CASE REPORT

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Chorionic Villus Sampling Prior to Pregnancy Termination, a Tool for Forensic Paternity Testing

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ABSTRACT: Chorionic villus sampling (CVS), prior to pregnancy termination (pre-termination CVS), is suggested as a tool for forensic paternity testing. Unlike the abortion material, which consists of ruptured tissues of fetal and maternal origin, extra-embryonic membranes obtained through CVS can provide an uncontaminated source of fetal tissue for genotyping. We discuss the possibility of confined placental mosaicism (CPM) and its implications on the polymerase chain reaction (PCR) based analyses of short tandem repeats (STRs) and the D1S80 loci.

KEYWORDS: forensic science, paternity testing, chorionic villus sampling, confined placental mosaicism, DNA typing, polymerase chain reaction, short tandem repeats, D1S80, CSF1PO, TPOX, THO1, F13AO1, FES/FPS, VWA, LPL, F13B

Chorionic villus sampling (CVS), a safe first trimester prenatal diagnostic technique, has become a primary tool for the diagnosis of fetal cytogenic, molecular and biochemical disorders (1). The procedure is performed at the eighth to the tenth week of gestation using either the transcervical or the transabdominal approach (1). Chorionic villi, originating from fetal tissue, can be separated from the extracted decidual maternal tissue and processed for analysis. This early stage fetal analysis has also been applied to cases of disputed paternity using the DNA-variable number tandem repeats (VNTR) by restriction fragment length polymorphism (RFLP) based technology (2,3). Recent advances in molecular genetic technology have led to the use of the polymorphic short tandem repeat (STR) loci for identity and paternity testing (4,5). Here, we present a rape case in which we have applied pre-termination CVS and PCR-STR based technology for forensic use and discuss the importance and implications of this procedure for analyzing similar cases.

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Case Report

As a result of a statutory rape, a 14-year-old girl became pregnant. She named the alleged father, a man with whom she was acquainted. The alleged father was questioned and admitted to the possibility of being the baby's father.

Within the first trimester, the girl's pregnancy was terminated. However, prior to the abortion procedure, chorionic villi were sampled. The CVS, the abortion content and reference samples were sent to our Forensic Biology Laboratory for DNA analysis.

Materials and Methods

Sample Preparation and Genomic DNA Extraction

Chorionic villi were obtained at the ninth week of gestation using the transcervical approach (Fig. 1). This procedure was followed by a suction curattege abortion (6). Chorionic villi were rinsed in cold saline and the floating, whitish, fluffy branched material, representing fetal tissue, was separated under a stereoscope from the maternal decidual amorphic tissue (Fig. 2). This cleaning procedure was done in the Department of Human Genetics at Hadassah University Hospital, Jerusalem, Israel.

The abortion content (endometrial and implanted pregnancy) was placed into petri dishes and rinsed with cold saline. The fetus was isolated and washed repeatedly in saline to remove all adherent tissues. DNA was extracted from chorionic villi, dissected fetal tissue, and the peripheral blood of the mother and the alleged father by the phenol/chloroform and ethanol precipitation procedure (7).

STR Typing

DNA was PCR amplified using the Promega Geneprint™ Multiplex systems—CTT (CSF1PO, TPOX, THO1) and FFv (F13A01, FESFPS, vWA)—and using the single loci kits—LPL (Promega GenePrint™ STR System-LPL) and F13B (Promega GenePrint™ STR System-F13B)—by the conditions recommended by the manufacturer (Promega Technical Manual Geneprint™ STR Systems). The D1S80 locus was amplified as previously described (8), except for the addition of 160 μg/mL BSA to each PCR reaction tube. The PCR products of all the

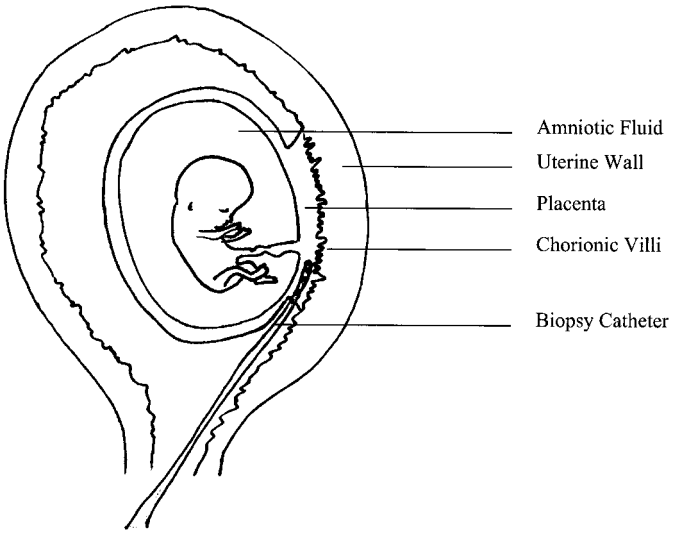

FIG. 1—*A schematic representation of the transcervical approach in chorionic villi sampling.*

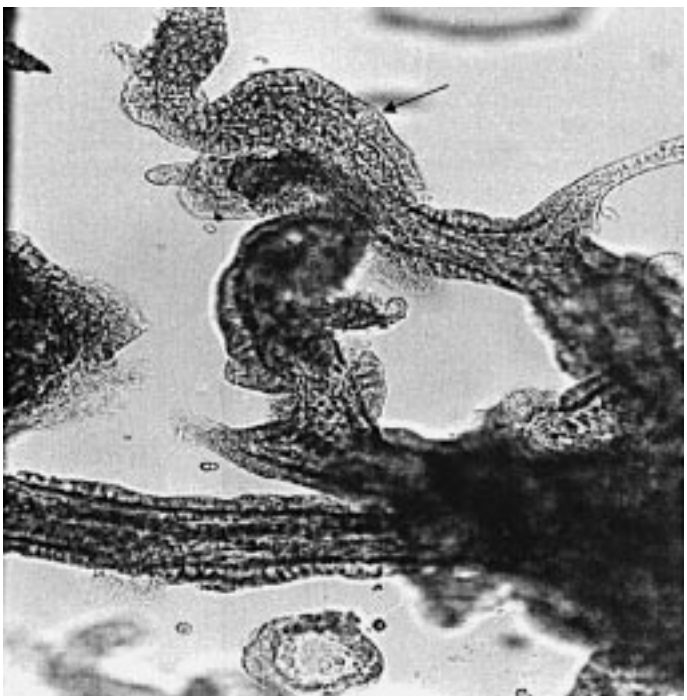

FIG. 2—*A photomicrograph of the chorionic villi after separation from the amorphic maternal tissue (×200).*

Promega STR loci were separated on a 4% denaturating polyacrylamide gel as recommended by the manufacturer. The PCR products of the D1S80 locus were separated on a neutral 6% polyacrylamide gel (8). The neutral and denaturated gels were silver stained as previously described (8,9), respectively.

Paternity and Statistical Analyses

The obligatory paternal alleles were determined from the maternal and fetal genotypes. The frequency of the paternal alleles was calculated using an Israeli database for the following loci: CSF1PO, TPOX, TH01, F13A01, FESFPS, vWA, D1S80. A U.S. Caucasian database was used for calculating the frequency of the alleles in the F13B and LPL loci. The F13B and the D1S80 loci are located on chromosome 1) Using a conservative approach, the results of one of these loci, the F13B locus, were not used in the calculation of the frequency of potential fathers and in the calculation of the paternity index. The combined paternity index was calculated using the product rule.

Results

The results of the PCR-based typing of the mother, the alleged father, the aborted fetus and the chorionic villus sampling are presented in Table 1. Based on these results the alleged father could not be excluded. The frequency of potential fathers is 1.81×10^{-4} (or one out of 5515 males). In other words, 99.982% of the males in the population can not be the possible father. The paternity index in our case was 2946, and the probability of paternity (assuming 50% prior probability) is 99.966%.

In the D1S80 locus both the alleged father and the fetus carried an allele that was out of range of the D1S80 allelic ladder (above the allele designated as 41 repeat units, >41). To ensure that both the alleged father and the fetus carried the same '>41' allele, the fetal and paternal PCR products were combined and characterized on a polyacrylamide gel. The '>41' allele in the alleged father coincided with the '>41' allele of the fetus (Fig. 3A).

The genotypes from the chorionic villi sampling and fetal tissue were compared and found to be identical in all loci. In a D1S80 gel, over-developed by silver staining, a very weak band of maternal origin was visible in the chorion villi sample and not in the fetal tissue sample (Fig. 3B). This weak band resulted from a minor amount of residual maternal tissue that remained after CVS separation.

Discussion

Short tandem repeat loci and the D1S80 locus are highly informative in identity testing. A panel of STR loci has also been validated for paternity testing (10,11). One of the goals in this analysis is to exclude a falsely accused man. The ability of these STR loci to do so, is expressed as the power of exclusion. The American Association of Blood Banks (AABB) has established an average power of exclusion of 95% to be the minimum standard for pater-

TABLE 1—*The genotypes (alleles) of the mother, the chorionic villi, the embryo, and the alleged father at nine STR loci. The obligatory alleles of all the potential fathers are listed along with their frequency in the population.*

Samples ⇒ Locus ⇓	Mother	Embryo and CVS	Alleged Father	Potential Fathers (PF)	Calculated Frequency of PF
CSF1PO	12,12	12,10	13,10	10	0.4630
TPOX	8,8	11,8	11,9	11	0.4088
TH01	9,7	7,7	9,7	7	0.2420
F13A01	7,5	7,5	6,5	7 or 5	0.8154
FESFPS	12,11	11,11	11,11	11	0.6619
vWA	17,16	17,17	17,16	17	0.5633
F13B	10,6	10,6	10,10	10 or 6	0.7480
D1S80	24,21	>41,21	>41,24	>41	0.0199
LPL	10,9	10,10	11,10	10	0.6543

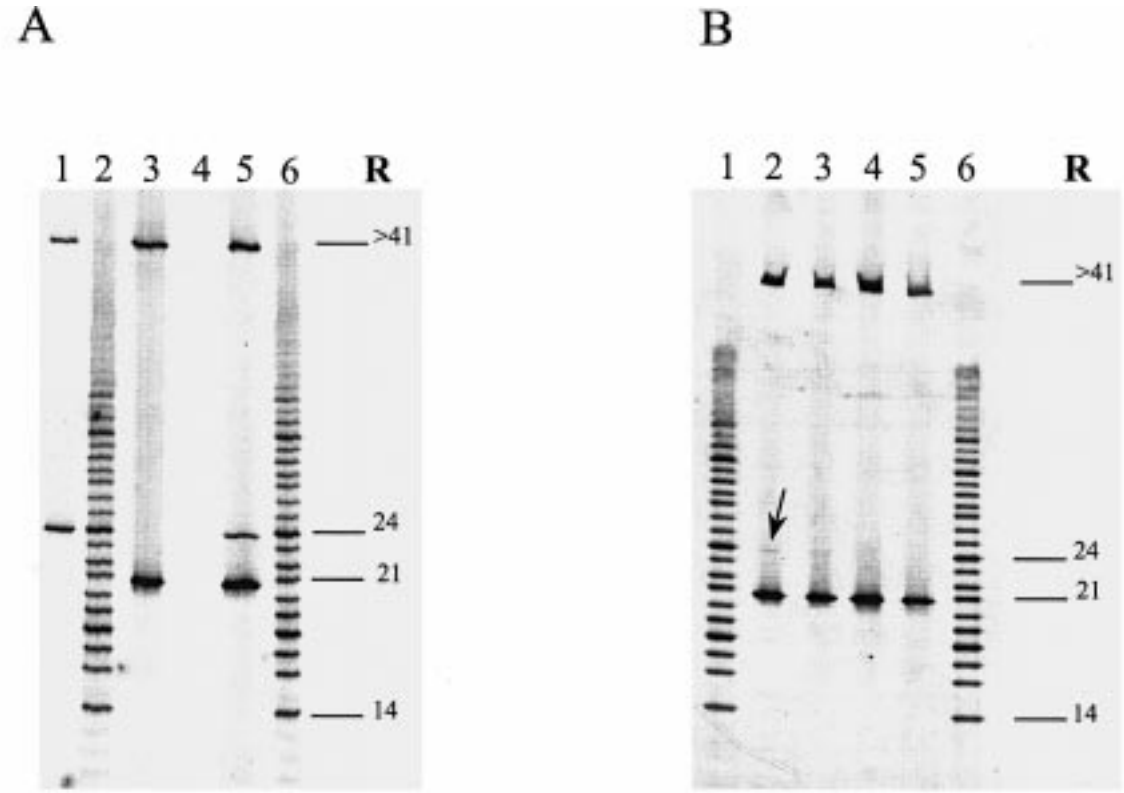

FIG. 3—*The DNA profile at the D1S80 locus of the following samples: A: lane 1—the alleged father, lane 3—the embryo, lane 4—reagent control, lane 5—the DNA mixture of the embryo and the alleged father, lanes 2 and 6—D1S80 ladder ranging from 14 to 41 repeat units. R, number of repeat units. B: lanes 2 and 3—Chorion villi sampling, lanes 4 and 5—embryo tissue from the abortion content, lanes 1 and 6—D1S80 ladder ranging from 14 to 41 repeat units. R, number of repeat units. The arrow indicates a weak band of maternal origin in the chorionic villus sample at the D1S80 locus.*

nity testing (12). The minimum combined power of exclusion for paternity testing of the nine loci used in this report is 99.98%, 99.91%, and 99.84% for the Black, Caucasian, and Hispanic populations, respectively (13).

Here, we present a case of paternity testing in a statutory rape case. Pre-termination chorionic villi were collected. The molecular analysis was carried out on chorionic villi obtained at the ninth week of gestation and on fetal tissue. Both analyses showed identical results in all loci. In our laboratory, in previous cases that resulted in pregnancies, only the aborted fetal material was sampled. The ruptured tissues of both maternal and fetal origin were grossly separated and taken for analysis. In a number of cases, the tissue originating from the fetus was contaminated with maternal tissue resulting in a mixed DNA profile. In such cases, tedious, repeated sampling was required to obtain separate profiles. Moreover, collection, separation and dissection of a fetus proved to be an emotionally distressing procedure. Alternatively, pre-termination CVS provides a clean source of fetal tissue without the need to deal with the fetus.

In the present case, the genotypes of the chorionic villi and the fetal tissue were consistent. However, a major concern in using CVS for prenatal diagnosis is the possible discordance between the chorionic villi analyses and the diagnosis of the fetus. These discrepancies can occur from either maternal tissue contamination or from true genetic differences between the fetus and the extraembryonic tissue known as confined placental mosaicism (CPM) (14).

Contamination of samples with residual maternal tissue, in most cases, occurs as a result of a small sample size that makes appropriate tissue selection difficult. In most experienced centers, adequate amounts of tissues are sampled and this problem is overcome (1). In cases like the one described in this report, chorionic villi sampling is followed by pregnancy termination. This makes it possible to extract a large CVS sample without concern about the outcome of the pregnancy. Following the extraction of large amounts of villi, the possibility of maternal contamination is minimized. In our case, a slight maternal contamination was evident in the D1S80 locus only (Fig. 3B).

Mosaicism is found in about 1%–2% of CVS cases (15) but is confirmed in the fetus in only 10%–40% of these cases (1). Discrepancies between the karyotype of the villus tissue and the actual fetal karyotype may occur as a result of CPM. In these cases, a cytogenic abnormality, most often trisomy, is confined to the placenta (1). The probability that aneuploid cells found in CVS will involve the fetus is related to the tissue source and the chromosome involved (16). It was demonstrated that autosomal mosaicism involving common trisomies (trisomies of chromosomes 21, 18, and 13) may be confirmed in the fetus in 19% of cases, whereas rare trisomies will result in fetal involvement in only 3% of cases (17).

CPM will always be evident once cytogenic analysis is carried out. However, PCR-based analysis will not identify CPM since it results from a post zygotic, mitotic event that is represented by the duplication of a paternal or maternal allele. Therefore, STR analysis for post zygote mosaicism confined to the placenta, will result in correct allele identification but with an increase of intensity of the duplicated allele. This variation in allele intensity is known to occur in the F13A01 locus with the K562 cell line DNA control in

which allele 5 appears more strongly than allele 4 by silver staining.

True pre-zygotic, meiotic aneuploidy may be rescued by a random chromosomal loss resulting in an embryonic diploid state. Some of these cases may result in uni-parental disomy (UPD) of a maternal origin. In maternal UPD, the paternal allele will not be present at one locus of the chromosome involved in this process and the alleged father may be erroneously excluded. This rare event may also occur when one examines the fetal tissues. In such cases, both the fetal tissues and the sampled extraembryonic tissues will be identical to the maternal genotype at this locus.

In 1991, the AABB recommended that when hypervariable single locus probes are used for paternity determination, exclusion of paternity should be based on exclusionary events at two or more loci. This recommendation was subsequently adopted and statistically validated for PCR-STR analysis in parentage testing (18). Exclusion based on two or more loci was suggested for genotyping of minisatellites, VNTR and STR loci, due to spontaneous mutations that may occur in the germline (19). As a result the true father may be excluded due to the identification of a mutated paternally transmitted allele rather than actual non-paternity. This is very unlikely to occur when exclusion is based on at least two loci (20). Because of the rare possibility of mosaicism followed by UPD, exclusion based on at least two loci, on different chromosomes, is essential. However, in the case reported above, the alleged father was not excluded in any of the nine loci examined, namely, D1S80, CSF1PO, TPOX, TH01, F13A01, FESFPS, vWA, F13B, and LPL.

CVS analysis has been shown to be a useful tool for determining disputed paternity in rape cases that result in pregnancy (20). In sexual assault cases, when termination of pregnancy is required, pre-termination transcervical CVS can become a minor part of pregnancy termination procedure required for the actual termination without undue medical risk or added discomfort to the patient (personal communication). In contrast to a simple CVS procedure, commonly used in prenatal diagnosis, pre-termination CVS is held in a sterile setup. This provides an environment that may protect the mother from rare CVS complications such as infection or bleeding. We suggest, therefore, that pre-termination CVS should be adopted as a standard procedure prior to pregnancy termination.

In conclusion, pre-termination CVS is a useful tool for forensic paternity cases. The chorionic villi provide a clean source of fetal tissue for genotyping. In rare cases of mosaicism, that result in UPD of a maternal origin, a misleading PCR-based exclusion for one locus may be obtained. Therefore, exclusion based on at least two loci, on different chromosomes, is essential. As forensic paternity testing using pre-termination CVS is a newly proposed technique, close collaboration should be encouraged between forensic laboratories and medical centers. The applicability of the proposed technique may vary between different countries.

References

1. Wapner RJ. Chorionic villus sampling. Fetal Diagn Ther 1997;24(1):83–110.
2. Mingjun L, Xin Z, Balazs I. Application of DNA profiling to paternity testing during early pregnancy. Hum Hered 1993;43:357–61.
3. Lobbiani A, Nocco A, Vedrietti P, Brambati B, Colucci G. Prenatal paternity testing by DNA analysis. Prenat Diagn 1991;11(5):343–6.
4. Alford RL, Hammond HA, Coto I, Caskey CT. Rapid and efficient resolution of parentage by amplification of short tandem repeats. Am J Hum Genet 1994;55:190–5.
5. Strom CM, Rechitsky S, Ginsberg N, Verlinsky O, Verlinsky Y. Prenatal paternity testing with deoxyribonucleic acid techniques. Am J Obstet Gynecol 1996;174(6):1849–53.
6. Kase NG, Wiengold AB, Gershenson DM. Principles and practice of clinical gynecology. Churchill Livingstone Inc. 2nd ed., 1990.
7. Comey CT, Koons BW, Presley KW, Smerick JB, Sobieralski CA, Stanley DM, et al. DNA extraction strategies for amplified fragment length polymorphism analysis. J Forensic Sci 1994;39:1254–69.
8. Sajantila A, Budowle B, Strom M, Johnsson V, Lukka M, Peltonen L, et al. PCR amplification of alleles at the D1S80 locus: Comparison of Finnish and a North American Caucasian population sample and forensic casework evaluation. Am J Hum Genet 1992;50:816–25.
9. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC. Analysis of the VNTR locus D1S80 by the PCR followed by high resolution PAGE. Am J Hum Genet 1991;48:137–44.
10. Alford A. Rapid and efficient resolution of parentage by amplification of short tandem repeats. Am J Hum Genet 1994;55:190–5.
11. Hammond HA, Jin L, Zhong Y, Caskey CT, Chakraborty R. Evaluation of 13 short tandem repeat loci for use in personal identification applications. Am J Hum Genet 1997;55:175–89.
12. Standards for Parentage Testing Laboratories. 2nd ed., 1994. Arlington, VA: American Association of Blood Banks.
13. Creacy SD, Charles M, Bever K, Bever RA. Application and utilization of STR multiplexes for parentage analysis. Proceedings from the sixth international symposium on human identification. Promega Corporation 1995.
14. Kalousek DK, Vekemans M. Confined placental mosaicism. J Med Genet 1996;33:529–53.
15. Ledbetter DH, Zachary ZL, Si MS, Golbus MS, Pergament E, Jackson L, et al. Cytogenetic results from the US collaborative study on CVS. Prenat Diagn 1992;12(5):317–45.
16. Mastroiacovo P, Botto LD. Chorionic villus sampling and transverse limb deficiencies: maternal age is not a confounder. Am J Med Genet 1994;53:182–6.
17. Phillips OP, Tharapel AT, Lerner JL, Park VM, Wachtel SS, Shulman LP. Risk of fetal mosaicism when placental mosaicism is diagnosed by chorionic villus sampling. Am J Obstet Gynecol 1996;174(3):850–5.
18. Chakraborty R, Stivers DN. Paternity exclusion by DNA markers: Effects of paternal mutations. J Forensic Sci 1996;41(4):671–7.
19. Jeffreys AJ, Royle NJ, Wilson V, Wong Z. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 1988;332:278–81.
20. Hammond HA, Redman JB, Caskey CT. In utero paternity testing following alleged sexual assault, a comparison of DNA-based methods. JAMA 1995;273(22):1774–7.

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