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Case report

Prenatal paternity testing with DNA analyses

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Summary. Two PCR amplified loci and 3 single locus DNA probes were applied in a paternity case in which a married woman became pregnant after being raped. DNA analysis were performed using samples from the woman, her husband and amniotic fluid cells taken during the 16th week of pregnancy. The combined probability of paternity for her husband was calculated as 0.999997107. The application of PCR analyses and single locus DNA probes were considered to be extremely informative in prenatal paternity testing.

Key words: Prenatal paternity testing – Amniotic fluid – Polymerase chain reaction (PCR) – Single locus DNA probes

Zusammenfassung. Zwei mit Hilfe der PCR amplifizierte Loci und 3 Single-Locus-DNA-Sonden wurden in einem Vaterschaftsfall angewandt, in welchem eine verheiratete Frau nach einer Vergewaltigung schwanger geworden war. Für die pränatal durchgeführte DNA-Analyse wurden Proben der Frau, ihres Mannes sowie in der 16. Schwangerschaftswoche gewonnene Ammnion-Zellen eingesetzt. Die kombinierte Vaterschaftswahrscheinlichkeit für den Ehemann wurde mit 0,999997107 berechnet. Die Anwendung der PCR-Analysen und der Single-Locus-DNA-Sonden erwies sich als extrem informativ für die pränatale Vaterschaftsuntersuchung.

Schlüsselwörter: Pränatale Vaterschaftsuntersuchung – Ammnionflüssigkeit – PCR – Single-Locus-DNA-Sonden

Introduction

Recently, DNA analyses have been applied to prenatal paternity testing using aborted fetal tissue or chorionic villus tissue [1, 2, 3, 4].

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In the present case, prenatal paternity testing was performed to determine whether the father was the rapist or the husband. Two amplified loci and 3 hypervariable DNA probes were applied using samples from the amniotic fluid, and were found to be informative for prenatal paternity testing.

Materials and methods

Case report. A married women became pregnant after she had been raped. Paternity testing between the fetus and her husband were requested from our laboratory. As it is not always safe to collect blood from a fetus, 25 ml amniotic fluid was collected by amniocentesis in the 16th week of pregnancy, of which 15 ml was used for standard blood group markers and PCR analyses. Culturing of the amniotic cells for Southern hybridization was carried out using the remaining 10 ml of amniotic fluid.

Standard blood group marker systems. Seven standard blood group markers were analyzed on the supernatant of amniotic fluid: ABO, haptoglobin (Hp), transferrin (Tf), group-specific component (Gc), α_1-antitrypsin (Pi), α_2-HS glycoprotein (α_2-HS) and inter-alpha-trypsin-inhibitor (ITI).

Table 1. Summary of blood group markers (phenotypes) typing in this case

	Supernatant of amniotic fluid	Mother	Husband
ABO*	A	A	A
Hp	Negative	2–1	2–2
Tf*	C2–1	C2–1	C1–1
Gc*	1F1S	1F1S	1F1S
Pi	Negative	M1–1	M1–1
α_2HS	1–1	2–1	1–1
ITI	Negative	2–2	2–1

* In ABO, Tf and Gc systems all samples have the same markers. In these cases, it is not certain that the amniotic types are really fetal. We could not determine Hp, Pi and ITI from the amniotic fluid

Table 2. Allele number of fragment size and probability of paternity (P.P) in this case

Marker	Allele number or fragment size			Frequency of the allele derived from the father[a]	P.P
	Husband	Amniotic cells	Mother		
D1S80	28, 15	28, 22	22, 21	0.1115	0.8177
ApoB	41, –	41, –	41, –	0.5149	0.6601
MR24/1	3.3, 2.5	6.5, 3.3	6.5, 5.4	0.0709	0.992958
3′-Globin	4.8, 3.6	4.8, 4.0	4.0, 2.3	0.1497	0.97093
pYNH24	2.45, 1.80	2.45, –	3.25, 2.45	0.0593	0.8939
Combined P.P from DNA analyses					0.999997107
α_2HS					0.5760
Combined P.P					0.999997870

[a] These frequencies were derived from the database from a Japanese population. These databases are as follows: D1S80, Kasai et al. (1992) Reports of the National Research Institute of Police Science. Research on forensic science 45:24–36 (in Japanese); ApoB, Nata et al. (1992) Res Pract Forensic Med 35:15–17 (in Japanese); MR24/1 and 3′-Globin: Yokoi et al. (1990) Z Rechtsmed 103:487–497; pYNH24, Yokoi et al. (1992) Jpn J Hum Genet 35:179–188. 123 bp DNA ladder (BRL, U.S.A.) was used for D1S80 and ApoB. The size was determined according to the method reported by Kasai et al. (1992). In MR24/1, 3′-globin and pYNH24, the size was determined according to the method reported by Yokoi et al. (1990, 1992)

Extraction of DNA from amniotic cells. A 15 ml aliquot of amniotic fluid was centrifuged at 2,000 rpm for 5 min, and the pelleted cells were collected. DNA was extracted from pelleted cells using standard techniques [5]. Approximately 2,8 μg of DNA was extracted from 15 ml of the amniotic fluid. DNA of the mother and her husband were isolated from peripheral blood using standard techniques [5].

PCR analysis. DNA samples were used in the polymerase chain reaction (PCR) [6] to amplify the Apolipoprotein B100 (Apo B) region [7] and D1S80 region [8].

Culture of amniotic cells and extraction of DNA from cultured amniotic cells. Ten ml amniotic fluid was cultured for 19 days using standard techniques [9]. DNA from cultured amniotic cells was extracted using standard technique [5], and approximately 25 μg of DNA was obtained.

DNA hybridization. The combinations of probes and restriction enzymes were as follows; MR24/1-*Hin*fI, 3′-globin-*Pvu*II, pYNH24-*Msp*I. Southern blot hybridization was performed using standard techniques [10].

Results

In the conventional systems, only α_2-HS was determined as a fetal marker and paternity could not be excluded (Table 1). In all 5 DNA systems the husband could not be excluded from paternity (Table 2). In the Apo B system, the mother, amniotic cells and the husband had the same homozygotic "41" allele. In D1S80 system, the "28" allele found in the amniotic cells could have come from the husband. The 3.3 kb allele in MR24/1-*Hin*fI system and the 4.0 kb fragment in the 3′-globin-*Pvu*II system could have also come from the husband (Fig. 1). The combined probability of paternity from DNA analyses and α_2-HS was 0.999997870 (Table 2).

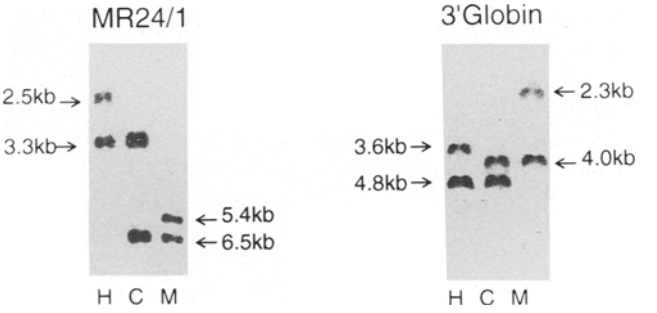

Fig. 1. Autoradiograms of the DNA hybridization analysis in this case (*(M)* mother, *(C)* cultured amniotic cell, *(H)* husband). The combinations of probes and restriction enzymes were MR24/1-*Hin*fI and 3′globin-*Pvu*II. The *arrowheads* indicate the size of the bands

Discussion

DNA analyses have already been applied to prenatal paternity testing using aborted fetal tissue after interruption of pregnancy [1, 2, 3] but could not be applied to cases where the pregnancy is not terminated. Ishiyama et al. [4] reported prenatal paternity test using chorionic villus tissue (wet weight 10 mg) without interruption of pregnancy. In the present case, prenatal paternity testing could be accomplished using amniotic fluid without interruption of pregnancy. It usually takes only a few days to obtain results from PCR analyses. In addition, about one month is necessary to obtain autoradiograms after culture of amniotic cells, extraction of DNA and Southern hybridization. PCR analysis was useful for prenatal paternity testing because of its rapidity. However, the usefulness of Southern hybridization analysis with

single locus DNA probes is not changed because it is informative and reliable.

As the prenatal diagnosis of chromosomal abnormalities and congenital metabolic disorders in prenatal paternity testing can cause ethical problems, it must be carried out with caution.

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