CASE REPORT

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Identification of Decomposed Human Remains by Deoxyribonucleic Acid (DNA) Profiling

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ABSTRACT: After routine methods failed to establish positive identification of a decomposed homicide victim, deoxyribonucleic acid (DNA) typing techniques using blood from the victim and putative parents of the victim were used. This is the first report in the literature of a case using DNA fingerprinting in a "parentage" context to establish identity of unidentified, decomposed human remains.

KEYWORDS: pathology and biology, human identification, deoxyribonucleic acid (DNA), paternity, DNA profiling, DNA typing, DNA fingerprinting, DNA analysis, maternity

This report covers efforts to establish the identity of a female homicide victim recovered from a suburban Seattle Washington park in February 1988. The body was in a moderate to advanced stage of decomposition and had been scavenged by animals. The postmortem interval based on the appearance of the body was estimated to be several days to two weeks. The ambient temperature was 9°C; ground temperature and core body temperature were equivalent at 6°C. The body had been scavenged by rats. Postmortem examination revealed four penetrating gunshot wounds: three to the head and one to the neck. Her death was attributed to these gunshot wounds. The manner was ruled homicide.

Routine methods of identification such as fingerprint analysis and X-ray comparisons to known missing persons failed to establish identity. In response to a released "reconstruction sketch" of the victim in the media, a tentative identification was made by family members. Visual confirmation of her identity was not possible due to the extent of decomposition. Efforts to obtain latent fingerprints from objects the deceased was known

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to have handled during life failed. Neither antemortem dental nor other body X-rays existed to establish positive identification.

A decision was made to explore the feasibility of using genetic markers to confirm her identity. Attempts to locate a hairbrush with possible hair root cellular material met with failure. A blood sample from the putative mother and father as well as postmortem blood and muscle from the deceased were sent to Lifecodes Corporation in New York for deoxyribonucleic acid (DNA) analysis.

Methods and Materials

From the time of discovery the body was refrigerated at 3°C. A peripheral blood sample from the body was collected in sodium fluoride at the time of autopsy and refrigerated. Samples of psoas muscle and a third molar were obtained almost three months later. These were forwarded to Lifecodes Corporation in New York for analysis.

After DNA was successfully isolated from the decedent's tissue samples, blood from the putative parents was collected in ethylenediaminetetraacetic acid (EDTA) and sent for analysis. At Lifecodes, DNA was isolated from the muscle and blood of the decedent and digested with restriction endonuclease *Pst I*. A DNA-PRINT pattern was developed using four independent genetic loci: D2S44, D17S79, DXYS14, and D14S1 and the Y-chromosome locus DYZ1.

Results

Sufficient high molecular weight DNA was isolated only from the three blood samples and the psoas muscle of the victim. The DNA-PRINT pattern generated for the loci DXYS14, D17S9, and D2S44 is shown in Fig. 1. The allele fragment sizes detected at each locus as reported by Lifecodes Corporation are shown in Table 1. Family studies have shown that the alleles segregate according to Mendelian laws of inheritance [*1*]. Also, the allele sizes and the clustering of alleles vary significantly between ethnic groups [*2*]. Based on these previous observations, the maternity and paternity index for each locus can be determined and are shown in Table 2.[4] Each locus will exclude a certain percentage of the population as the biological parent. In the instance of this alleged mother, the probability of maternity using the combination of the four genetic loci is greater than 99.9% as compared to an untested woman in the North American population, assuming a prior probability of 0.5. The putative father is excluded as the biological father of the victim because he lacks all fragments present in the child's pattern which are absent in the mother's pattern.

Discussion

This report illustrates the application of DNA typing to identification of decomposed human remains. Recent legislation in California, Colorado, and Washington States has brought to the fore many practical questions regarding the use of DNA typing. Availability of DNA technology to the forensic science community is limited at this time to a few commercial companies and is not part of the conventional crime laboratory repertoire. These companies each use various probes, and no standards of comparison have been established from one lab to another. Admissibility of DNA typing in courts is still subject to pretrial hearings on a case-by-case basis. These issues plus the cost of the technique

[4]I. Balazs, M. Baird, M. Clyne, and E. Meade, "Human Population Genetic Studies of Five Hypervariable DNA Loci," Lifecodes Corporation, Valhalla, NY, personal communication, 1988.

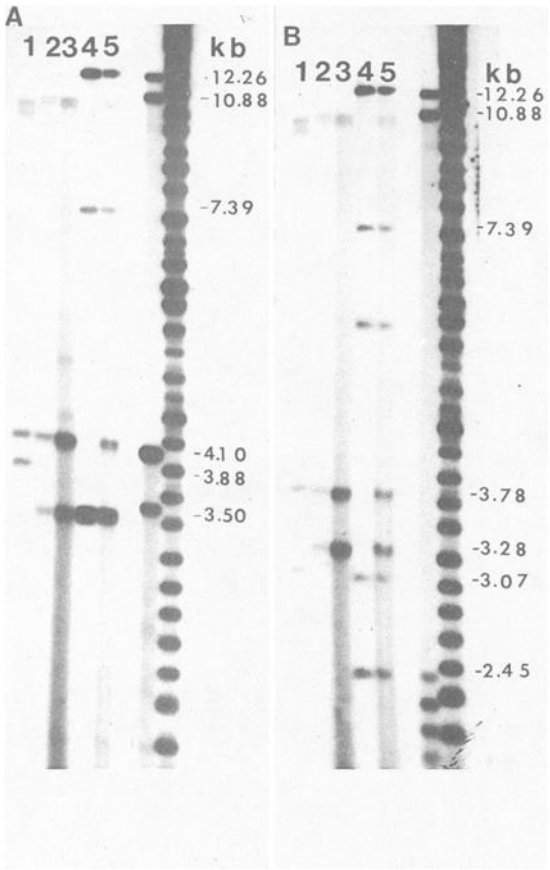

FIG. 1—*DNA profiles for Probes D2S44* (a and b top), *D17S79* (a, bottom), *and DXYS14* (b, bottom). *Lane 1 = alleged mother, Lane 2 = unknown blood, Lane 3 = unknown psoas muscle, Lane 4 = alleged father, and Lane 5 = mixture of unknown psoas muscle and alleged father.*

TABLE 1—*DNA fragment sizes from blood standards. (Allele fragment size measurements, expressed in kilobase pairs have a standard deviation of 0.6%. Fragments with measurements that are within appropriately 2% of each other [3 standard deviations or 99.7% confidence level] are considered indistinguishable and their average size reported.)*

	Loci			
Sample	D14S1	D2S44	D17S79	DXYS14
Blood from alleged mother	3.92, 3.82	10.62, 10.20	4.10, 3.88	3.78, 3.14
Blood from decedent	3.92, 3.82	10.88, 10.62	4.10, 3.50	3.78, 3.28
Blood from alleged father	5.04, 4.53	12.26, 7.39	3.50, 3.50	3.07, 2.45, 1.92

TABLE 2—*Indices for alleged parents. Based on the results of the DNA-PRINT test using four independent genetic systems, the combined maternity index is 1820 for black and 4829 for Caucasian populations (calculated by multiplying the individual maternity indices, M). The probability of maternity is greater than 99.9% as compared to an untested woman in the North American population, assuming a prior probability of 0.% (calculated as* M/+M).

Ethnic Group	D14S1	D2S44	D17S79	DXYS14	Combined Maternity/ Paternity Index
			MATERNAL		
Black	5.62	4.59	3.78	18.67	1820
Caucasian	3.96	3.89	7.60	41.25	4829
			PATERNAL		
Black	0.00	0.00	7.56	0.00	...
Caucasian	0.00	0.00	15.20	0.00	...

(approximately $1200 for the case described here) pose problems for the forensic science community at large.

Although still in early stages of application, DNA typing has been heralded as a major breakthrough for forensic biology [*3,4*]. Forensic science applications are twofold: identification [*5*] and disputed parentage [*6–8*]. Some suggest identifications could be aided by compiling antemortem databanks of DNA prints for military personnel and sex offenders [*9,10*]. Identical matches could then be sought with unidentified persons and suspects. Forensic science applications of DNA typing usually involve comparisons of markers present or absent in forensic science evidence to markers present or absent in suspects or victims. Typical cases are those that involve disputed paternity [*6–8*] and sexual assaults [*11*].

DNA typing has shown promise when applied to bloodstains [*12,13*], semen [*11*], and hair roots [*3,14*]. Gill et al. [*3*] have isolated sperm DNA from vaginal cell debris. Although not reported in the forensic science literature, formaldehyde-fixed paraffin blocks from hospital biopsies might serve as a source of DNA [*4*]. Ribonucleic acid (RNA) harvested from such blocks has been reported, but the authors did not indicate the type of RNA isolated [*15,16*]. DNA polymerase chain reactions may be used to amplify DNA that is quantitatively insufficient for variable number tandem repeat/restriction fragment length polymorphism (VNTR-RFLP) analysis [*17*].

Identification can be facilitated through comparisons of DNA profiles of blood relatives. Kuo [*18*] applied similar logic to determine whether conventional genetic markers in blood of a missing person's parents demonstrated parental inclusion of a putative bloodstain of the missing individual. Judicially acceptable probabilities for maternity (paternity) can be obtained from conventional markers such as human leukocyte antigen (HLA) [*19*] and DNA [*20*].

Decomposition is often encountered in unidentified remains. Certain levels of DNA predigestion resulting from changes of decomposition may not appreciably affect mini-probe hybridizations. Successful restriction of partial digests, yielding high molecular weight DNA, has been reported [*3,11,21*]. Bacterial contamination will not affect hybridization when using human probes [*3,22*].

Perry et al. [*23*] analyzed human bone marrow over varying postmortem intervals and demonstrated that DNA degradation took place more slowly under low humidity conditions. Dryer conditions are much more conducive to DNA stability. Paabo [*24*] recovered small amounts of DNA from a 2400-year-old mummy. The amount was 5% of that expected from fresh muscle. Higuchio obtained 1% of the expected amount of DNA from 140-year-old, dried muscle of an extinct horse (*Equus quagga*) [*25*].

TABLE 3—*Detectability of DNA over time in various tissues.*

Tissues	Time Limit	Refs
Dried blood	3–4 years	*15*
Bone marrow	84 days	*23*
Dried sperm	3–4 years	*15*
Third molar pulp	weeks	*24*
Dried muscle	up to 2400 years	Footnote 4

Much current research on the use of DNA typing in forensic medicine has concentrated on the effects of the postmortem interval on DNA degradation in various tissues. To date, sperm and blood have been tissues of major interest [*3*], but other tissues have been examined including bone [*23*] and third molar pulp.[5] With third molar pulp, Orrego has obtained mitochondrial DNA from teeth soaked in water at room temperature for several weeks.[5] Gill [*3*] has shown that DNA can be recovered from dried blood and semen stains after periods in excess of three years. McNally et al. isolated high molecular weight DNA of sufficient quality from bloodstains on evidentiary material with an unknown environmental history.[6] Their study included stains of different sizes on a variety of surfaces, for example, plastic, denim, and carpet. The quality of the stains also varied and included stains with evidence of putrefaction.

Conclusion

Identification of deceased individuals is a two-part process. First, information must be established as to the possible identity. Second, the hypothesized identity must be confirmed. In the absence of fingerprints, antemortem dental charts or X-rays, or other body X-rays, alternate means of identification must be pursued. Application of genetic markers to the identification problem is an option. This case demonstrates that DNA comparison of a deceased with that of suspected relatives is a valid option for identification.

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References

[*1*] Baird, M., Wexler, K., Clyne, M., Meade, E., Ratzlaff, L., et al., "The Application of DNA-PRINT for the Estimation of Paternity," in *Advances in Forensic Haemogenetics*, Vol. 2, Springer-Verlag, New York, 1987.

[*2*] Baird, M., Balazs, I., Guisti, A., Miyazaki, L., Nicholas, L., et al., "Allele Frequency Distribution of Two Highly Polymorphic DNA Sequences in Three Ethnic Groups and Its Application to the Determination of Paternity," *American Journal of Human Genetics*, Vol. 39, 1986, pp. 489–501.

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

[5]C. Orrego, personal communication, School of Public Health, University of California, Berkeley, CA, 1988.

[6]L. McNally, R. C. Shaler, A. Guisti, M. Baird, I. Balazs, et al., "The Effects of Environment and Drying Surfaces on DNA: The Use of Casework Samples from New York City," Lifecodes Corporation, Valhalla, NY, and John Jay College of Criminal Justice, New York, personal communication, 1988.

[4] Goelz, S. E., Hamilton, S. R., and Vogelstein, B. V., "Purification of DNA from Formaldehyde Fixed and Paraffin Embedded Human Tissue," *Biochemical and Biophysical Research Communications*, Vol. 130, 1987, pp. 118–126.

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

[6] Melvin, J. R., Kateley, J. R., Oakes, M. K., Simson, L. R., and Maldonado, W. E., "Paternity Testing," in *Forensic Science Handbook, II*, R. Saferstein, Ed., Prentice-Hall, New York, 1988, pp. 273–346.

[7] Walker, R. H., Ed., *Inclusion Probability in Parentage Testing*, American Association of Blood Banks, Arlington, VA, 1983.

[8] Salmon, D. and Brocteur, J., "Probability Testing Exclusion When Relatives Are Involved," *American Journal of Human Genetics*, Vol. 30, 1978, pp. 677–690.

[9] Newmark, P., "DNA Fingerprints Go Commercial," *Nature*, Vol. 321, No. 6066, May 1986, p. 104.

[10] Hicks, J. W., "Letter to the Editor," *American Academy of Forensic Sciences, Academy News*, Vol. 18, No. 3, May 1988, p. 2.

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

[12] Tyler, M. G., Kirby, L. T., Wood, S., Vernan, S., and Ferris, J. A. J., "Human Blood Stain Identification and Sex Determination in Dried Blood Stains Using Recombinant DNA Techniques," *Forensic Science International*, Vol. 31, 1986, pp. 267–272.

[13] Southern, E., "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," *Journal of Molecular Biology*, Vol. 98, No. 3, Nov. 1975, pp. 503–517.

[14] Burgess, R. M., Sutton, J. G., and Whitehead, P. H., "An Improved Means of Enzyme Typing of Hair Roots Using Isoelectric Focusing," *Journal of Forensic Sciences*, Vol. 24, No. 2, April 1979, pp. 329–396.

[15] Dubeau, L., Myerson, D., Leary, J. J., Spalholz, S. Z., Travis, C. K. Y., et al., "Southern Blot Analysis of DNA Extracted from Formalin-Fixed Pathology Specimens," *Cancer Research*, Vol. 46, 1986, pp. 2964–2969.

[16] Rupp, G. M. and Locker, J., "Purification and Analysis of RNA from Paraffin-Embedded Tissues," *Biotechniques*, Vol. 6, No. 1, 1988, pp. 56–60.

[17] Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B., and Erlich, H. A., "Analysis of Enzymatically Amplified Betaglobin and HLA-DQ Alpha DNA with Allelespecific Oligonucleotide Probes," *Nature*, Vol. 324, pp. 163–166.

[18] Kuo, M. C., "Linking a Bloodstain to a Missing Person by Genetic Inheritance," *Journal of Forensic Sciences*, Vol. 27, No. 2, April 1982, pp. 438–444.

[19] Di Lonardo, A. M., Orrego, C., Darlu, P., King, M., and Baur, M., "Human Genetics and Human Rights," *The American Journal of Forensic Medicine and Pathology*," Vol. 5, No. 4, 1984, pp. 339–347.

[20] Jefferys, 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, 1985, pp. 818–819.

[21] Kanter, E., Baird, M., Shaler, R., and Balaz, I., "Analysis of Restriction Length Polymorphisms in DNA Recovered from Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 403–408.

[22] Dabbs, D., "The Use of DNA Profiling in Linking Serial Murders," *Medico-Legal Bulletin*, Vol. 37, No. 6, Nov.–Dec. 1988.

[23] Perry, W. L., Bass, W. M., Riggsby, W. S., and Sirotkin, K., "The Autodegredation of Deoxyribonucleic Acid (DNA) in Human Rib Bone and Its Relationship to the Time Interval Since Death," *Journal of Forensic Sciences*, Vol. 33, No. 1, Jan. 1988, pp. 144–153.

[24] Paabo, S., "Molecular Cloning of Ancient Egyptian Mummy DNA," *Nature*, Vol. 34, April 1984, pp. 644–645.

[25] Higuchi, R., Bowman, B., Freiberger, M., Ryder, O. A., and Wilson, A. C., "DNA Sequence from Quaggamn an Extinct Form of Horse," *Nature*, Vol. 312, 1984, pp. 282–284.

[26] Spitz, W. U. and Fisher, R. S., *Medicolegal Investigation of Death,* Charles C Thomas, Springfield, IL, 1980, p. 18.

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