CASE REPORT

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Parentage Determination on Aborted Fetal Material Through Deoxyribonucleic Acid (DNA) Profiling

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ABSTRACT: After a rape, women who are pregnant often elect to abort the fetus. The authors describe ten cases in which deoxyribonucleic acid (DNA) typing was performed on the aborted fetal material to provide evidence of the genetic constitution of the suspect. The problems encountered with this new technique are discussed.

KEYWORDS: pathology and biology, criminal sex offenses, genetic typing, deoxyribonucleic acid (DNA), DNA typing

In some rape cases, sperm is not available from vaginal swabs and, unfortunately, the resulting type of evidence can be a fetus. With parentage determination it is possible to link the crime to the perpetrator. In fact, genes from the suspect become part of the genetic makeup of the fetus.

In the literature, genetic testing is most conclusive when performed on infants over 6 months of age. In cases of abortion, Reisner et al. [*1*] propose a method using genetic markers such as red cell enzymes to link the biological father to the fetus. However, reports of parentage testing on aborted material are relatively rare in the forensic science literature [*2*,*3*].

The aim of this study is to stress the possibility of identifying a probable biological father through deoxyribonucleic acid (DNA) typing of abortion material. This type of testing has been capable of producing compelling evidence [*4*–*7*].

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Materials and Methods

DNA typing was applied to tissues from 10 abortuses [*8*]. The fetal material ranged in gestational age from 8 weeks to 5 months (Tables 1 and 2). All the pregnancies were alleged to have resulted from rape. Abortions were performed by suction technique for fetuses from 8 to 11 weeks old; older fetuses were delivered by treatment with prostaglandins.

As noted in Table 1, the tissues used in the test were fetal or maternal cells, or both, from abortion material, which included chorionic villus material in two cases (Cases 1 and 3).

Table 2 shows that the tissues used from older fetuses included white blood cells (Case 6), fetal lung, quadriceps muscle, or rib cells. The tissues were cut into small fragments with scissors and tweezers in a lysis buffer [8*M* urea, 2% sodium dodecyl sulfate (SDS), 10m*M* ethylenediaminetetraacetic acid (EDTA), 0.3*M* sodium chloride (NaCl), 10m*M* tris(hydroxymethy)aminomethane (Tris) buffer, at pH 8.0] and incubated overnight at 37°C. Afterwards, tissues and whole blood samples from the victims, suspects, and fetuses were treated using a standard technique for DNA typing published elsewhere [*9*].

Briefly, restriction endonuclease digestion with PST1 was performed according to the specifications of the manufacturer (Boerhinger, Mannheim, Germany). Digested DNA was electrophoresed through 0.9% agarose gels during 65 h. The gels were stained with ethidium bromide, photographed, and briefly exposed to ultraviolet illumination. The gels were then treated with 1.5*M* NaCl/0.5*M* sodium hydroxide (NaOH), neutralized in 0.5*M* Tris/1.5*M* NaCl at pH 7.2, and blotted onto nylon membranes (Nylon N$^+$, Amersham, France). The blotting buffer was 10× standard saline citrate.

DNA probes [YNH 24 (D2 S44, pAC 255), with an allele size range of 3 to 13 kb; V1 (D17 S79, pAC 256), with an allele size range of 6.7 to 14 kb; and MLJ 14 (D14 S13, pAC 225), with an allele size range of 2.5 to 5 kb (Lifecodes Corp., Valhalla, New York)] were labeled with phosphorus-32 (^{32}P) by the random oligonucleotide method using the random primed DNA labelling kit (Amersham). The probe specific activities were >1

TABLE 1—*Tests of fetal tissues used to obtain genetic evidence in rape trials—fetuses aged less than 12 weeks.*

Case No.	Fetal Age, weeks	Amount of Treated Tissue, g	Fetal Profile	Presence of Chorionic Villi	Suspect Inclusion Probability
1	8	7.65	mother-fetus, mixture	no	0.9699
2	9	8.40	yes	yes	0.9992
3	11	5	mother-fetus mixture	yes	0.9987
4	10	79	yes	no	0.9996

TABLE 2—*Tests of fetal tissues used to obtain genetic evidence in rape trials—older fetuses.*

Case No.	Fetal Age, months	Amount of Treated Tissue	Fetal Tissue Tested	Suspect Inclusion Probability
5	4	3 g	lung	0.9729
6	5	1 mL	blood	0.9982
7	3	0.60 g	quadriceps muscle	0.9824
8	5	2.7 g	quadriceps muscle	0.9945
9	3	1.76 g	ribs	0.9972
10	5	1.50 g	quadriceps muscle	no suspect

$\times 10^8$ counts per minute (cpm)/μg. Nylon membranes were hybridized overnight at 65°C with 1×10^6 cpm/mL of probe. Washes were performed under stringent conditions (the final wash at 65°C).

Autoradiography of the hybridized filters was performed by exposure to Kodak XAR film at −80°C.

Since the abortion method using prostaglandins resulted in the delivery of intact fetuses, providing us with more fetal tissue than was available with the suction method, testing could be performed on heart blood cells or on fetal tissues. When no blood was available or if bacterial contamination or fetal maceration was observed, the testing procedure for fetal tissues was then performed on muscles (quadriceps), lungs, or brain tissue.

Results

Results are shown in Tables 1 and 2.

After visual interpretation indicated that DNA restriction fragments from the suspect and the fetus matched, inclusion of the suspect was confirmed by computer-assisted image analysis (Bioimage Systeme). This provides an objective approach to visual evaluation. As recommended by Budowle [*10*], our tolerance window was empirically derived by repeated measurements of both ideal and nonideal samples; the size of some DNA fragments can vary by +2.5%. The probability of inclusion required by the judges was calculated according to the method described by Morris et al. [*11*]; the database used was established from a randomly allocated French population (N = 300), constituted according to the criteria of Baird et al. [*12*].

In Case 1 (Fig. 1), we could not clearly separate maternal from fetal tissues. We obtained a three-band pattern with single-locus probes. Yet, in this case, comparison of the fetal-maternal sample with the maternal reference sample gave us a powerful indication of the paternal band. In the other cases, DNA analysis indicated an overwhelming probability that the suspect contributed the paternal fragment. For fetuses 8 to 11 weeks old, chorionic villus material provided fetal material without maternal contamination. In

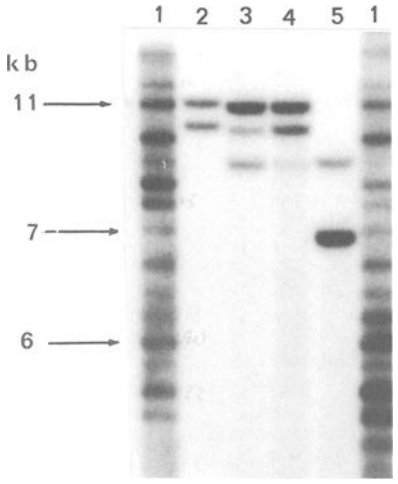

FIG. 1—*Autoradiograph of samples hybridized to single-locus DNA probe YNH 24: Lane 1, molecular weight marker, Lane 2, pattern of the mother, Lanes 3 and 4, pattern of the fetal-maternal mixture, and Lane 5, pattern of the suspect. Alleles 1 or 2 of Lane 3 can be inherited from Allele 1 or 2 belonging to the mother; Allele 3 matches the upper suspect's allele.*

the last case (Case 10 in Table 2), no conclusion could be drawn, due to the lack of a suspect.

Discussion

Our experience leads us to suggest that it is possible to use fetal tissues for parentage determination using DNA typing. Many technical problems may be encountered. The sample size and the tissue type, as well as the time elapsed after the abortion, are critical factors in determining the success or failure of the method. The type and size of the fetal samples depend on the abortion method employed. Reisner et al. [*1*] pointed out that the optimal sample consists of fetal blood obtained by heart puncture, which is only possible in older fetuses.

Unfortunately, most very young fetuses are aborted using suction procedures, which generally do not produce intact material or material whose origin is easily defined. In most cases, suction techniques provide a mixture of maternal and fetal cells. The mixed tissues often yield valuable information (Case 3), but analysis must be approached cautiously. In such cases, the analysis of chorionic villi may provide the fetal pattern without maternal contamination. The crucial need to have for tissues free of bacterial contamination in order to perform DNA typing makes it necessary to deliver the tissue to the testing laboratory as soon as possible after the abortion. The tissues should be transported on ice, and at no time should they be treated with preservatives.

If significant maceration is noted, we have found that the fetal lungs and brain tissue then become more suitable for DNA testing. In each case, greater numbers of tissue samples allowed us to obtain high-molecular-weight DNA suitable for DNA analysis. On the other hand, if the DNA were degraded, no valid results could be expected.

Although we did succeed in obtaining specific results with DNA typing in 9 out of 10 cases, in one case we obtained a mixture of fetal and maternal tissue, which lowered the discriminatory power of the test but still allowed one to draw conclusions as to inclusion or exclusion of the suspect.

In the last case, the allele inherited from the father was easily recognized, but the suspect had not been arrested at the time of the test [*13*].

The experimental nature of the procedure and its possible lack of results need to be understood by all involved. However, if the rape victim decides to abort, parentage determination using DNA typing is now possible.

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