CASE REPORT

David Sweet,[1] D.M.D., Ph.D.; Dean Hildebrand,[2] Ph.D.; and Don Phillips,[3] R.T.

Identification of a Skeleton Using DNA from Teeth and a PAP Smear

REFERENCE: Sweet D, Hildebrand D, Phillips D. Identification of a skeleton using DNA from teeth and a PAP smear. J Forensic Sci 1999;44(3):630–633.

ABSTRACT: Identification of unknown living or deceased persons using dental treatment records is an established forensic technique. However, some cases remain unidentified, especially when antemortem dental records are not available for comparison to postmortem dental records. Cytological smears have been previously reported to be potential sources of DNA reference samples which can be compared to DNA recovered from found human remains. The case described here involves an adult skeleton which exhibited extensive, complex dental restorative treatment. A putative identification of the found skeleton as a missing woman was established using circumstantial evidence found at the scene. However, it became important to establish a positive identification using reliable scientific methods. When it was discovered that antemortem dental records were not available because the treatment was completed in another country and the treating dentist could not be found, cytological smears stained with Papanicolaou (PAP) stain obtained from the putative decedent's medical records were used as a reference DNA sample. DNA was recovered from the teeth of the skeleton using cryogenic grinding. Comparison of the genotypes resulted in the conclusion that the DNA originated from the same source. The use of PAP smears in this way is seen as a valuable resource in cases where positive identification using traditional dental and medical records is not possible.

KEYWORDS: forensic science, human identification, DNA typing, PAP smear, cryogenic grinding, dental identification, teeth, AmpFLSTR Blue, Profiler Plus

In cases of found bodies in which dental treatment is present, comparison of antemortem and postmortem dental records is a common identification technique. Often, a putative identification is established using circumstantial evidence. This allows investigators to search missing persons databases for information and dental records for individuals who potentially match the characteristics of the found body. Unfortunately, dental records are not available in all missing persons cases. Therefore, some cases remain unidentified when limited antemortem data are available.

Previous investigators have reported the use of stained cytological smears as sources of forensic DNA evidence (1,2). Sensitive PCR-based DNA typing procedures provide an opportunity to compare the genotype of cells from stained smears to other biological evidence. It is possible to use smears treated with Papanicolaou (PAP) stain from a known individual as a reference DNA source (3).

In the case reported here, antemortem dental records were not available for comparison to dental restorations found at postmortem examination. Therefore, archival PAP-stained cytological smears were compared to biological evidence recovered from the skeletal remains. Using these data, a positive identification of the decedent was established.

Case Circumstances

Skeletal remains of an adult female were discovered by two people walking their dog in a wooded park. Certain personal effects, presumed to be the decedent's, in addition to a back pack and clothing were also discovered by police. Two empty aspirin bottles and another bottle determined to be from prescription medication were found at the scene. Investigators compared the available postmortem anthropological, odontological, and circumstantial evidence to a computer database of missing persons. Due to the presence of extensive dental restorations found in the upper and lower teeth (see Fig. 1), it was presumed that any potential matches between these data and that of reported missing persons could be confirmed with dental records.

Putative identification of the decedent as a 40-year-old woman named "HK" was established using circumstantial evidence. HK had been reported missing 39-months earlier. She had recently moved to the area from Japan. This is apparently where her dental treatment had been completed. She was described by family and mental health professionals as suffering from depression approximately six months prior to her disappearance. She had expressed a suicidal tendency to her mother.

Unfortunately, it was learned that no antemortem dental records from the treating dentist were available for comparison. A search for antemortem medical records was undertaken in an attempt to locate radiographs which may be used for comparative identification purposes. Although medical records were not available either, it was learned that three cytological (PAP) smears were produced

[1] Director, Bureau of Legal Dentistry, 146-2355 East Mall, Vancouver, BC, Canada V6T 1Z4.

[2] Research Associate, Bureau of Legal Dentistry, 146-2355 East Mall, Vancouver, BC, Canada V6T 1Z4.

[3] Section Head, Analytical and Quantitative Cytology Laboratory, British Columbia Cancer Agency, 600 West 10 Avenue, Vancouver BC, Canada V5Z 4E6.

Received 2 July 1998; and in revised form 4 Sept. 1998; accepted 8 Sept. 1998.

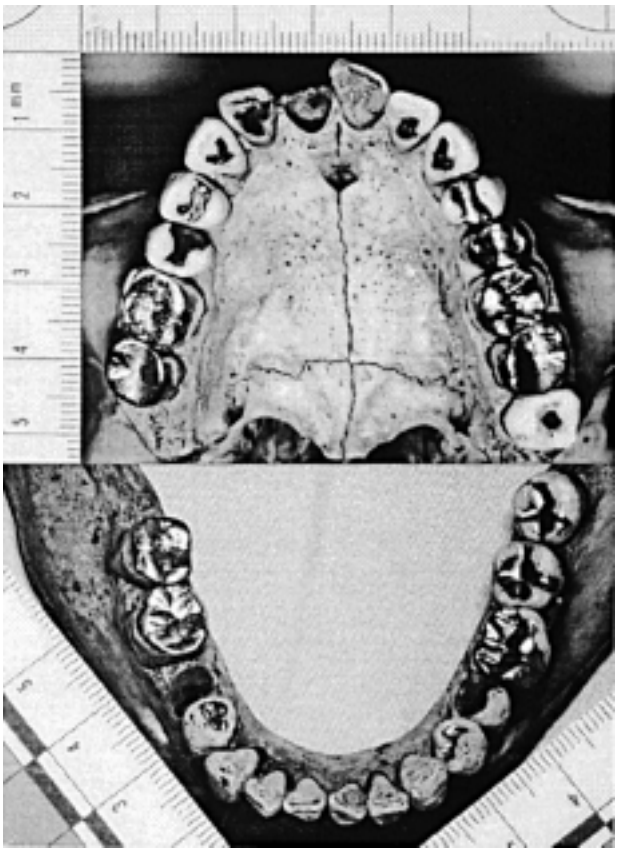

FIG. 1—*Extensive dental restorations were present in the upper and lower teeth; however, no dental records for HK (putative decedent) could be located.*

several years earlier. Inquiries about the availability of microscopic slides containing these smears revealed that they were archived in secure storage in a laboratory at a provincial cancer agency.

It was decided that these PAP smears, two of which were known to have originated from HK in 1993 and one in 1994, would be used as known reference DNA samples. Short tandem repeat (STR) genotypes produced from these reference samples were compared to questioned samples of DNA recovered from the teeth of the skeleton.

Material and Methods

Questioned Samples

Two posterior teeth (lower left second and third permanent molars) were removed from the mandible, decontaminated in bleach for 20 min, rinsed with sterile water, then soaked in sterile water for 20 min, rinsed with ethanol, and air dried at room temperature under a 256 nm ultraviolet light source (Phillips TUV 30 watt, Microzone Corp., Nepean, Ontario) for 20 min (rotated after 10 min) (4). A 6700 freezer mill (SPEX Sample Preparation, Metuchen, NJ) was used to cryogenically grind each tooth. The electromagnetic chamber was precooled for 10 min in liquid nitrogen. The teeth were ground for 5 min at an impactor rate of 150 strokes per min.

One mL of lysis buffer (10 mM Tris-HCl (pH 8.0), 10 mM EDTA, 50 mM NaCl, 2% (w/v) SDS) and 15 μL of proteinase K (10 mg/mL) were added to approximately 0.5 g of tooth powder in a sterile 1.5 mL eppendorf tube. This was incubated overnight at 56°C. The samples were extracted using buffer-saturated phenol-chloroform-isoamyl alcohol (25:24:1) (5) in a phase lock gel tube (5′-3′ Inc., Boulder, CO) and microconcentrated in AMICON-100 tubes (Millipore Canada, Toronto, ON) (6). The sample was resuspended in TE buffer (10 mM Tris (pH 8); 1 mM EDTA). An aliquot of 8 μL of each extract was amplified in a final volume of 25 μL using the AmpFLSTR-Blue triplex system (loci: D3S1358, vWA, FGA) optimized with Amelogenin (PE Applied Biosystems, Foster City, CA). One μL each of the Amelogenin forward and reverse primers (10 mM stock) were added to the standard 25 μL reactions. Subsequently, 10 μL of template DNA was amplified using the Profiler Plus STR system (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820 plus Amelogenin) (PE Applied Biosystems, Foster City, CA). Amplicons were visualized on an ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA).

Known Samples

The microscope slides containing the PAP smears were soaked in xylene for five days to remove the cover slips. The cellular material on the slide was embedded in Mount-Quick mounting media (No. 6270A, Newcomer Supplies, Middleton, WI) and polymerized using the heat from a standard desk lamp for 3 h. The slides were incubated for 30 min in dH_2O at 37°C to soften the mounting medium. The media containing the cellular material was then peeled from the slide and divided in two sections. One section was preserved for the medical record. The other section was incubated in 3 mL of lysis buffer (10 mM Tris-HCl pH 8.0), 10 mM EDTA, 50 mM NaCl 50, 2% (w/v) SDS) and 15 μL of proteinase K (10 mg/mL) overnight at 56°C. The liquid sample was extracted using a one-step organic method and microconcentrated as previously described. The extracts were quantified using slot-blot hybridization with the human-specific D17Z1 probe (7). Aliquots containing 800 pg of target DNA were amplified using the AmpFLSTR-Blue system optimized with Amelogenin followed by the Profiler Plus system.

Genotype Frequency

Statistical data and a computer application (STRquest II, Dr. G. Carmody, Ottawa, Canada) provided by the Royal Canadian Mounted Police were used to calculate the frequency of the genotype in the general Canadian population.

Results

The samples extracted from the PAP smears contained approximately 7.0 ng of human DNA (3.5 ng in one section equal to approximately one-half the total cellular material present). STR profiles produced using the AmpFLSTR-Blue plus Amelogenin and Profiler Plus systems were the same for the DNA recovered from the PAP smears and for the DNA recovered from the teeth. The electropherograms from the AmpFLSTR-Blue plus Amelogenin system are shown in Fig. 2. The allele calls for all of the loci tested are shown in Table 1. Not all loci amplified in the Profiler Plus reactions. Alleles from D7S820 and D18S51 did not appear in the profiles. Calculation of a conservative estimate of the frequency of the resulting genotype at 7 loci in the Canadian population results in a value of 4.52×10^{-9}.

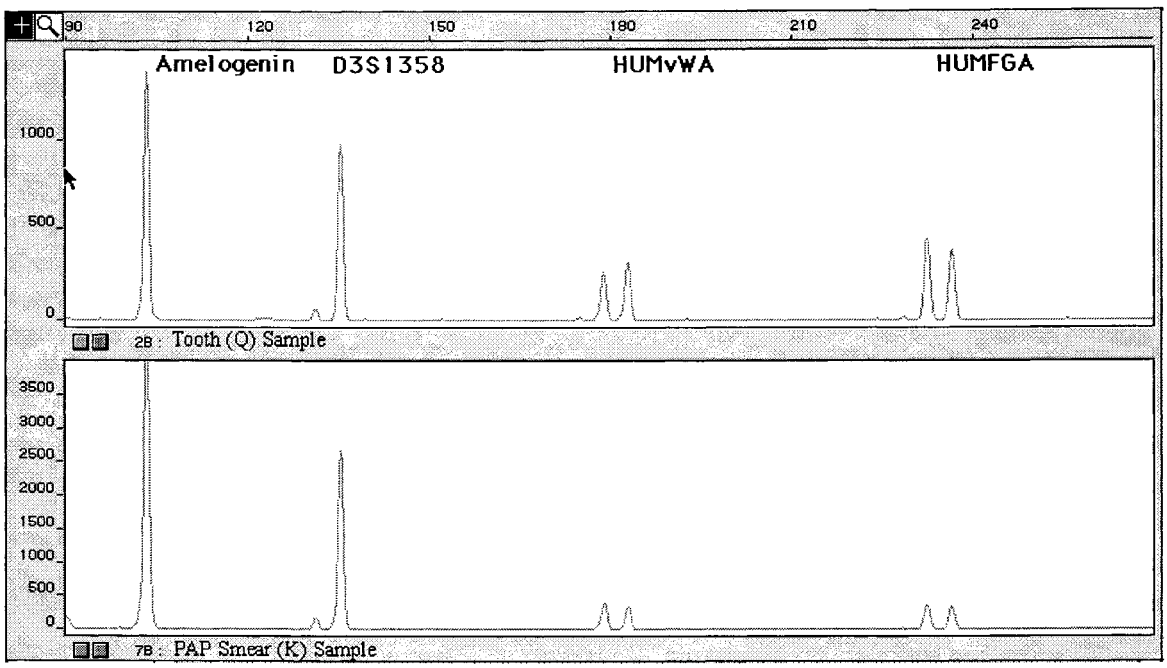

FIG. 2—*Electropherograms showing comparison of dental DNA (questioned sample) to DNA from PAP smear (known sample) at 3 STR loci plus the Amelogenin gender locus.*

TABLE 1—*Allele calls for nine STR loci plus Amelogenin gender locus comparing DNA from the PAP smear to DNA from teeth, including fragment length in base pairs.*

Locus	DNA from PAP Smear	DNA from Teeth	Fragment Size (bp)
Amelogenin	X, X	X, X	103.39, 103.39
D8S1179	10, 16	10, 16	131.49, 158.12
D3S1358	18, 18	18, 18	135.40, 135.40
D5S818	11, 12	11, 12	148.19, 152.68
vWA	17, 18	17, 18	179.00, 182.96
D21S11	29, 32.3	29, 32.3	204.75, 218.64
D13S317	10, 12	10, 12	213.47, 221.53
FGA	22, 23	22, 23	232.56, 236.74
D7S820	*	*	*
D18S51	*	*	*

* Allelic drop-out was seen in both samples at STR loci D18S51 and D7S820 (longest fragment lengths) using the Profiler Plus system.

Discussion

Unfortunately, the identity of many deceased individuals remains unknown when antemortem dental or medical records are not available. This eliminates the opportunity to compare the unique dental traits and characteristics which can establish positive identification.

The teeth survive most postmortem events and changes, and are valuable potential sources of forensic DNA evidence (4,8). Cases in which it is not possible to compare antemortem and postmortem dental records may be solved by comparing DNA extracted from the teeth found in the remains to a DNA reference sample. Recovery of dental DNA from recently extracted human teeth by cryogenic grinding has been shown by the authors to be successful (4). This paper reports the successful application of this technique to a forensic case.

In this case, there was circumstantial evidence found at the scene which provided information about the possible identity of the victim. It was decided by the coroner in charge of the investigation that the genotype frequency calculated from the triplex (5.79×10^{-3}) was sufficient to establish positive identification when taken into account with this other evidence.

It is often very difficult to locate a DNA reference sample that is known to come from the putative decedent. This case illustrates the need for investigators to consider, for at least one-half of the population, the use of cytological stained smears as potential reference samples. DNA was extracted from smears that were approximately 5-years-old. Information made available to the authors indicate that these smears are usually archived for decades and can potentially be found in various laboratories and medical agencies. Similarly, the case illustrates the suitability of the cryogenic grinding method to extract DNA from teeth exposed to ambient conditions on the ground for a period of approximately 3.5 years.

Acknowledgments

The assistance of Coroners T. E. Newell and D. Snider of the British Columbia Coroners Service, Burnaby, BC, Dr. J. LeRiche and E. Hilland of the Analytical Cytology Laboratory, British Columbia Cancer Agency, Vancouver, BC is gratefully acknowledged.

References

1. Gall K, Pavicic D, Pavelic J, Audy-Jurkovic S, Pavelic K. PCR amplification of DNA from stained cytological smears. J Clin Pathol 1993; 46:378–9.

2. Roy R, Reynold R. Amplitype PM and HLA DQa typing from pap smear, semen smear, and postcoital slides. J Forensic Sci 1995;40(2):266–9.
3. Dimo-Simonin N, Grange F, Brandt-Casadevall C. PCR-based forensic testing of DNA from stained cytological smears. J Forensic Sci 1997;42(3):506–9.
4. Sweet DJ, Hildebrand DP. Recovery of DNA from human teeth by cryogenic grinding. J Forensic Sci 1998;43(5):1199–202.
5. Walsh DJ, Corey AC, Cotton RW, Forman L, Herrin GL, Word CJ, Garner DD. Isolation of deoxyribonucleic acid (DNA) from saliva and forensic science samples. J Forensic Sci 1992;37(2):387–95.
6. Hochmeister MN, Budowle B, Borer UB, Eggman U, Comey CT, Dirnhofer R. PCR-based typing of DNA extracted from compact bone from human remains. J Forensic Sci 1991;(36)6:1649–61.
7. Waye JS, Presley LA, Budowle B, Shutler GG, Fourney RM. A simple and sensitive method for quantifying human genomic DNA in forensic science specimen extracts. BioTechniques 1989;7(8):852–5.
8. Sweet DJ, Sweet CHW. DNA analysis of dental pulp to link incinerated remains of homicide victim to crime scene. J Forensic Sci 1995;40(2): 310–4.

Additional information and reprint requests:
Dr. David Sweet, Director
Bureau of Legal Dentistry, 146-2355 East Mall
Vancouver, BC Canada V6T 1Z4
Telephone: (604) 822-8822, fax: (604) 822-8884,
e-mail: boldlab@interchange.ubc.ca