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Parentage testing following an infanticide case using fetal DNA from archival fixed tissues

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Abstract

Genetic investigation of an old infanticide case was conducted using DNA from archival fixed tissues in an attempt to provide independent evidence of the parents identity. The initial PCR assays using DNA extracted from paraffin embedded tissues failed because of possible inhibitors, requiring additional purifying steps. STR profiling was conducted on nine loci plus Amelogenin using PAGE gels. Comparison of the fetal DNA profile with the DNA profiles of the putative mother and her husband revealed the compatibility of the fetal DNA profile with the maternal profile and excluded the man from the paternity.

Keywords: Paraffin-embedded tissues; Infanticide; Maternity; Identification

1. Introduction

In a small town, the body of a female newborn child packed up in a plastic bag was found accidentally in a dustbin. The police investigations and the clinical forensic examination indicated, without succeeding in proving it, that the putative parents were a young woman and her lover, both living in the neighborhood of the town. The woman's jealous husband was incriminated as the child's murderer. One year later, DNA analysis was requested by the prosecution in an attempt to provide independent evidence of the child's parents identity. The objective of the DNA analysis was to compare the putative mother and her husband's DNA profiles (PCR-STR) with the fetal DNA profile, obtained from the archival fixed tissues and from bloodstains on the plastic bag.

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2. Materials and methods

Blood samples were collected from the putative mother and her husband and then extracted using Wizard Genomic® purification kits (Promega). Bloodstains from the plastic bag were processed using a Chelex®100 (Sigma) protocol: initial wash step, 56 °C incubation in 20% resin suspension for 1 h, 8 min boiling in water bath, quick spin, dialysis and concentration of the supernatant on Microcon®100 device (Amicon) [1,2].

After 1 year of storage, the paraffin-embedded tissues (myocardial tissue, thyroid gland, brain cortex, lung tissue and kidney) sampled from the autopsy of the child were re-examined microscopically, in order to specify the degree of tissue degradation. No significant tissue degradation was detected. The DNA extraction from paraffin-embedded tissues was performed using approximately 300 mg of microtome sections 10 μm thick, from each paraffin block. Paraffin was completely removed by two incubations in xylene at room temperature on a rocking platform, centrifugation at high speed, two washes with 95% ethanol (1 ml) and 70% ethanol (1 ml) followed by drying at 37 °C under strict conditions to avoid cross-contamination [3,4]. The tissue pellets were extracted using a proteinase K/phenol–chloroform protocol [3,4]. Fresh aliquots of proteinase K were added every 16 h up to full tissue digestion. DNA was finally precipitated with ethanol and suspended in 100 μl storage buffer (10 mM Tris–HCl, 0.5 mM EDTA) [3,4]. After the PCR failure, a second DNA organic extraction from the paraffin-embedded tissues was done by replacing the ethanol precipitation with a dialysis and concentration step using Centricon®30 device (Amicon) [5,6] and by performing an additional purification step with Chelex®100—same protocol mentioned for blood stains previously [2].

PCR was performed using the GenePrint® STR System-Silver Stain Detection (Promega): Silver STR III System (D7S820, D13S317, D16s359); CSF1PO, TPOX, TH01 Multiplex; F13A01, FESFPS, vWA Multiplex and Amelogenin. All samples were amplified according to Promega recommended protocols [7] and in the presence of BSA (Sigma) in the amplification mix (16 μg/100 μl) in order to overcome inhibition [2,5]. To determine if PCR failure was caused by inhibitors, aliquots of the problematic samples were added to the positive controls [2] and also 1/10 sample dilutions were used [2,5].

All amplified STR fragments were separated by 6% PAGE (carried out on precast GenGel Hyres—Amersham Pharmacia Biotech) and detected by silver staining (DNA Silverstaining kit—Pharmacia Biotech). Statistical evaluation of non-exclusion was performed by calculating the Maternity Index (MI) and the Probability of Maternity (*W*), according to Essen–Moller's formula. Allele frequencies were based on Promega's published data for Caucasian populations [7].

3. Results

From the paraffin-embedded tissues, DNA was extracted in high quantities (31.2–76.5 μg detected on spectrophotometer), but only the kidney sample was successfully amplified.

The woman's husband did not share any alleles with the newborn baby on the vWA, D7S820, D13S317 loci. His paternity was excluded.

On the investigated loci, the putative mother shared the following alleles with the newborn child: CSF1P0 (9 and 10), TP0X (8), TH01 (6), D16S359 (11 and 12), D7820 (11), D13S317 (8), vWA (15), F13A01 and FESFPS (inconclusive). Therefore, the mother of the newborn baby was not excluded. MI = 686.033; $W = 99.854\%$. Conclusion: maternity practically proven.

4. Discussion

In this old infanticide case, the biological material belonging to the newborn child represented the reference sample for the parentage investigation. As we supposed, the bloodstain analysis from the plastic bag showed the presence of mixtures: maternal blood or placenta together with fetal blood. In these circumstances, the DNA isolated from the paraffin-embedded tissues was a crucial step in the process of DNA profiling.

We tried to correlate the degradation of fetal tissues with the duration of the postmortem period knowing its influence on DNA integrity [8]. The case history mentioned only the low environmental temperatures at the site where the newborn baby was found. As the microscope examination revealed no significant degradations in the newborn baby's tissues, we concluded that paraffin-embedded tissues were suitable samples for DNA analysis.

Samples extracted from paraffin gave an apparently acceptable DNA concentration, but did not amplify. The failed amplification was thought to be due to the poor quality of the DNA or by the inability to remove inhibitors during the extraction process. The replacement of the ethanol precipitation and an additional Chelex extraction after the organic procedure [9] removed some of the potential inhibitors from the paraffin-embedded samples. Amplification with diluted, re-extracted DNA was finally successful, but only from the kidney sample. As mentioned by Faulkner and Leigh [10], elevated temperatures during the paraffin-wax process can cause significant changes to the DNA: DNA can be complexed with proteins and often nicked, giving short fragments. The high rate of PCR failure makes paraffin-embedded tissues a less than ideal source of template [10].

Performing PCR with unknown inhibitory substances requires good controls, which allows the assays sensitivity estimation.

The case emphasized problems critical for the criminal investigation and the fact that samples initially stored for other purposes could be processed by DNA technology. Because fetal fixed tissues were preserved, maternal identity was proven for this old infanticide case.

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