TECHNICAL NOTE

Masamitsu Honma,[1] M.S.; Tomio Yoshii,[1] M.S.;
Ikuo Ishiyama,[1] M.D.; Kohnosuke Mitani,[2] M.S.;
Ryo Kominami,[3] M.D.; and Masami Muramatsu,[2] M.D.

Individual Identification from Semen by the Deoxyribonucleic Acid (DNA) Fingerprint Technique

REFERENCE: Honma, M., Yoshii, T., Ishiyama, I., Mitani, K., Kominami, R., and Muramatsu, M., **"Individual Identification from Semen by the Deoxyribonucleic Acid (DNA) Fingerprint Technique,"** *Journal of Forensic Sciences,* JFSCA, Vol. 34, No. 1, Jan. 1989, pp. 222-227.

ABSTRACT: For individual identification from semen, the deoxyribonucleic acid (DNA) fingerprint technique was used. In a blind trial, we succeeded in determining the semen donors among several volunteers comparing the DNA fingerprints of the blood and semen samples, respectively. Thereafter, we examined semen in a condom left beside a naked female dead body. The DNA fingerprint of the semen was recognized to be identical to that of the blood from a suspected man arrested later. This is the first report that the DNA fingerprint technique was practically used in a criminal investigation in Japan.

KEYWORDS: criminalistics, human identification, semen, deoxyribonucleic acid (DNA), DNA fingerprinting

It is important for criminal evidence to recognize semen in cases of sex assault. Obviously, the evidence of spermatoza, prostatic acid phosphatase, or γ-seminoprotein is necessary, but it indicates nothing more than the occurrence of sexual activity [*1-3*]. For individual identification from semen, ABO blood group typing has been conventionally used. However, because this examination gives little information, it has often been difficult to exclude a suspected man or to confirm by a high probability.

Deoxyribonucleic acid (DNA) polymorphisms, that is, DNA sequence differences between

Received for publication 18 March 1988; revised manuscript received 9 May 1988; accepted for publication 10 May 1988.

[1]Postgraduate student, junior lecturer, and professor, respectively, Department of Forensic Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

[2]Postgraduate student and professor, respectively, First Department of Biochemistry, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

[3]Professor, First Department of Biochemistry, Faculty of Medicine, University of Niigata, Niigata, Japan.

individuals, can be visualized by Southern blotting and DNA hybridization as size differences in DNA fragments when DNA is digested by restriction endonuclease (restriction fragment length polymorphisms [RFLPs]) [*4*]. Some genetic markers of DNA polymorphisms are used in forensic science [*5–8*]. Particularly the "DNA fingerprint" technique, which was first described by Jeffreys et al. [*9*], is very useful for individual identification [*10–12*]. This is because the resulting Southern-blot profile consists of a complex set of large and highly variable DNA fragments and has extraordinary individualizing power. The DNA fingerprint technique has also been used in paternity determination [*13*], based on the fact that each fragment shown by this technique is segregated in Mendelian fashion.

In our previous papers [*14,15*], we have reported that a synthesized DNA probe for DNA fingerprints is indeed of great use to prove paternity. Recently, we encountered the chance of identifying semen which had been left at the scene of murder. This article describes a practical case of individual determination from semen by means of the DNA fingerprint technique.

Materials and Methods

DNA Isolation of Blood and Semen Samples

Fresh blood samples were diluted with ten volumes of blood lysis solution (0.2% sodium chloride [NaCl]). The white cells were pelleted by centrifugation at 600 × *g* for 10 min and resuspended in TNE buffer (10m*M* Tris-hydrochloric acid [HCl] [pH 7.5], 100 m*M* NaCl, 1m*M* ethylenediaminetetraacetate [EDTA]). Proteinase K (Merk Co.) and sodium laurylsulfate (SDS) added up to a final concentration of 100 μg/mL and 1.0%, respectively. The sample solution was incubated overnight at 50°C and extracted twice with phenol, once with chloroform, and then dialyzed against two changes of a thousandfold excess of TE buffer (10m*M* Tris-HCl [pH 7.5] and 1m*M* EDTA). DNA was precipitated with 2.5 volumes of ethanol overnight at −20°C, pelleted by centrifugation, and washed with 80% ethanol. DNA was dissolved in a small volume of TE buffer. Semen samples were suspended directly in TNE buffer containing dithiothreitol (DTT) at a concentration of 50m*M* and treated as described above for the blood samples. The amount of DNA recovered was measured in an ultraviolet (UV) spectrophotometer at 260 nm.

Southern-Blot Analysis

DNA samples (5 μg) were digested with ten units of *Hinf* I (Takara Co.) at 37°C for 3 h and recovered by ethanol precipitation. Digested DNA samples were dissolved in 5 μL of TE buffer plus 1 μL of gel-loading buffer (30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol) and loaded onto an agarose gel (0.8% agarose in 40m*M* Tris-acetate, 20m*M* sodium acetate, 1m*M* EDTA, 0.5 μg/mL ethidium bromide, pH 8.3; gels 0.7 cm thick by 14 cm long). The gel was electrophoresed at 40 V for 20 h until all DNA fragments less than 1.0 kb long had been electrophoresed off the gel. DNA in the gel was denatured in situ and transferred to a nylon membrane filter (Du Pont Co.) by blotting [*16*]. The filter was irradiated with ultraviolet rays for fixing DNA and then incubated for 12 h at 62°C in 5 × SSC (SSC is saline sodium citrate: 150m*M* NaCl and 15m*M* sodium citrate [pH 7.0]), 1m*M* EDTA, 1% SDS, and 10 μg/mL yeast ribonucleic acid (RNA). The hybridization buffer was the same. After incubation the filter was hybridized to a ^{32}P-labeled single-strand minisatellite probe for 12 h at 62°C. The DNA probe was labeled by the nick translation procedure (nick translation kit, Boeringer Co.) [*17*]. After hybridization the filter was washed at 62°C for 1 h with 1.5 × SSC, 1% SDS. Autoradiography was carried out at −80°C with an intensifying screen for one day.

Minisatellite Probe

A minisatellite "Myo" probe, which has been reported to be derived from a tandem repetitive segment of human myoglobin gene [*18*], was synthesized by the DNA synthesizer and molecular cloning technique. It is comprised of 15 repeats of a 33-bp sequence. The probe repeat unit is:

GACCGAGGTCTAAAGCTGGAGGTGGGCAGGAAG

Results

Blind Trial Case of Identification from Semen

We examined two semen and eight blood samples; the two semen samples were taken from the male volunteers donating the blood. The DNA fingerprints of the blood from eight volunteers and from the semen are shown in Fig. 1. Each DNA was digested with *Hinf* I and subjected to Southern blotting and DNA hybridization by the minisatellite probe "Myo." Lanes 1 through 8 show the DNA fingerprints of the blood and Lanes A and B those of the semen. Comparison of these DNA fingerprints reveals that each of the seven clear bands longer than 2.0 kb in Lanes A and B correspond to the bands in Lanes 5 and 6, respectively.

Therefore, we can easily determine that Semen A and B are from Volunteers 5 and 6, respectively.

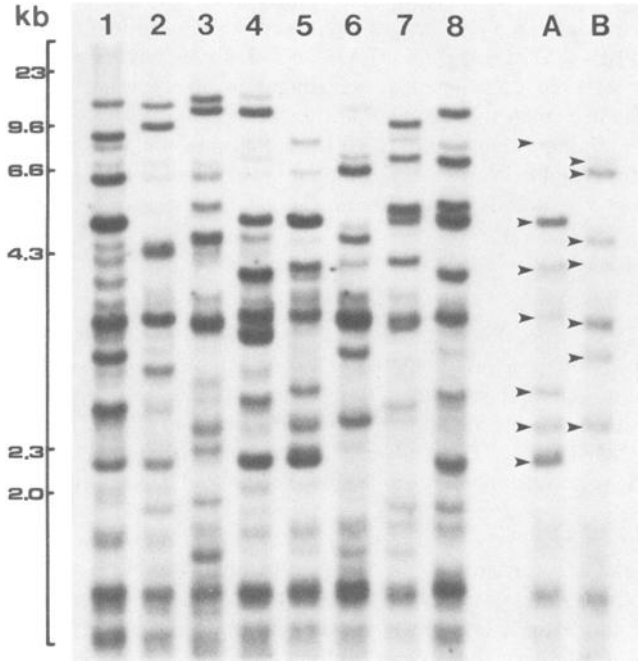

FIG. 1—*DNA fingerprints in a case of a blind trial of individual identification from semen. Two of the eight volunteers are semen donors. Lanes 1 through 8 show the DNA fingerprints of blood and Lanes A and B those of semen. Arrowheads (➤) indicate clear bands longer than 2 kb in Lanes A and B. Hinf I digested DNA from blood or semen was hybridized to the* ^{32}*P-labeled minisatellite probe "Myo."*

A Practical Case

A naked female dead body was found in a hotel room in Tokyo. The woman was thought to have been dead for one day. The cause of death was shown to be asphyxia by strangulation with a ligature. It was found that a condom which contained semen was left beside her. Considering the circumstances of the scene of murder, it was expected that the murderer had killed the woman after sexual intercourse and left the scene.

Later, a man suspected of the murder was arrested. We were requested to examine the semen in the condom and the blood of the suspected man. Figure 2 shows the DNA fingerprints of the semen (S) and blood of the man (X). U1 and U2 in Fig. 2 are from blood DNA of unrelated men. The DNA fingerprint of the semen, which contains 19 clear bands longer

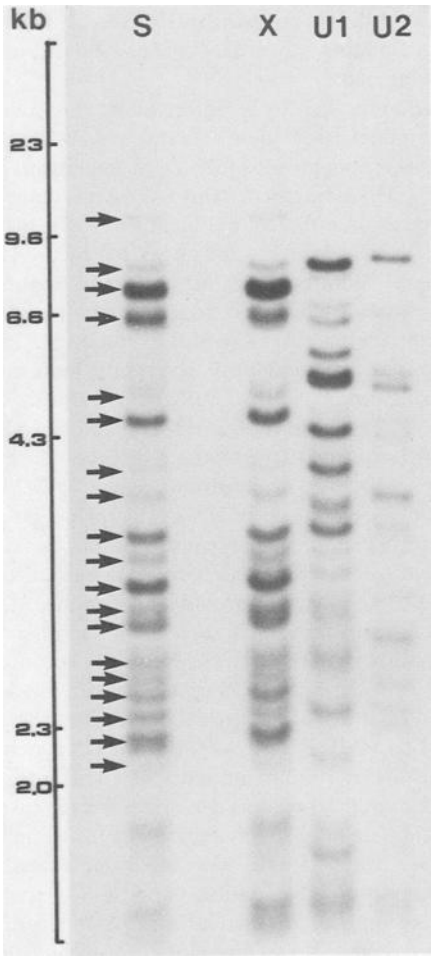

FIG. 2—*DNA fingerprints from semen in a condom left beside a naked female dead body (Lane S). The pattern, which contains 19 clear bands (→) longer than 2 kb, is the same as that obtained from blood of a suspected man arrested later (Lane X). Unrelated men's DNA fingerprints from blood are shown in Lanes U1 and U2. Hinf I digested DNA from blood or semen was hybridized to the ^{32}P-labeled minisatellite probe "Myo."*

than 2.0 kb, is completely identical to that of the blood from the suspected man. This indicates that the semen is the suspected man's.

Discussion

In this practical case, we examined semen fluid in a condom. Because the semen was not contaminated with vaginal materials, we could obtain DNA derived from only the sperm of the semen. Furthermore, the semen was not old, so that most of the isolated DNA was of high molecular weight. Such good conditions allowed us to make the DNA analysis with ease. This was very fortunate for the criminal investigation.

Often in the sexual assaults semen is left in the vagina of the victim, and so the semen is contaminated with vaginal celluar debris. With time, sperms in the vagina disintegrate. Thus, it is difficult to obtain not only correct ABO blood group typing but also the DNA fingerprint of the sole rapist [*19*]. It is noteworthy that recently Guisti et al. [*8*] and Gill et al. [*11,20*] have succeeded in isolating sperm DNA from semen-contaminated vaginal swabs using the deferential lysis method.

Practically, an examination for identity is required to have a high probability to exclude a suspected person from a criminal case. While only typing the ABO blood group gives us little information for discrimination, the profile of the DNA fingerprint consists of a complex set of large and highly variable DNA fragments and has extraordinary individualizing power. Therefore, it is possible to carry out individual identification using only this technique.

The minisatellite "Myo" probe, which was synthesized by the DNA synthesizer and the molecular cloning technique, contains the "Core" sequence reported by Jeffreys et al. [*9*] and cross-hybridizes to loci that are detected by Jeffrey's polycore probe. But the resulting Southern-blot profile is not identical. Testing of this probe on a limited number of people and performing the appropriate calculations on the results indicate that the probe also has extraordinary individualizing power. So the Southern-blot profile by the probe can also be termed a "DNA fingerprint" [*14*]. But it is so difficult to determine the number and frequency of bands in DNA fingerprints that we cannot calculate the affirmative probability of identity exactly. We have reported in our previous papers [*14,15*] that the probability in which an identical band longer than 2 kb in DNA fingerprints between two unrelated persons appears by chance is from 0.2 to 0.3. This value is similar to that shown by Jeffreys et al. [*10*]. In this case we recognized the complete correspondence of both of the DNA fingerprints, which both consist of 19 bands. The probability of chance association is calculated to $(0.2)^{19}$ or $(0.3)^{19}$, that is 5.2×10^{-14} or 1.2×10^{-10}. On the other hand, Jeffreys et al. [*21*] mentioned that the probability that DNA fingerprints of two unrelated individuals being identical is given as $(1 - 2X + 2X^2)^{n/x}$. The value X is a mean band-sharing probability and n is the number of bands in each DNA fingerprint. In this case, the probability can be calculated to 1.23×10^{-16} or 1.04×10^{-15}. Anyway, the chance match is virtually eliminated.

It is most important to adopt similar amounts of DNA from each sample for DNA fingerprinting. In the case of a blind trial we could not obtain complete identical patterns from the semen samples and from the suspected men's blood samples because the applied amounts of DNA from these semen samples were too little. Nevertheless, we could identify the pattern easily, since there is no incompatible band in the pattern of the semen.

If high molecular DNA is obtained, we can use the DNA fingerprint technique on criminal materials such as blood and semen. It can give more decisive information than that offered by conventional markers. We hope that this new technique will be generally used for criminal investigation in the near future.

Acknowledgment

We thank Dr. Y. Okada for preparation of this manuscript.

References

[1] Dahlke, M. B., Cooke, C., Cunnane, M., Chawla, J., and Lau, P., "Identification of Semen in 500 Patients Seen Because of Rape," *American Journal of Clinical Pathology*, Vol. 68, No. 6, 1977, pp. 740-748.

[2] Schuman, G. B., Badawy, S., Peglow, A., and Henry, J. B., "Prostatic Acid Phosphatase. Current Assessment in Vaginal Fluid of Alleged Rape Victims," *American Journal of Clinical Pathology*, Vol. 66, No. 12, 1976, pp. 944-952.

[3] Hara, M., "Forensic Immunological Studies on Human Body Fluids," *Japanese Journal of Legal Medicine*, Vol. 28, No. 3, May 1974, pp. 164-176.

[4] Botstein, D., White, R. L., Skolnich, M., and Davis, R. W., "Construction of a Genetic Linkage Map Using Restriction Fragment Length Polymorphisms," *American Journal of Human Genetics*, Vol. 32, No. 3, May 1980, pp. 314-331.

[5] Olaisen, B., Mevag, B., Jonassen, R., Paulsen, G., Thorsby, E., and Teisberg, P., "Identification of Blood Stains Through DNA Typing with C4 and HLA-DR Probes," *Zeitschrift für Rechtmedizin*, Vol. 99, No. 1, 1987, pp. 55-64.

[6] Baird, M., Balazs, I., Giusti, A., Miyazaki, L., Nicholas, L., et al., "Allele Frequency Distribution of Two High Polymorphic DNA Sequences in Three Ethnic Groups and Its Application to the Determination of Paternity," *American Journal of Human Genetics*, Vol. 39, No. 4, Oct. 1986, pp. 489-501.

[7] Kanter, E., Baird, M., Shaler, R., and Balazs, I., "Analysis of Restriction Fragment Length Polymorphisms in Deoxyribonucleic Acid (DNA) Recovered from Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 403-408.

[8] Guisti, A., Baird, M., Pasquale, S., Balazs, I., and Glassberg, J., "Application of Deoxyribonucleic Acid (DNA) Polymorphisms to the Analysis of DNA Recovered from Sperm," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 409-417.

[9] Jeffreys, A. J., Wilson, V., and Thein, S. L., "Hypervariable 'Minisatellite' Regions in Human DNA," *Nature*, Vol. 314, No. 6006, March 1985, pp. 67-73.

[10] Jeffreys, A. J., Wilson, V., and Thein, S. L., "Individual Specific 'Fingerprints' of Human DNA," *Nature*, Vol. 316, No. 6023, July 1985, pp. 76-79.

[11] Gill, P., Jeffreys, A. J., and Werrett, D. J., "Forensic Application of 'DNA Fingerprints'," *Nature*, Vol. 318, No. 6046, Dec. 1985, pp. 577-579.

[12] Gill, P. and Werrett, D. J., "Exclusion of a Man Charged with Murder by DNA Fingerprinting," *Forensic Science International*, Vol. 35, No. 2/3, Oct./Nov. 1987, pp. 145-148.

[13] Jeffreys, A. J., Brookfield, J. F. Y., and Semeonoff, R., "Positive Identification of an Immigration Test Case Using Human DNA Fingerprints," *Nature*, Vol. 317, No. 6040, Oct. 1985, pp. 818-819.

[14] Honma, M. and Ishiyama, I., "DNA Fingerprints: The Importance in Forensic Medicine (1). Application to Paternity Testing by Minisatellite DNA Probes," *Japanese Journal of Legal Medicine*, Vol. 41, No. 3, June 1987, pp. 236-241.

[15] Ishiyama, I., Yoshii, T., Honma, M., Mukaida, M., and Yamaguchi, T., "DNA Fingerprints: The Importance in Forensic Medicine (2). The Significance of Examining DNA Polymorphisms of Placental Tissues for the Purpose of Paternity Determination During the Early Stage within the First Trimester," *Zeitschrift für Rechtmedizin*, Vol. 99, No. 4, 1988, pp. 241-248.

[16] Southern, E. M., "Gel Electrophoresis of Restriction Fragments," *Methods in Enzymology*, Vol. 68, 1979, pp. 152-176.

[17] Rigby, P. W. J., Dickman, M., Rodes, C., and Berg, P., "Labeling Deoxyribonucleic Acid to High Specific Activity In Vitro by Nick Translation with DNA Polymerase I," *Journal of Molecular Biology*, Vol. 113, No. 1, June 1977, pp. 237-251.

[18] Weller, P., Jeffreys, A. J., Wilson, V., and Blanchetot, A., "Organization of the Human Myoglobin Gene," *EMBO Journal*, Vol. 3, No. 2, 1984, pp. 439-446.

[19] Yoshioka, N. and Takahashi, K., "ABO Blood Grouping on Seminal Fluids Contaminated with Vaginal Secretions," *Acta Criminologiae et Medicinae Legalis Japonica*, Vol. 52, No. 6, 1986, pp. 236-242.

[20] Gill, P., Lygo, J. E., Fowler, S. J., and Werrett, D. J., "An Evaluation of DNA Fingerprinting for Forensic Purpose," *Electrophoresis*, Vol. 8, No. 1, 1987, pp. 38-44.

[21] Jeffreys, A. J. and Morton, D. B., "DNA Fingerprints of Dogs and Cats," *Animal Genetics*, Vol. 18, No. 1, 1987, pp. 1-15.

Address requests for reprints or additional information to
Masamitsu Honma
Department of Forensic Medicine
University of Tokyo
Hongo, Bunkyo-ku
Tokyo, 113 Japan