PROVING PATERNITY USING FORMALIN FIXED, PARAFFIN EMBEDDED TISSUE SAMPLES FROM A DECEASED FATHER

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INTRODUCTION

DNA testing has had a major impact in forensic medicine, particularly in identification of individuals from crime scene samples and in paternity analysis. Additional convenience for DNA testing can be obtained by using the polymerase chain reaction (PCR) (1) for amplification of DNA. The use of PCR facilitates paternity analysis from old and degraded blood or tissue samples, where conventional serological analysis cannot be performed.

Here we describe a paternity case of a deceased father who had died of ventricular adenocarcinoma 13 months before the analysis.

MATERIALS AND METHODS

DNA extraction

From the putative father DNA was extracted from formalin fixed, paraffin-embedded samples from normal esophageal and from ventricular adenocarcinoma tissue. DNA was extracted by first removing the paraffin with xylene, afterwhich the samples were treated with absolute alcohol, and lysed in the presense of proteinase-K and dithiothreitol (2).

From the mother and the child DNA was extracted from EDTA-blood samples using the Chelex^R method (3).

Analysis of DNA samples

Four minisatellite loci (D1S80, apoB, D17S30, col2A1) and two microsatellite loci (vWA, HumTH01) were analyzed. The analysis was performed using the amplified fragment length polymorphism (AMP-FLP) technique (4). In AMP-FLP analysis the hypervariable mini- and microsatellite loci were first amplified by PCR using the primers described in the litterature. The amplified alleles were then separated according to their size in a high resolution polyacrylamide gel electrophoresis (PAGE), and visualized using simple silver staining (5).

RESULTS

Four minisatellite loci (D1S80, apoB, D17S30, col2A1) and two microsatellite loci (vWA, HumTH01) were successfully typed for the mother-child-putative father trio (Table 1). The AMP-FLP results from both normal esophageal and ventricular adenocarcinoma tissues from the putative father were consistent in all mini/microsatellite loci (Table 1). Paternity index (6) was 104, providing strong evidence for paternity, and corresponding to the force of evidence of >99 %. An example of AMP-FLP analysis of minisatellite D17S30 and microsatellite HumTH01 is given in Figure 1.

CONCLUSIONS

Our earlier study has shown that genetic typing from soft tissue samples can be successfully performed using AMP-FLP technique (8). Our present results show that formalin-fixed, paraffin embedded tissue samples can also serve as source of DNA for genetic typing in various applications. In paternity cases where the putative father is deceased, analysis of such tissue samples may be the only alternative to prove paternity. The present case is the first paternity case in Finland, where the court has accepted DNA typing from biological sample other than blood as evidence for paternity.

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TABLE 1. Results of the AMP-FLP analysis. M = mother, C = child, PF = putative father, B = blood sample, N = normal esophageal tissue, Ca = ventricular adenocarcinoma tissue.

SAMPLE	D1S80	D17S30	apoB	col2A1	HumTH01	vWA
M, B	18-18	3-12	36-48	11-23	6-10	5-8
C, B	18-24	4-12	46-48	11-23	6-7	5-5
PF, N	24-31	1-4	31-46	19-23	6-7	5-6
PF, Ca	24-31	1-4	31-46	19-23	6-7	5-6

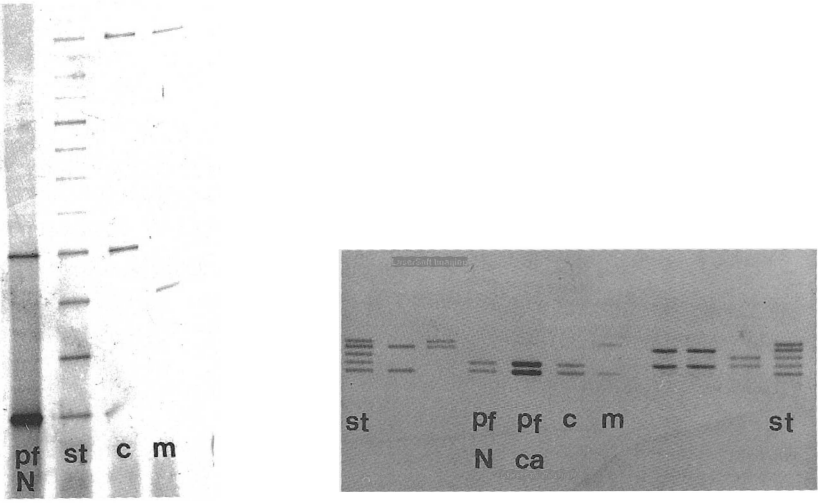

FIGURE 1. A. AMP-FLP analysis of minisatellite D17S30. B. AMP-FLP analysis of microsatellite HumTH01. M = mother, C = child, PF = putative father, B = blood sample, N = normal esophageal tissue, Ca = ventricular adenocarcinoma tissue, L = allelic ladder constructed of known alleles in the Finnish population as previously described (7).