DNA FINGERPRINTING IN PATERNITY ANALYSES

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INTRODUCTION

Most cases of disputed paternity can be adequately resolved by testing 26 blood group, serum and enzyme systems, ev. completed by testing the HLA-System. In some cases however, the probability of paternity according to Essen-Möller is lower than 99,80%, which is the limit required by Swiss courts. In a few cases, exclusions, suggestive of a possible mutation or a null allele, make the analyses inconclusive.
A set of such cases was analysed by DNA fingerprinting (1, 2, 3, 4). In each case an unequivocal conclusion was reached.

MATERIAL AND METHODS

Leucocytes were collected from 10ml EDTA treated blood after centrifugation. Contaminating erythrocytes were lysed by addition of 10 volumes of 50mM KCl and incubation at 37°C for 15 minutes. After recovering the leucocytes by centrifugation, the lysis in 50mM KCl was repeated.
High molecular weight DNA was isolated according to Dykes (5) with some modifications: the leucocytes were resuspended in 9ml of 75mM NaCl, 25mM EDTA, 10mM Tris-HCl pH 7.6; then 0.9 ml 10% SDS and 100µl Proteinase K (10mg/ml in 10mM Tris-HCl pH 7.6, 1mM EDTA) were added.
After protein digestion, the peptides were precipitated by addition of 3ml of saturated NaCl solution and pelleted by centrifugation. The DNA was precipitated from the supernatant by addition of 2 volumes of absolute ethanol and was resuspended in 10ml Tris-HCl pH 7.6, 1mM EDTA.
DNA samples of 11µg were digested with restriction endonuclease Hinf I (Boehringer Mannheim, FRG) or Hae III (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.
After ethanol precipitation the DNA fragments were subjected to gel electrophoresis (0.8% agarose gel in Tris-borate buffer, at 40V for 40h). Then the DNA was transferred to a nylon membrane (Hybond N, Amersham, Buckinghamshire, UK) either by capillary transfer or by vacuum blotting.
Hybridization to the probe 33.15 (Cellmark Diagnostics, Abingdon, UK) labelled with ^{32}P by primer extension was carried out in 10% dextran sulfate, 0,5M NaCl, 1% SDS, 100µg/ml denatured salmon sperm DNA for 16h at 60°C. The membranes were washed twice in 2xSSC*, 0.1% SDS and twice in 1xSSC*, 0.1% SDS at 60°C and then autoradiographed at -70°C with AGFA-CURIX RP1L film (Agfa-Gevaert, Leverkusen, FRG).
Hybridization to the biotin-labelled probe MZ 1.3 (Biotest, Frankfurt, FRG) and subsequent colour development was carried out according to the manufacturer's instructions.
In some cases, the probe was removed from the filter by incubation in 0.4M NaOH at 42°C, followed by neutralization in 0.2M Tris-HCl pH 7.6, 0.1xSSC*, 0.1% SDS. Then the filter was hybridized again as described above.
*(20xSSC = 3M NaCl, 0.3M Na citrate)

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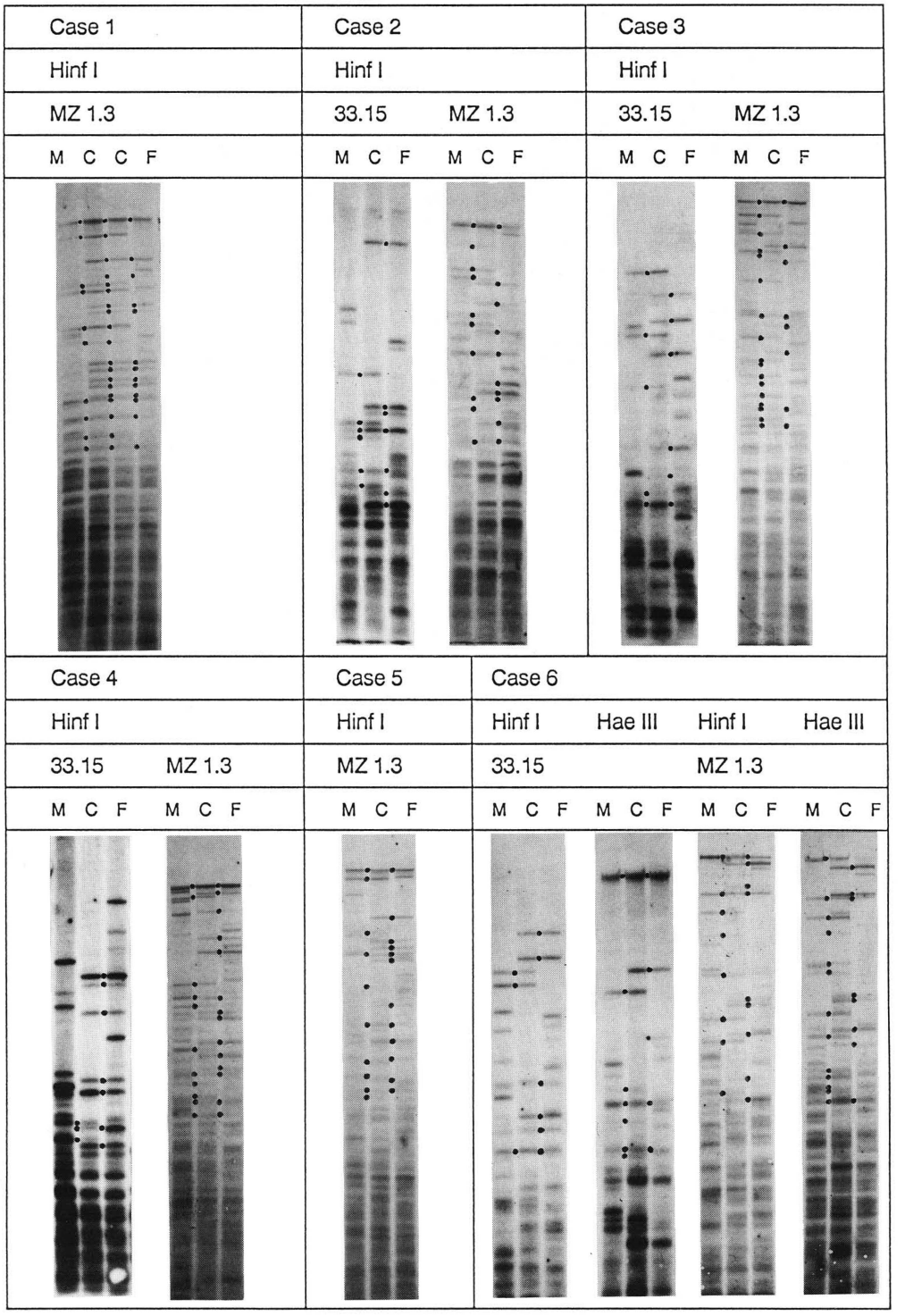
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
ABO Rhesus MNSs, P_1 Kk,Fy,Jk	no exclusion	no exclusion	Fy(a-b+)/ Fy(a+b-)	no exclusion	no exclusion	no exclusion
Hp-S, Gc Gm(afxbg) Km(1), PLG Ag(x,y), C3 Bf, Tf, Pi	no exclusion	no exclusion	no exclusion	PLG 2/ PLG 1	no exclusion	no exclusion
ACP, PGM_1 ADA, Ak GPT, ESD Gt, GLO	no exclusion	no exclusion	no exclusion	GLO 1/ GLO 2	Gt Oron-1/ Gt 1 (6)	no exclusion
HLA-A,B,C	not done	not done	no exclusion	no exclusion	no exclusion	no exclusion
Additional tests	none	none	none	C6, α_2HS ORM_1, F XIIIB no exclusion	none	Lu^a, Co^b F XIIIB no exclusion
Bio-statistics	95,80%	91,32%	88,89%; with HLA 99,97%	with HLA 91,5%	>99,9999%	with HLA 99,993%; with add.tests 99,9998%
Conclusion after DNA-finger-printing	paternity confirmed	paternity confirmed	paternity confirmed; proband orignates from north Africa	paternity confirmed; silent alleles in PLG- and GLO-System	paternity confirmed; mutation to Oron-type	paternity confirmed; the accused man sub-mitted an expert evi-dence that he was infertile



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