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# DNA Fingerprints: The Importance in Forensic Medicine

## II. The Significance of Examining DNA Polymorphisms of Placental Tissues for the Purpose of Paternity Determination During the Early Stages Within the First Trimester*

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**Summary.** Investigation of genomic polymorphisms detected by a minisatellite named tentatively "Myo", which is expected to correspond to the minisatellite in human myoglobin gene of Jeffreys et al., gives distinct and different aspects of chorionic villus and the decidual membrane in the same placenta. The chorionic villus, which is regarded as the extraembryonal tissue, represents the essential embryonal DNA fingerprint pattern, while the decidual membrane reveals the maternal one. A comparison between the DNA fingerprints from the chorion villus and from the blood sample of the suspected father provides the possibility of setting a paternity determination which can be achieved during the first trimester of a pregnancy.

**Key words:** DNA fingerprinting, paternity determination, DNA polymorphism

**Zusammenfassung** Die genomischen Eigenschaften der Plazenta wurden mit Hilfe von einer Sonde "Myo" gegenüber dem Minisatellit, der im Bereich vom humanen Myoglobinen vorhanden ist, analysiert. Der DNS Fingerabdruck von Chorionzotten stimmt völlig mit dem des Fötus überein, während der Fingerabdruck der Dezidua die mütterliche Herkunft darstellt. Die Analyse von verschiedenen DNS Fingerabdrücken aus Fötus, Chorionzotten, Mutter und Vater bei einer künstlichen Schwangerschaftsunterbrechung hat gezeigt, daß der DNS Fingerabdruck von Chorionzotten, die gewöhnlich bei der klinischen Chorionzottenbiopsie entnommen werden können, die

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entscheidende Auskunft darüber gibt, das Bestehen der Vaterschaft vollständig zu beweisen.

**Schlüsselwörter:** DNS Fingerabdruck, Vaterschaftsfeststellung, DNS Polymorphismus

## Introduction

Jeffreys et al. [1–4] have reported that hypervariable "minisatellite" regions, which are dispersed in the human genome, show restriction fragment length polymorphisms (RFLPs) due to allelic differences in the number of tandem repeats containing the core sequence, and the Southern blot hybridization using minisatellite core probes produces variable band patterns, which are completely individual-specific and inherited in a Mendelian fashion. It is, therefore, expected that RFLPs thus obtained provide the utmost decisive information in diagnosing individuality and paternity identification in forensic medicine. Based on these findings, these authors have shown that DNA fingerprints are very useful in the diagnosis of twins with respect to their zygosity (i.e., monozygosity and heterozygosity) determination [5, 6].

We have examined this method and have confirmed that the DNA fingerprints obtained by this minisatellite method offer a very reliable probability in paternal determination [7]. Furthermore, we have investigated the DNA fingerprints of the chorion villus and the decidual membrane in the same placenta at an early stage of gestation and have found that the cytogenetic mosaic conformation of this organ can be demonstrated by using the minisatellite probe. In addition, we have noticed that this method provides the possibility of paternity identification of the fetus by examining the genomic expression of chorion villus (the extraembryonal mesodermal tissue).

Recently, we have analyzed the DNA fingerprints of the fetus, the chorion villus, the decidual membrane, and the blood samples of the mother and father in a case of a therapeutic abortion and have found that the paternity determination can be achieved by estimating the DNA fingerprints of the chorionic villus and the blood of the suspected father. Our results will be reported in this short communication.

## Materials and Methods

### *Minisatellite Probe*

The probe was kindly supplied by Prof. R. Kominami (First Dept. of Biochemistry, Tokyo University). The minisatellite "Myo" was prepared in the following manner: DNA with a 33 basic pair of GACCGAGGTCTAAAGCTGGAGGTGGGCAGGAAG and its complementary pair of GCTCCAGATTTCGACCTCCACCCGTCCTTCCTG were synthesized chemically with the aid of synthesizers.

They were linked to each other enzymatically to conform the double strand DNA with tandem repeats of about 15 times (0.5 kb).

The probe against these repeats was produced by cloning. It was expected to correspond to the minisatellite in human myoglobin gene reported by Jeffreys et al. [1].

*Materials*

Placentas in various early stages of gestation were collected. One case of a therapeutic abortion at the gestation stage of 10 weeks, in which our experimental results will be reported in this communication, was found to be satisfactory for our investigation; i.e., the placenta, the fetus, and the blood samples of both the mother and the father could be collected simultaneously. Consanguinity was completely excluded in this marriage and this was also confirmed in our experiment. All materials were cooled by ice immediately and brought to our laboratory. In the case of the placenta, the chorion villus and decidual membrane were isolated for macroscopic observation. The tissue was then dissected into small pieces of about 1 mm and used for the extraction of DNA.

*Extraction of DNA*

The samples were incubated with a buffer solution (10 m*M* Tris-HCl of pH 7.5, 5 m*M* EDTA, 100 m*M* NaCl, 1% SDS) containing 100 µg/ml Proteinase K (Merck Co.Ltd.) at 50°C for 12 h. The aliquot was washed twice with phenol, once with chloroform, and the DNA was then precipitated with ethanol. The DNA was washed with cooled 80% ethanol, dried, and dissolved in a TE buffer solution (10 m*M* Tris-HCl of pH 7.5, 0.1 m*M* EDTA), after which the DNA content was estimated photometrically by measuring its absorbance at 260 and 280 nm.

*Restriction Enzyme Digestion and DNA Blotting*

*DNA Digestion.* DNA (20 µg) was mixed with the restriction enzyme Hinf 1 (Boehringer Co. Ltd.) in 36 U, and the total volume of the reaction mixture was brought to 200 µl with a buffer solution (100 m*M* NaCl, 50 m*M* Tris-HCl of pH 7.5, 10 m*M* MgCl, 1 m*M* Dithiothreitol). The digestion was continued for 3 h at 37°C and stopped by adding EDTA. The DNA fragments thus obtained were precipitated with ethanol, dried, and dissolved with 20 µl of TE.

*Electrophoresis of DNA Fragments.* An aliquot containing 5 µg of DNA fragments was mixed with a 1/10 volume solution containing 50% glycerin, 0.25% xylenecyanol FF (Chroma-Gesellschaft) and 0.25% bromophenol blue (Wako Co. Ltd.) and charged onto a 1% agarose gel in a thickness of 0.6 cm and electrophoresed in the medium containing 40 m*M* Tris-HCl, 5 m*M* sodium acetate, and 1 m*M* EDTA (pH 7.7) at 3 V/cm for 22 h. After this electrophoresis, the gel plate was treated with 150 ml of a solution containing 0.2 *N* NaOH and 0.6 *M* NaCl for 1 h at room temperature (DNA denaturation) and neutralized with 150 ml of a solution containing 0.5 *M* Tris-HCl (pH 7.4) and 0.9 *M* NaCl for 1 h.

*Transfer of the DNA Fragments on the Nylon Sheet.* The DNA fragments which had been separated by electrophoresis, were transferred to a Nylon sheet (Gene Screen Plus: DuPont Co. Ltd.) by the method described by Southern [8]. After this transfer, the sheet was dried and irradiated with a UV-light (short length) for 3 min to fix the DNA fragments onto the sheet.

*Prehybridization.* This was performed by treating the Nylon sheet with a prehybridization buffer solution containing 5 × SSC, 10 m*M* Tris-HCl of pH 7.4, 2 m*M* EDTA, 1% SDS, 5 µg/ml tRNA for 3 h at 60°C.

*Hybridization*

The prehybridized sheet was placed in a heat-sealable plastic bag and a few milliliters of the prehybridization buffer solution were added. The minisatellite "Myo" probe was labeled with $\alpha$-($^{32}$P)dCTP by Nick translation system (Nick translation kid, Boehringer Co. Ltd.). The isotope-labeled "Myo" probe was isolated by filtration through a Biogel p-60 column, treated at 100°C for 10 min. Of this probe 2 µCi per single sheet was added to the bag and incubated at 62°C overnight. The sheet was then washed with a solution containing 2 X SSC and 1% SDS

for 30 min at 62°C, after which the sheet was placed between paper towels and squeezed to extract any excess solution, wrapped with a plastic paper, and autoradiographed to a XRP-1 film (Kodak Co. Ltd.) backed by a Lightening Plus intensifying screen (DuPont Co. Ltd.) at −60°C for 1 day.

## Experimental Results

### *Comparison of the DNA Fingerprints of the Placental Tissues (the Chorionic Villus and Decidual Membrane), the Fetus, and the Blood Samples of the Mother and Father*

Figure 1 represents the patterns of DNA fingerprints of various kinds of materials. As expected, the DNA fingerprint of the decidual membrane demonstrated the identical pattern found in the maternal blood, while that of the chorionic villus was identical with the fetal image. This indicates distinctly that the DNA fingerprint of this extraembryonal tissue of embryonal origin duplicates completely the genetic information of the fetus. The comparison of the

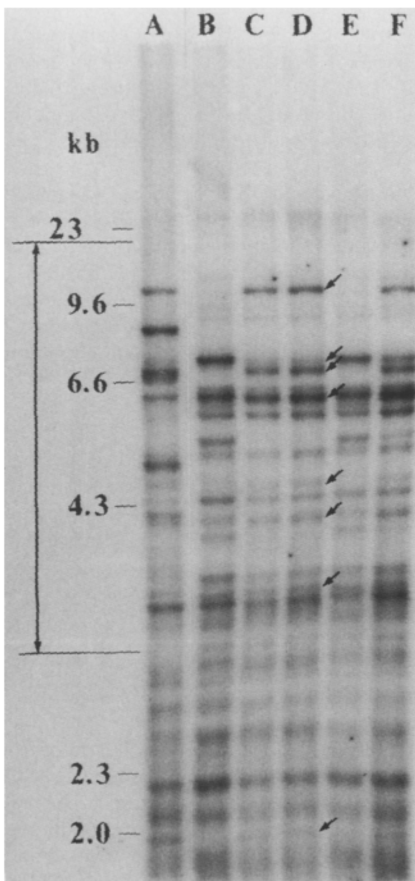


**Fig. 1.** DNA fingerprints of various kinds of samples in a case of therapeutic abortion at the gestational age of 10 weeks.
*A* Paternal blood, *B* maternal blood, *C* fetus, *D* chorionic villus, *E* decidual membrane, *F* coagulum found at the surface of the placenta (this sample represents the DNA fingerprint image from mixing the maternal blood and fetal tissue). *kb:* kilobases. *Small arrows* indicate the bands in the DNA fingerprints from the fetus and chorionic villus, which are similarly found in the paternal DNA fingerprint, but not in the maternal one. *Large arrows* at the kb show the regions of the highly hypervariability

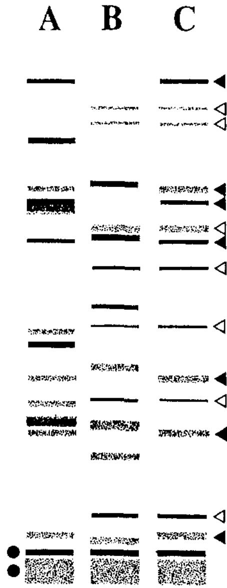


**Fig. 2.** Distinct DNA fragment bands found in the samples A (paternal), B (maternal), and C (embryonal DNA fingerprint) in Fig. 1 and original autoradiographic film. Distinct bands in the DNA fingerprints appearing in the highly hypervariable regions (3–20 kb) were represented.
—— : Bands discerned distinctly in Fig. 1.
▒▒▒ : Bands discerned hardly in Fig. 1, but detected distinctly in the original film.
*Triangles* at the lane C (embryonal DNA fingerprint) indicate the bands which show the similar electrophoretic mobility and autoradiographic intensity in the maternal (white) and paternal (black) DNA fingerprints, respectively. *Dots* represent the bands, which are found similarly in both the maternal and paternal DNA fingerprint

embryonal DNA fingerprint with that of the maternal (the maternal blood and placental decidual membrane) and paternal samples offers some important factors as to the paternity determination. As indicated in Fig. 2, all fragments in the embryonal (fetal and/or chorionic) DNA fingerprint pattern could be scored either in the maternal or in the paternal RFLPs, while no bands unrelated in the parental images were detected. This finding confirms conclusively that the polymorphic feature of the genome in the fetus is derived from the parental genomic polymorphisms. Statistical estimation of the paternity can be calculated in the following manners: there are eight distinct bands, which are found in the parental DNA fragment pattern in a similar manner and not in the maternal one. According to Jeffreys et al. [2], the mean probability that a fragment in the DNA fingerprint of one person is present in a second individual selected at random is approximately 0.2 for Northern Europeans. Now, if this value (examination of 12 Japanese unrelated individuals revealed the similar tendency) is applied in our case, the chance that the fetus is unrelated to the father but still happens to share all eight fragments is, therefore, $0.2^8 = 2.56 \times 10^{-6}$, so that the paternity estimation value calculates out to $1–2.56 \times 10^{-6} = 0.999993$, which would indicate that the paternity really exists. The paternity probability can be calculated in another way. Figure 2 indicates the distinct genomic bands in these three samples. Sixteen and fifteen bands were detected in the highly hypervariable regions between 3 to 20 kb, respectively, when the maternal and paternal DNA fingerprints were observed. Of these, only two bands with a similar electrophoretic mobility and autoradiographic intensity were found in both samples. Hence, the probability that a fragment in one individual is matched by a band of similar electrophoretic mobility and autoradiograph intensity in a second random person is expressed by $(2/15 + 2/16)/2 = 0.129$. We were able to detect, however, seven similar bands in the paternal and the embryonic DNA finger-

**Table 1.** The yield of highly molecular mass ($M_r$) ungraded DNA from the chorionic villus and decidual membrane

| Tissue | Gestation stage (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | 6 | 6–7 | 8 | 10 | 10 | 14 |
| Chorion | 2,400 | 1,200 | 2,900 | 1,600 | 2,010 | 2,300 |
| Decidua | 1,800 | Ø | 1,000 | 950 | 1,700 | 1,500 |
| Fetus | Ø | Ø | Ø | Ø | 1,650 | Ø |

The values were expressed by μg/0.5 g (wet wt).
Ø: Investigation was not achieved

prints. Thus, the probability of the paternity is overwhelmingly expressed as $1-0.129^7 = 0.9999996$. If the resolution of electrophoresis in analyzing the DNA fingerprint could be improved to such levels as Jeffreys et al. have shown [3], then a far higher paternal probability would be attained.

### *The Yields of DNA Extracted from the Placental Tissues*

As the DNA amount extracted from the tissue sample is the essential factor of this procedure, we have compared the yields of DNA in the placentas in their early gestation stages. Table 1 represents the results.

From this, it is estimated that the yield of DNA from 10 mg of tissue in wet weight is ca. 24–58 μg in the case of decidual membrane. The yields of DNA from the chorion villus in our analysis exceed slightly those of Quaife and Liu [9] giving the average of 17.9 μg in 10 mg of the tissue.

### *The Comparison of the Experimental Results of Decidual and Chorionic DNA Fingerprints*

We have conducted six cases of placental analyses as seen in Table 1, the results of which will be reported in subsequent papers. Our investigations have shown so far that the difference in the genetic control in the placenta can be ascertained distinctly.

## Discussion

As the investigation in determining the individual polymorphism using multiple red-cell antigens and enzymes is able to be performed for the paternity and individuality diagnosis in forensic medicine, accuracies of the identification of the order over 99.9% may be achieved but only if the relevant allele frequencies in the population are known. Similarly, analysis of multiple restriction enzyme site polymorphisms with several different DNA probes produces a substantial percentage of "false positive" diagnosis of identification. Minisatellite probes lack this drawback because of the large number and substantial variability of the hypervariable DNA segments that they detect, which is so great that hybridiz-

ing minisatellite fragments are seldom shared between randomly selected individuals [1–5]. In this respect, application of the minisatellite probes in forensic medicine should be regarded as the utmost important problem. In addition, it should be stressed that the paternity determination by the use of RFLPs is achieved even at the immature stages of individuals, at which the manifestation of genetic markers in phenotype are hindered completely.

The analysis of the placenta using DNA fingerprinting, gained by the application of the "Myo" probe, has provided decisive information for determination of paternity during pregnancy. A paternity determination can be accomplished when the chorion villus tissue to be sampled has a wet weight of 10 mg, which is usually enough for a DNA fingerprint and recommended for the extraction of DNA in various laboratories [9]. In this respect, the present interests and stand points of this chorion villus sampling in the clinical medicine should be considered.

It has been noticed that the chorion villus sampling provides the promise of the prenatal diagnosis of certain chromosomal, genetic, and metabolic disorders before the first trimester, thus allowing termination at the earlier gestation than is currently possible with most other parental diagnostic techniques [10]. The failures in losing fetus are expected in only 2.4% of the attempted sampling with figures ranging from 10.6% to nil in more than 100 cases. These results suggest that the variation is due to the two factors of relative aggressiveness in seeking an analyzable sample and the method of sampling used [11]. Thus, the data indicate that the procedure is applicable to almost all candidate patients and that a high percentage of sampling attempts will be successful in experienced hands. The risk of failure therefore appears to be small. Recent advances in chorion villus sampling concerning the techniques and applications were collected in the review of Liu et al. [12].

In reflecting these backgrounds concerning the chorion villus sampling in clinical medicine, our experiments indicate distinctly that paternity determination can be performed during the early stages of pregnancy, as the same technique used for the diagnosis of congenital malformations in the prenatal medical care can be applied directly in diagnosing the paternity determination. For example, the procedure for the extraction of DNA from the chorion villus, which is reported by Quaife and Liu [9] in the research field of obstetrics, satisfy thoroughly all conditions for the investigation of paternity in our experiment.

From the view of theory and practice, we have confirmed that the matter of paternity can be determined conclusively even in the gestation stages as early as 8–10 weeks, though getting the DNA fingerprint of the chorion villus mainly depends on technically improving the method of obtaining the chorion villus sampling without endangering the fetus.

In certain instances, some benefit may be attained by examining the placental tissue; i.e., conflicts over paternity, which should arise after the delivery of newborns, could have been avoided by the determination of paternity in the first trimester of pregnancy.

Our method, however, may be considered as too controversial in view of present medical and social ethics. For now, we suggest that the determination is still accomplished by using a method of sampling chorion villus, which does not

damage the fetus, but the application of this method should be controlled by a reasonable sociomedical consensus.

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