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DNA investigations on fetal material from paternity cases

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Summary. Three paternity cases have been investigated where DNA was extracted from fetuses (age: 8–10 weeks old) after interruption of pregnancy. In each case it was possible to clearly identify the putative father using 5 or 6 single locus probes (SLP's). Fetal bands (SLP) could be clearly identified from mixtures of placental and fetal DNA by comparison with the maternal and paternal bands. However, it was very difficult to resolve the fragment patterns of tissue mixtures with one multi locus probe (MLP), because of band overlap. Another advantage of using SLP's was that biostatistical calculations could be carried out and very informative Essen-Möller values for the probability of paternity were obtained.

Key words: VNTR-Polymorphisms – Single locus probes – Multi locus probes – Fetal DNA – Paternity

Zusammenfassung. Untersucht wurden 3 Paternitätsfälle, in denen nach Abort aus 8–10 Wochen alten Foeten DNA extrahiert worden war. In allen Fällen konnte unter Verwendung von jeweils 5–6 Singlelocus Sonden (SLS) der Putativvater eindeutig identifiziert werden. So konnten auch bei Vorliegen von plazentalem und foetalem Mischgewebe durch Vergleich mit dem mütterlichen bzw. PV-Fragmentmuster, die foetalen Banden mit SLS's zweifelsfrei zugeordnet werden. Mit einer Multilocus Sonde (33.15) hingegen ließ sich ein Mischgewebe-Fragmentmuster aufgrund tw. überlappender Bandenpostionen nicht mehr auswertbar auflösen. Ein weiterer Vorteil der SLS's bestand darin, daß biostatistische Berechnungen durchgeführt werden konnten, die zu sehr aussagekräftigen Essen-Möller-Werten führten.

Schlüsselwörter: VNTR-Polymorphismen – Singlelocus Sonden – Multilocus Sonden – Fetale DNA – Paternität

Introduction

Many investigations of filiation on fetal material have been previously reported using classical systems (e.g. Sulzman 1964; Siejskal et al. 1973; Eriksen 1983) but results with high evidential value could only be obtained from a few systems such as PGM, AK, ADA, EAP (Madea et al. 1986). These methods of investigations are basically limited and considerably complicated by the presence of tissue mixtures (e.g.placenta-blood, placenta-tissue and fetal tissue with maternal cells). The value of the results is also diminished by concentration differences between maternal and fetal tissue and blood components and by the incomplete expression of erythrocyte antigens in 8 to 10-week-old fetuses.

The investigation of DNA polymorphisms does not suffer from the same limiting factors and reliable evidence can also be obtained from placental tissue.

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Materials and methods

Blood samples were collected in EDTA, haemolyzed with 0.05 *M* KCl and digested with proteinase K (2 mg/ml, Pharmacia, FRG) overnight at 37°C. DNA was extracted twice with phenol/chloroform (1:1), once with chloroform/isoamylalcohol (24:1) and precipitated by addition of 1/10 vol sodium acetate (pH 5.2) and 2½ vol absolute ethanol.

Where it was possible to identify fetal fragments (case 3) these were separated from the tissue mass and extracted separately. In both other cases, however, no clear distinction could be seen and the tissue was extracted in toto.

Fetal and placental tissue samples were extracted by homogenising in buffer A (10 m*M* Tris pH 8.0, 10 m*M* EDTA, 10 m*M* NaCl, 0.6% SDS), digested with proteinase K for 4 hr at 56°C followed by phenol/chloroform extraction and ethanol precipitation as described above. DNA concentrations were measured fluorimetrically and controlled electrophoretically by side-by-side comparison with known concentrations of Lambda DNA. Aliquots of DNA (3 µg) were digested with HinfI (30 U, Gibco, BRL; UK) and the degree of digestion was checked by electrophoresis.

Separation of the DNA fragments was carried out by electrophoresis in 0.7% agarose gels (length 23 cm) for 24 hr and 80 V at 4°C. The DNA was denatured, transferred to nylon membranes (Hybond-N, Amersham, FRG) by Southern blot (20 × SSC) and the resulting blots were baked at 80°C for 6 hr. The probes MS1, MS8, MS31, MS43, G3 and 33.15 were obtained from Cellmark Diagnostics UK; YNH24 was obtained from Promega Corporation WI.

Labelling of the probes was carried out by random priming (Feinberg and Vogelstein 1983) with an average radioactive incorporation of 2×10^8 cpm/µg for MLP's and 4×10^8 cpm/µg for SLP's

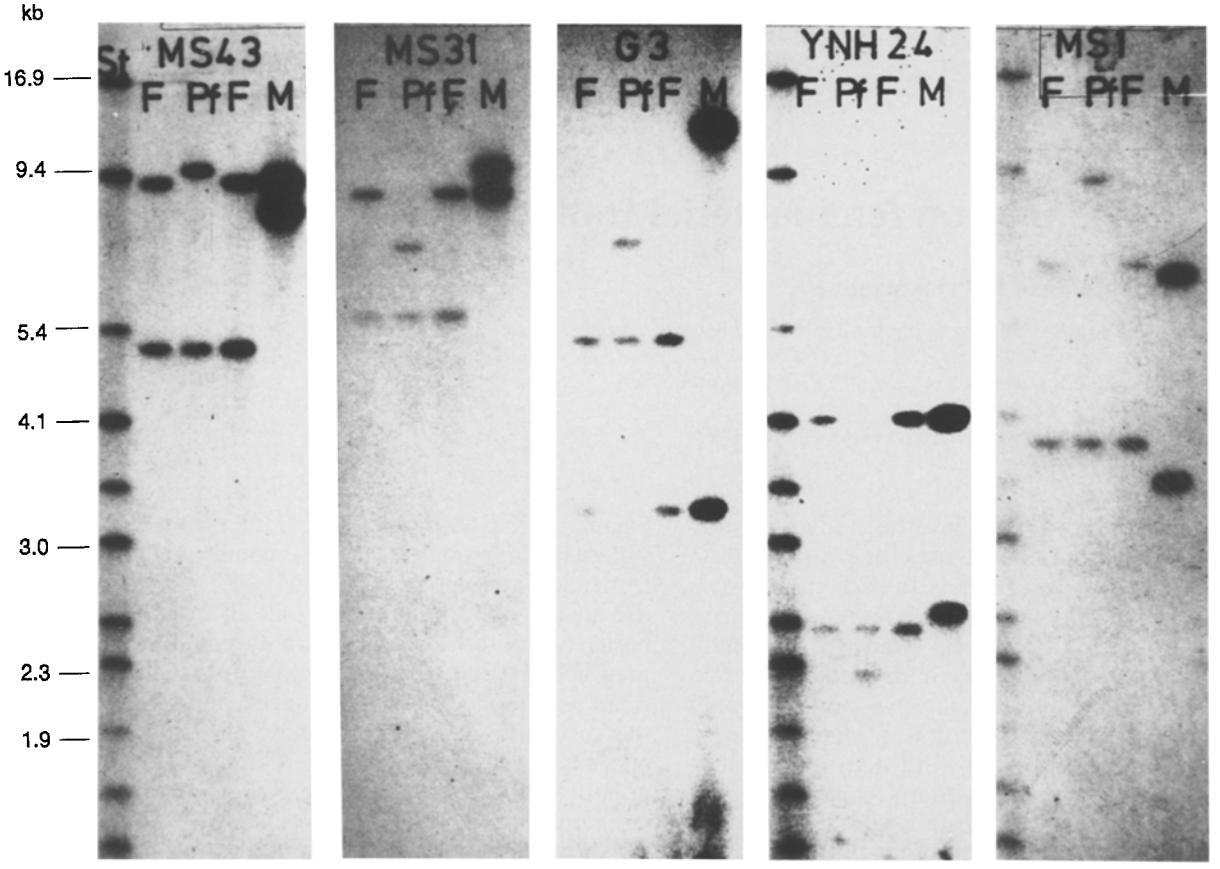

Fig. 1. Autoradiograph of 5 SLP's (YNH24, MS31, MS1, MS43, G3) for case 1 (see text). Restriction enzyme: HinfI. St = Phage DNA standard (MW100; Collaborative Research Incorporated); Pf = putative father; F = fetus; M = mother

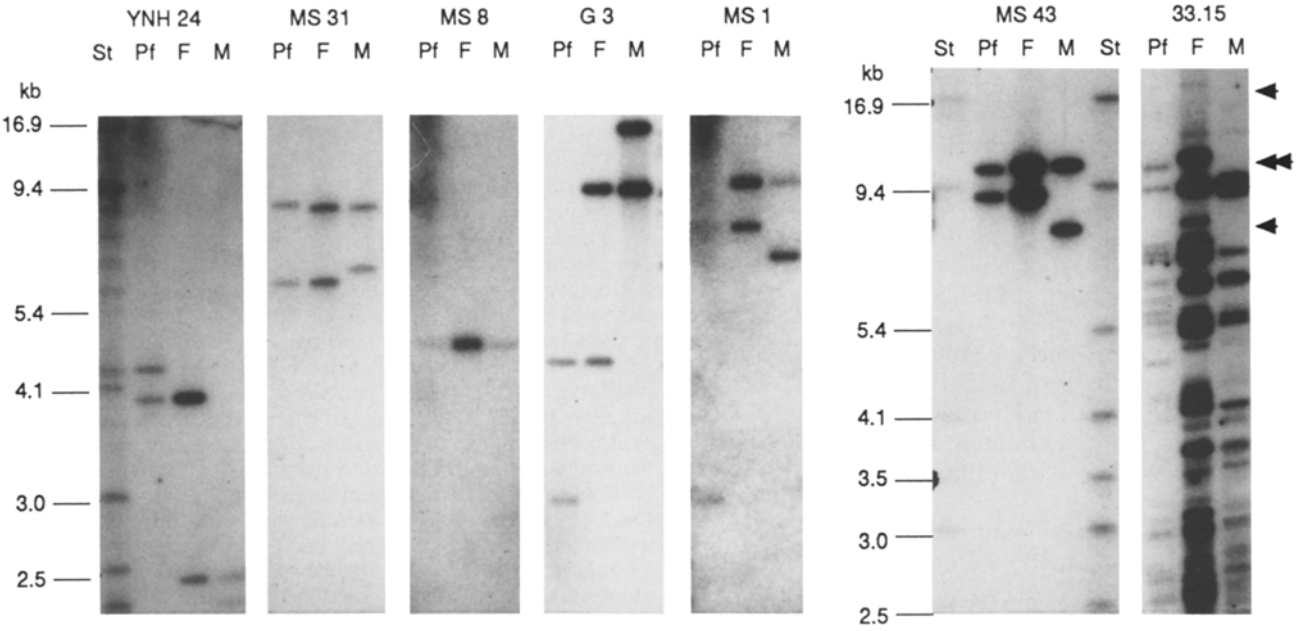

Fig. 2. Autoradiograph of 6 SLP's (MS43, MS31, G3, MS1, MS8, YNH24) and 1 MLS (33.15) for case 2. Restriction enzyme: HinfI. St = Phage DNA standard (MW100 + 102; Collaborative Research Incorporated); Pf = putative father; F = fetus; M = mother

(α ^{32}P dGTP for MLP's, α ^{32}P dCTP for SLP's, Amersham, 3000 Ci/mmol). Nylon membranes were prehybridised in the appropriate hybridisation solution (MLP: 0.2% BSA, 0.2% Ficoll 400, 0.2% PVP, 6% PEG 6000, 0.1% SDS, 50 µg/ml degraded herring sperm DNA; SLS: 5 × Tris, 10% dextran sulphate, 1 *M* NaCl, 30% formamide, sonicated salmon sperm DNA 10 µg/ml) at 62°C for MLP's and 50°C for SLP's, followed by hybridisation overnight under the same conditions.

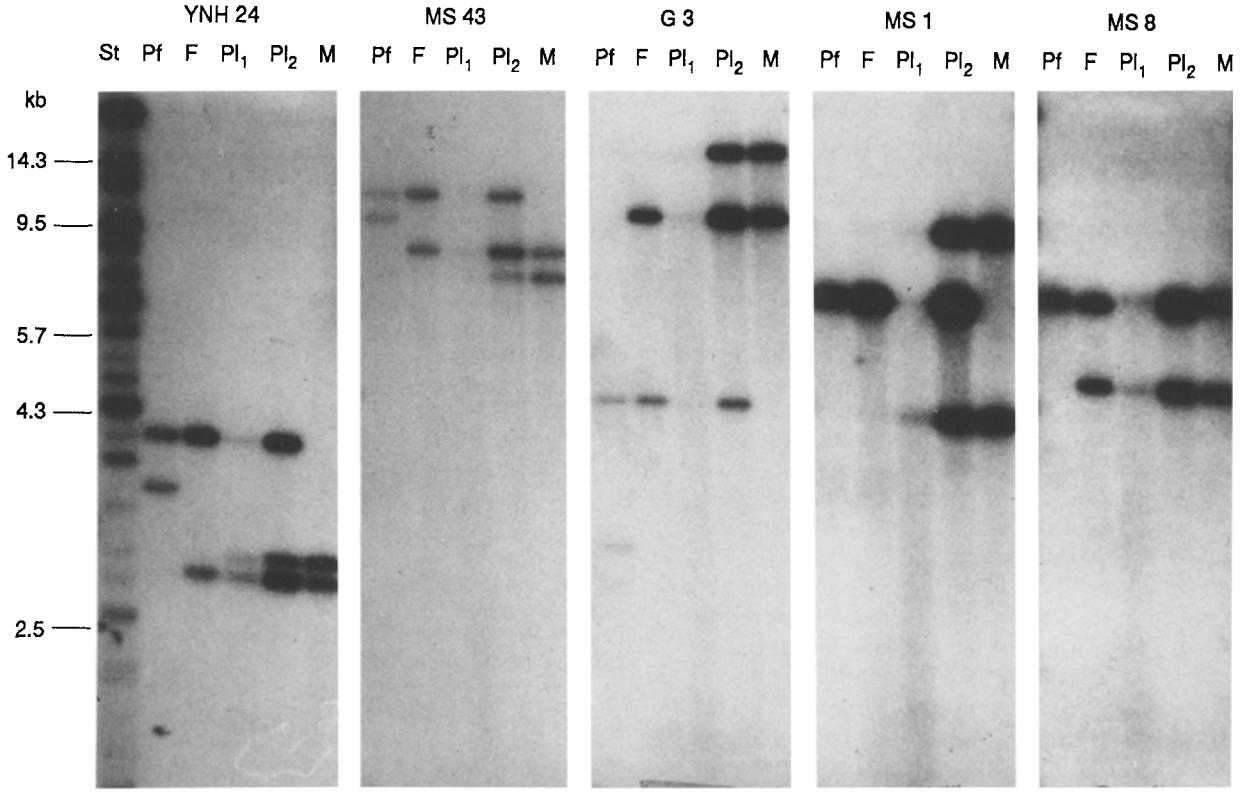

Fig. 3. Autoradiograph of 5 SLP's for case 3. Restriction enzyme HinfI. St = Phage DNA Standard (Analytical Marker DG1911; Promega); Pf = putative father; F = fetus; Pl1 = Placental tissue; Pl2 = Placental blood; M = mother

For MLP's the nylon membranes were washed 2–3 times at 62°C for 30 min in the wash solution (20 × SSC, 50 µg/ml degraded herring sperm DNA, 10% SDS) and once for 1 min in 3 × SSC.

For SLP's the membranes were washed once at 20°C (10 min in 2 × SSC) and twice at 65°C (30 min in 0.1 × SSC, 0.2% SDS).

Autoradiography was carried out at −80°C for 1–10 days with intensifying screens.

Multiple rehybridisation was carried out on a single membrane but the sequence of probes varied from case to case.

The frequencies of band patterns were calculated using a sliding window approach (Gill et al. 1990) with a range of +/− 3 SD of measurement error. The frequency data was derived from a population study of the Münster area.

Results

All 3 cases involved sexual abuse by the stepfather. The termination was carried out in the 8–10 week of pregnancy. In each case the putative father could not be excluded with 5–6 single locus DNA-probes.

Case 1. Using 5 single locus probes (MS1, MS31, MS43, G3 and YNH24) all the fragments which could not be attributed to the mother (i.e. paternal fragments) in the pattern of the fetus were also present in the putative father. Conspicuous here was an apparent "exclusion" between mother and child using MS1 (Fig. 1e). The combination of 5 SLP's led to an Essen-Möller value of W = 99.9998%.

Case 2. The putative father could not be excluded using 6 single locus probes (MS1, MS8, MS31, MS43, G3, YNH24). The Essen-Möller value for 6 SLP's was W = 99.9994%. An additional investigation with the multi locus probe 33.15 (Fig. 2g) gave less clear-cut results: 3 fetal bands (arrowed) could not be attributed to either the mother or the putative father.

Case 3. In this case DNA could be extracted from fragments of fetal tissue as well as from placental blood and tissue. Heterozygote band patterns were obtained for all 5 single locus probes (MS1, MS8, MS43, G3, YNH24; Fig. 3a–e) and the putative father could not be excluded. The 3 band pattern of the placental DNA was clarified by the identification of a maternal band which allowed the assignment of the paternal fragments without the presence of pure fetal DNA. In this case also an apparent mother-child "exclusion" with MS1 was found (Fig. 3d). The Essen-Möller value for the SLP's was calculated to be 99.9918%.

Discussion

There have been various reports on the use of DNA probes for the investigation of fetal tissue and mixtures of placental and fetal cells in paternity cases (Rittner et al. 1989; Sprecher et al. 1990; Henke et al. 1991). In the cases reported here the abortions were carried out using the vacuum method so that the material available for examination was relatively homogeneous. For this reason histological examination and identification of the tissue fragments was not feasible. In one case, however, it was

possible to macroscopicaly identify fetal fragments (see case 3) which could be isolated and extracted.

When the fetal and placental tissues were extracted separtely, a clear assignment of the DNA bands from the placenta could be made by comparison with the maternal and paternal bands (Fig. 3). In case 2 (Fig. 2) the results of the multi locus probe 33.15 were conspicuous in that 3 fetal bands (arrowed) were found which matched neither the mother nor the putative father. This could be a quantitative effect due to a weaker labelling of the fragments from the putative father than those from the mother and fetus, but in the case of the second non-matching band (double arrowed) there seems to be a shift between the paternal and the fetal band. The 2 fetal bands in this region are of approximately equal intensity as are the 2 paternal bands, although the paternal pattern is altogether weaker. Furthermore, artefacts due to methylation (extraction from fetal tissues), which have been recognised using HinfI (Budowle et al. 1990) and the possible formation of additional bands by somatic mutation (Jeffreys et al. 1990) must also be considered.

In such cases an exclusion of the putative father must be considered as an alternative, but a complete match with 6 SLP's would seem to negate this argument. This is supported by an Essen-Möller value of 99.9994% and by a band sharing of 56% with 33.15 which according to Jeffreys et al. (1985) could not be found in a collective of non-fathers. These results demonstrate that the use of multi locus probes in abortion cases can cause problems especially when only mixtures of fetal and placental DNA are present. Conclusive results can not always be obtained by the use of multi locus probes because of substantial overlapping of bands in the maternal/fetal mixture.

The apparent mother/child exclusions found with MS1 in 2 cases described here can clearly be classified as new mutations because no exclusions were found in all other systems. The rate of new mutations for MS1 is approximately 3% (Smith et al. 1990) and for this reason it should be avoided in standard paternity diagnostics (Brinkmann et al. 1989).

The 3 cases presented emphasize problems which can occur in paternity investigations but with the assistance of DNA technology it is possible to identify the father in cases of termination of very early pregnancies, even when mixtures of tissues are present.

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