluded men
NUR: No Useful Result (Mother and child showing identical band patterns)

a : see Table 2.

Table 2. Apparent exclusions from hMF1 testing

	PI from conventional tests	No. of apparent excluding bands
Case 1	155	1
Case 2	20333	1

In Case 1. the child's DNA was only partially digested and the profile showed one exclusion band and one maternally specific band, both of which dissappeared when the test was repeated. (See Fig. 1).

In Case 2, the child showed one very clear exclusion band which did not appear in either parent. The mother's DNA was only partially digested with a high background signal. When repeated the mother's DNA still did not digest to completion but the apparent exclusion band from the child was now visible in the mother.

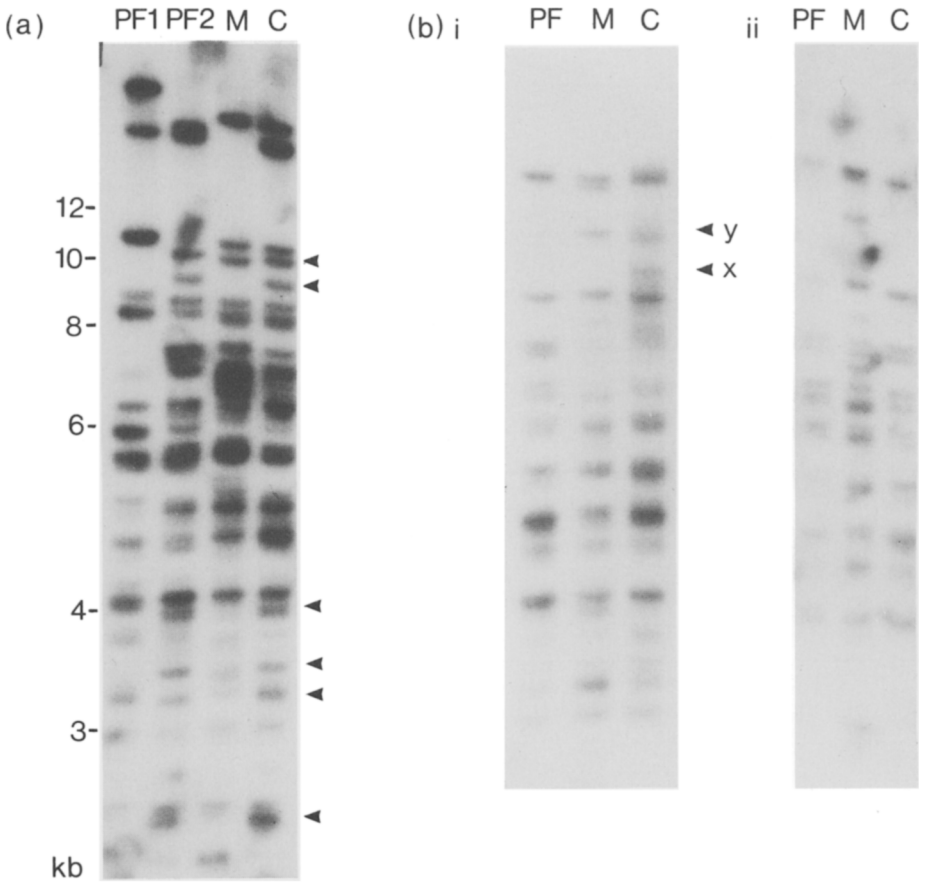

Fig. 1 (a) DNA profiles of a family in a case of disputed paternity involving 2 putative fathers (PF1, PF2), mother (M) and child (C). Paternally derived bands present in the child and PF 2 only are arrowed. These bands exclude PF1 from paternity and the large number of paternally specific bands showed by PF2 and the child strongly suggest that he is the true biological father. (b) DNA profiles of a family showing an apparent exclusion on hMF1 testing where no exclusions were found by conventional tests. In the first test (i), the child appears to show no exclusion band (x) and a maternally specific band (y), both of which are missing when the test was repeated (ii). The fuzzy background signal on the autoradiograph and the presence of DNA of higher than expected molecular weight on the gel (when visualised by ethidium bromide staining (UV transillumination) are indicative of the partial digest responsible for these artefacts.

2. Families Showing Exclusions from Paternity on Conventional Tests

Table 3. Results of families showing exclusions on convetional Tests

	Conventional tests	hMF1 tests		
	Ex	Ex	Non-Ex	NUR
No. of families	11	6	2	3

Ex : Excluded Man
Non-Ex : Non-excluded men
NUR : No Useful Result

Table 4. Cases showing exclusion from paternity on conventional tests but no exclusion by hMF1 testing.

	Conventional systems showing exclusions	No. of paternal bands shared by man and child
Case 1	PGM (1st Order)	1
Case 2	No exclusion from 17 systems Only subsequent HLA testing showed exclusion.	6

In Case 1, where man excluded by conventional tests, only 1 band seen shared by child and PF. In Case 2, the putative father was excluded only by HLA testing and not by any of the other 17 conventional systems used. The HLA genes A2 or Ax, B27 and CW1 present in the child and of paternal origin were lacking in the putative father.

The presence of 6 bands of known paternal origin shared by the child and the putative father is surprising. The possibility of the true father being a close relative of the excluded man could explain this finding.

3. Estimation of the Power of Exclusion of hMF1

By construction of false families by using unrelated individuals on the same gel as mother and child as putative fathers, the database for exclusion statistics can be extended.

From a total of 100 families, an exclusion rate of 79% was calculated for hMF1.

SUMMARY

The results obtained using the probe hMF1 are very largely in agreement with those obtained by conventional testing.

The cases where partial digests result in false exclusions are of obvious importance and highlight the relevance of testing in parallel with conventional systems and need for careful monitoring of digestion.

The estimated exclusion rate of 79% makes hMF1 a useful tool in parentage testing, particularly in conjunction with conventional systems.

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