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CASE REPORT

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Incestuous Paternity Detected by STR-typing of Chorionic Villi Isolated from Archival Formalin-fixed Paraffin-embedded Abortion Material Using Laser Microdissection

ABSTRACT: Microscopic examination of a blood clot expelled by a physically and mentally disabled woman taken to the emergency room because of genital bleeding revealed the presence of chorionic villi encircled by decidua, hemorrhage, and necrosis. In order to identify the father of the product of conception, sections of formalin-fixed, paraffin-embedded abortion material were subjected to laser microdissection: DNA extraction from chorionic villi selectively isolated from the surrounding tissues allowed successful STR-typing of fetal cells, which was otherwise prevented by excess maternal DNA. The large number of homozygous genotypes in the fetal profile suggested incestuous paternity. Analysis of reference DNA samples from male relatives excluded the woman's father, paternal grandfather, and maternal grandfather, whereas the obligate paternal alleles of the fetus were constantly present in the genotypes of the woman's brother, clearly demonstrating brother–sister incest (probability of paternity $>99.99999\%$).

KEYWORDS: forensic science, laser microdissection, abortion material, chorionic villi, paternity testing, short tandem repeat

STR-based paternity testing on chorionic villi found in abortion material is hindered by the concurrent presence of abundant maternal tissue, as preferential amplification of excess maternal DNA often prevents detection of fetal genotypes. Preparatory histological screening of the sample in order to select areas rich in fetal tissue (1) and manual trimming of chorionic villi under microscopic control (2) can reduce the ratio of co-isolated maternal DNA. By these conventional methods, however, the occurrence of mixed extracts with the potential to interfere with STR analysis is frequent and fetal DNA typing may even be impossible when chorionic villi are rare amid maternal tissue.

Laser microdissection was originally developed to isolate selected cell populations in tumor tissues for research and clinical purposes (3). The technique has recently found interesting applications in forensic casework, such as isolation of residual chorionic villi from the endometrial mucosa after therapeutic abortion (4) and individual collection of sperm in postcoital slides (5). In this case, a laser microdissection-gravity force recovery (LMD-GFR) system was used to selectively recover chorionic villi from archival formalin-fixed, paraffin-embedded abortion material, allowing subsequent paternity testing.

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Case Report

A 21-year-old woman with severe physical and mental disabilities was taken to the emergency room because of genital bleeding. A spindle-shaped blood clot ($10 \times 3 \times 2$ cm) collected during medical examination was processed for histology. Microscopic examination revealed a small number of fibrotic chorionic villi surrounded by maternal decidua, blood, and necrosis (Fig. 1*a*). Since it was evident, due to the patient's serious psychiatric disability that pregnancy was the consequence of sexual abuse, the medical staff reported the case to the public prosecutor, who ordered DNA analysis on the archival formalin-fixed paraffin-embedded abortion material in order to identify the father of the product of conception.

Materials and Methods

Two 5 μm sections of the formalin-fixed, paraffin-embedded abortion material were mounted on polyethylene membranes fixed to special metallic frames. Sections were heated for 20 min at 37°C and for 20 additional min at 60°C, deparaffinized in xylene twice, for 1 min and 30 sec, respectively, and then stained with hematoxylin and eosin. Five chorionic villi (each comprising about 20 cells) were isolated from each section by means of the LMD-GFR system DM LMD (Leica Microsystem, Wetzlar, Germany) coupled to a DM LA microscope (Leica Microsystem). The UV-pulsed laser microbeam, whose track could be followed on a computer monitor and controlled by a mouse, was directed to cut out areas of interest from tissue sections (Fig. 1*b*) and selected cells fell by gravity into 200 μL tubes containing 20 μL of 1X PCR

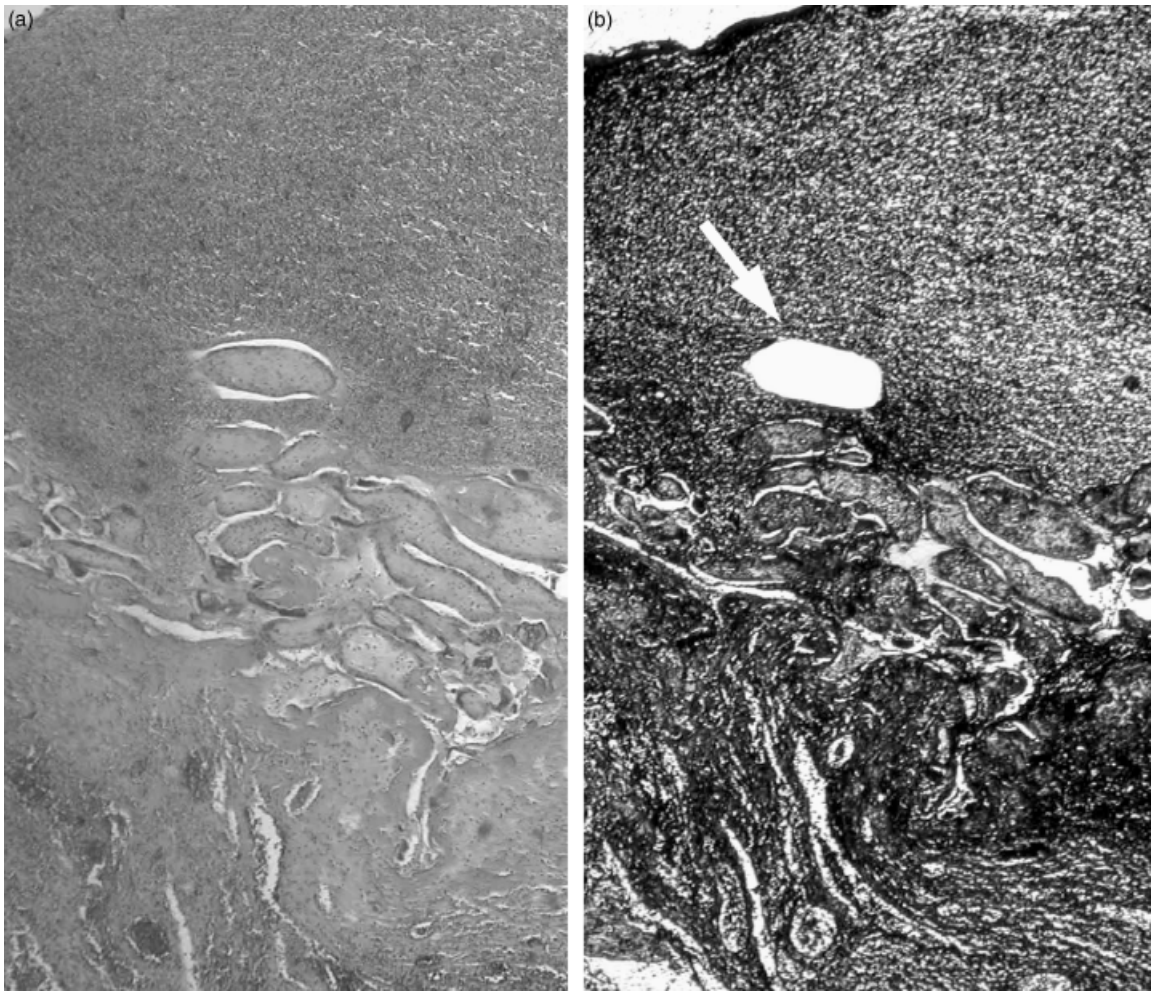

FIG. 1—*(a) Abortion material expelled by the victim showed chorionic villi surrounded by maternal decidua, hemorrhage, and necrosis (H&E × 4). (b) A tissue section, contiguous to that shown in (a), was prepared for microdissection: the arrow indicates the void area previously occupied by a villus that was selectively cut and recovered by gravity in PCR tube (H&E × 4). Note the different light refraction of the tissue due to the absence of mounting media and coverslide in the microdissected section.*

TABLE 1—*STR profiles of the victim, fetal tissue, and victim's male relatives.*

Locus	Victim	Fetal Tissue	Victim's Brother	Victim's Father	Victim's Paternal Grandfather	Victim's Maternal Grandfather
D8S1179	15	15	15	13, 15	13, 15	10, 13
D21S11	28, 29	29, 30	30	29, 30	27, 29	28, 31.2
D7S820	10	10	10, 12	8, 10	8	10, 12
CSF1PO	11, 12	11	10, 11	11	11	10, 12
D3S1358	15	15	15	15	15,16	16, 18
TH01	8,9	8	8,9	9	6,9	6, 8
D13S317	11, 12	12	12, 13	11, 13	9, 11	12
D16S539	9, 11	9, 14	9, 14	11, 14	11	9,12
D2S1338	17, 20	17, 18	18, 20	20	20, 24	18, 20
D19S433	16, 16.2	14, 16.2	14, 16.2	14, 16	12, 14	13, 15
vWA	14, 17	17	17, 18	14, 18	14, 17	17, 19
TPOX	8, 9	8, 11	8, 11	8, 9	9, 10	8, 11
D18S51	10, 22	10, 15	10, 15	15, 22	14, 15	13, 14
Amelogenin	X, X	X, X	X, Y	X, Y	X, Y	X, Y
D5S818	13	13	13	13	11, 13	13
FGA	20, 24	20, 21	21, 24	20, 21	21	21, 23

buffer (Tris-HCl 20 mM pH 8.4, KCl 50 mM) fortified with 1% Tween 20 and 2 μL of proteinase K (20 mg/mL). For each tissue section, the five microdissected villi were combined in a single tube. Fetal cells were digested O/N at 56°C. Following enzyme heat inactivation (10 min at 95°C), the whole content of each tube was directly analyzed in two separate amplification reactions, each containing 10 μL of lysate (so that a total of four replicate PCR analyses of fetal DNA extract were performed).

Reference DNA of the victim and her male relatives was isolated from blood samples using the NucleoMag 96 Blood kit

(Macherey-Nagel, Dueren, Germany) consisting of cell lysis with chaotropic reagents followed by binding of the DNA to silica-coated paramagnetic beads. Washing steps and elution of pure nucleic acids were then performed on the automatic magnetic separator KingFisher mL (Thermo Labsystems, Vantaa, Finland).

Fifteen microsatellites and the Amelogenin locus were analyzed by PCR using the Identifiler amplification kit (Applied Biosystems, Foster City, CA), following the manufacturer's recommendations. Fluorescent PCR products underwent electrophoresis on an ABI Prism Genetic 310 Analyzer (Applied Biosystems) and were analyzed by means of software GeneScan 3.7 and Genotyper 3.7 (Applied Biosystems). The allele calling threshold for STR loci based on peak heights was 50 relative fluorescence units (rfu).

The probability of consanguineous mating was calculated from the formulas described by Wenk et al. (6). The probability of paternity on the assumption of sister–brother incest was determined as suggested by Tamura et al. (7). Allele frequencies for STR loci used in the calculations were derived from data available in the literature on the Italian population (8–10).

Results

In all the replicated amplification reactions with DNA isolated from the microdissected villi, a reproducible female genetic profile was obtained for the 15 STR loci tested. At each locus this profile shared one allele with the victim's genotype, so that it could be unambiguously attributed to fetal tissue (Table 1). No additional alleles (>50 rfu) ascribable to contamination with maternal DNA were detected. No evidence of additional peaks (>50 rfu) suggesting the possibility of allelic imbalance or allelic dropout, often described in association with low amounts of DNA template (11) or the DNA-degrading effects of formalin fixation (12), was observed in replicate PCR analyses of apparently homozygous genotypes. Control amplifications of DNA extracted from a whole section of abortion material tissue exclusively generated genetic profiles identical to the mother's reference sample.

The observation of eight homozygous genotypes in the fetal profile suggested that pregnancy might have been a consequence of incest. The likelihood ratio (LR) for incestuous paternity versus random mating was determined through comparison of maternal and fetal genotypes. The calculated LR of 9.228 was in accordance with the hypothesis of incest ($p = 90.22\%$). Therefore, all the victim's male relatives (father, brother, paternal grandfather, and maternal grandfather) were asked to provide biological samples for DNA testing; their profiles are reported in Table 1. Genetic analysis demonstrated that the woman's father, paternal grandfather, and maternal grandfather could be clearly excluded as possible fathers, due to multiple allelic incompatibilities. The victim's brother's genotypes, on the contrary, included at each locus the obligate paternal allele of the product of conception. The probability of paternity on the assumption of sister–brother incest was therefore calculated, and gave a likelihood ratio of 3×10^7, corresponding to a probability of paternity $>99.99999\%$ (prior probability of paternity 50%).

Discussion

Mixtures of body fluids from different individuals are a frequent occurrence in forensic casework. By standard DNA extraction and STR-typing, it is usually difficult—if not impossible—to detect the minor component of a mixed stain when it is present at approximately less than one tenth of the major component (13). Various techniques, such as differential lysis in mixed traces containing semen (14) or Y-STR-typing in male/female mixtures (15), can be used to solve this problem, according to the circumstances.

LMD-GFR is a valuable technique for the analysis of mixed forensic traces, when cells from different contributors can be morphologically discriminated under microscopic control. In this unusual case, the accuracy of laser-induced computer-assisted tissue dissection, coupled to the sensitivity of PCR-based STR analysis, allowed DNA typing from a small number of chorionic villi found in archival formalin-fixed paraffin-embedded abortion material that, for the most part, was made up of maternal tissue. Selective cell recovery enabled the amplification of fetal genotypes, otherwise masked by the overwhelming presence of maternal DNA in the sample: with DNA isolated from as few as 50 microdissected fetal cells, it was possible to generate a reproducible multilocus profile including 15 STR markers for subsequent paternity testing.

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