APPLICATION OF DNA POLYMORPHISMS IN PATERNITY TESTING IN GERMANY: SOLUTION OF AN INCEST CASE USING BACTERIOPHAGE M13 HYBRIDIZATION WITH HYPERVARIABLE MINISATELLITE DNA

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INTRODUCTION

More than 25 blood, serum, and enzyme polymorphisms have been introduced into paternity testing in Germany in recent years (Rittner, 1975). If a "no" decision is defined by exclusion, and a "yes" decision requires a probability of 99.73 % or more, more than 90 % of court cases can be solved in this respect. A few cases not being clarified by a standard expertise include:

1) Cases with more than one alleged man if the men and/or the mother and the men are related.
2) Some cases where the putative father is deceased, and neither the parents nor the legitimate offspring are available for the study.
3) Cases where possible exclusion in a given polymorphic system interferes with an overall evidence in favor of paternity of the alleged man. The "exclusion" could then be traced back to a mutation.

Even some of such cases can sometimes be led to a conclusion, particularly if the HLA antigens can be determined. We have shown recently that a sixteen weeks old fetus could reliably be typed for HLA, thereby providing the paternity of the father of the child's mother with a confidence of 99.936 % probability (Rittner et al., 1986).

In another case (H./H., see Fig. 1) even after the inclusion of more than 30 blood, serum, and enzyme polymorphisms, including HLA-A,B,C, we were unable to distinguish PV_1 - the brother of the mother - from PV_2 - the father of the mother - as putative fathers of her child.

To obtain further information, we applied the DNA technology based on the detection of so-called hypervariable mini-satellite DNA, more widely known as "DNA fingerprinting" (Jeffreys et al., 1985). Such non-coding, highly repetitive DNA "core" sequences are scattered over the genome with unknown chromosomal localization. Therefore, the allelic relationships of their restriction fragments could not yet be elucidated. The repetitive intron sequences are phylogenetically highly conserved as simple repeats. So it was not surprising that similar sequences could be detected with oligonucleotide probes specific for "GAT/CA" repeats (Ali et al., 1986).

In 1987, Vassart et al. reported that the single-stranded bacteriophage M13, generally used as cloning vector for DNA sequencing, also detects a distinct set of hypervariable minisatellites in human and animal DNA, provided that herring sperm DNA

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

is omitted as competitor for unspecific binding during hybridization. Therefore, we applied this procedure with some modifications to solve the incest case.

MATERIAL AND METHODS

Genomic DNA was purified from peripheral blood cells according to standard procedures. Individual DNA samples of 10 µg each were digested with 50 U of restriction endonuclease Hinf I (Pharmacia) in a total volume of 300 ul according to manufacturer's instructions. After digestion, the samples were extracted once with an equal volume of 1:1 phenol/chloroform, precipitated in ethanol and resuspended in 10 mM Tris, 1 mM EDTA, pH 6.8. Agarose gel electrophoresis was carried out in a 0.8 % gel using a 26 mM Tris, 13 mM Sodiumacetate, 1.3 mM EDTA, pH 7.7 electrophoresis buffer. The gels (20 x 20 cm) were run at a constant voltage of 30 V for 20 hours. After completion, the gels were treated with 250 mM HCl for 15 min., then twice with 0.5 M NaOH, 1.5 M NaCl for 30 min., and finally twice with 0.5 M Tris, 1.5 M NaCl pH 7.4 for 30 min.. The gels were blotted onto nitrocellulose filters (Schleicher & Schüll, BA 85) overnight in 16x SSC.

Prehybridization was carried out for 4 hours in a hybridization mixture as described by Johnson et al. (1984). The blots were hybridized with the M13 probe in the same solution for 15 hours. As hybridization probe, the double-stranded form of M13mp18 (Boehringer, Mannheim) was labeled by nick translation with ^{32}P-dCTP to a specific activity of 2 x 10^8 cpm/ug, and used at a concentration of 10^6 cpm/ml hybridization mixture. After hybridization, the filters were washed once for 15 min. in 2x SSC, 0.1 % SDS at room temperature, twice for 15 min. in 2x SSC, 0.1 % SDS at 50°C, four times for 30 min. in 2x SSC, 0.1 % SDS at 65°C, once for 30 min. in 1x SSC, 0.1 % SDS at 65°C, and once for 30 min. in 1x SSC at 65°C. The filters were exposed to Fuji RX film at -70°C with intensifying screens.

RESULTS

We used the procedure described above in a number of experiments with related and unrelated individuals, including monozygotic twins (Rittner et al., unpublished). We confirm that unrelated individuals rarely share given kb fragments, whereas monozygotic twins do not differ in their RFLP patterns.

The resulting DNA patterns of family H. are shown in Fig. 2. As could be expected for intrafamilial analysis, a number of fragments can be detected in all individuals, mainly in the size range of 20 to 25 kb. Some bands show segregation from the grandfather and the grandmother, respectively, to the offspring. To differentiate both putative fathers, only those informations are suited which are present in the grandmother and not in the grandfather (PV2), and do not segregate to their daughter, the

mother of the child. Vice versa, all informations should be accounted for which are not derived from the grandmother, but from the grandfather (PV2), which are again not present in the mother, but in the child, and may be missing in the brother (PV1) of the mother. By careful inspection, only fragments of the first category can be ascertained: fragments of 12.5, 5.8, and 3.5 kb do occur in the grandmother, but not in the grandfather (PV2), are missing in the mother, but are present in the child and the brother (PV1) of the mother. No fragment of the second category was found.

By definition, all restriction fragments segregate in a dominant fashion. Thus, one of the parents must possess a given fragment observed in the child's DNA. On the basis of these findings it could be concluded that the grandfather cannot be the true father of the child K.

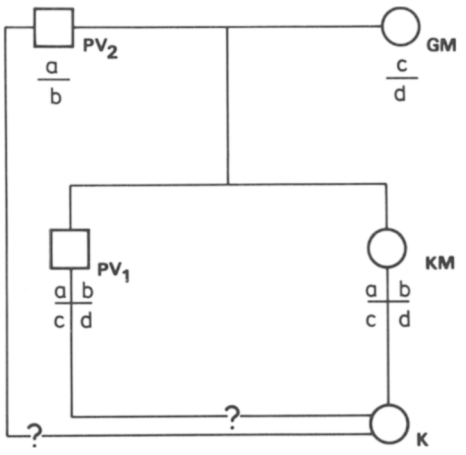

Fig. 1. Pedigree of family H.

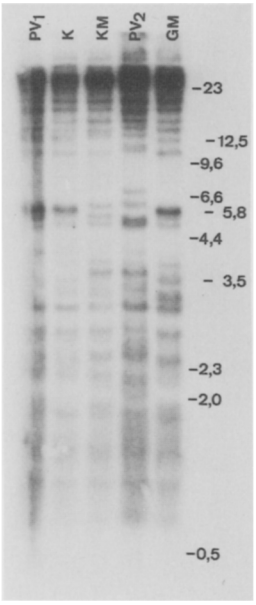

Fig. 2. Autoradiogram of a Southern blot of family H after digestion of familial DNA with Hinf I and hybridization with M13.

DISCUSSION

The detection of highly polymorphic mini-satellite DNA by the commercially available phage M13 provides a new tool for the solution of incest and other problematic cases, which cannot be solved by conventional blood group expertise, even after the inclusion of the HLA system. The analysis of repetitive intron DNA is also well suited to detect a single individual out of a number of possible offenders on the basis of trace evidence (Gill et al., 1985), and in a number of further forensic applications

like identification of bodies or parts of a body, provided some antemortem material can be used for comparison.

However, we do not expect a general introduction of this technique into paternity testing in Germany in the near future. As has been stated already, "yes" or "no" decisions can be made in the majority of cases using conventional typing. In most cases, the true father is alleged. This results in non-exclusion in all polymorphic systems studied. If the man is not excluded, German legislation strictly demands a calculation of the probability for fatherhood based on the Essen-Möller approach. For a system being included in the blood group expertise, the following prerequisites must be fulfilled:

1) estimation of allelic frequencies in the German population,
2) proof of dominant inheritance by segregation analysis,
3) negligible occurrence of mutations or other genetic events.

These conditions are not yet given in the analysis of minisatellite DNA. Estimates of mutation rates are as high as 1/240 (Jeffreys et al., 1985); haplotype frequencies cannot be calculated due to the lack of information about chromosomal localization, allelic relationship, and independence as basis of combinational probabilities. Thus, neither the combined exclusion chance nor the total probability in favor of the fatherhood of a given man can yet be addressed to the Court.

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Acknowledgement: This work was supported in part by Biotest GmbH, Frankfurt, FRG.