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Typing of Deoxyribonucleic Acid (DNA) Extracted from Compact Bone from Human Remains

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ABSTRACT: The application of deoxyribonucleic acid (DNA) typing methods for the potential identification of unknown human remains was investigated. DNA was isolated from compact bone tissue from badly decomposed bodies and from known and unknown human remains, using a decalcification and ion wash procedure. Restriction fragment length polymorphism (RFLP) analysis of variable number of tandem repeats (VNTR) loci yielded results in some cases, but more often the DNA was too degraded to produce RFLP patterns. No RFLP profiles could be obtained from putrified soft tissues.

However, DNA extracted from compact bone tissue of human remains up to eleven years old was successfully amplified using the polymerase chain reaction (PCR) for the VNTR loci D1S80, D17S5, COL2A1, and APO B, as well as the HLA-DQ alpha locus. This is especially significant, since PCR results were obtained from those samples whose DNA had been degraded substantially and had yielded no RFLP patterns. All DNA types determined from the compact bone tissue from decomposed bodies whose identification had been established first by other means (and whose parents or offspring were available for typing) demonstrated mendelian inheritance of the alleles of the loci analyzed. These results suggest that amplification and typing of DNA extracted from compact bone of human remains could be useful in establishing the identity of a person, as well as in excluding possible false identifications.

KEYWORDS: forensic science, genetic typing, human identification, deoxyribonucleic acid (DNA), compact bone tissue, polymerase chain reaction (PCR), paternity testing

The examination of human remains can be very informative in establishing identity. However, if a body is decomposed to such a degree that the facial structures are destroyed, no fingerprints exist, dental records are uninformative, X-ray comparisons are useless, or, in some cases of children, unique features are not available, it is probable that positive identification will not be made.

The detection of deoxyribonucleic acid (DNA) polymorphisms by restriction fragment length polymorphism (RFLP) analysis has had a major impact on identity testing. Yet, DNA may degrade rapidly in cadaver blood and soft tissues even in the early postmortem

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period. Bär et al. [*1*] observed good DNA stability in brain cortex, lymph nodes, and psoas muscle over a period of three weeks postmortem. In some cases, however, they noted that DNA degradation in soft tissues was prominent even after a short period of time, which might be a consequence of rapid bacterial growth in decomposing bodies, especially at high temperatures.

A more stable tissue and at times the only physical evidence of human remains is bone. Lee et al. reported successful typing of ABH antigens in bone tissue and were among the first to suggest the use of RFLP typing of DNA derived from bone [*2*]. The autodegradation of DNA in human rib bone samples, incubated under sterile conditions, in relation to the time interval since death was investigated by Perry et al. [*3*]. They concluded that high humidity conditions and temperatures are factors that affect the degradation rate of DNA and suggested that field studies of decaying bodies be conducted. Although DNA may be more stable in bones than in other tissues, it still can be degraded to such a degree that it is no longer suitable for RFLP analysis, particularly when typing the variable number of tandem repeats (VNTR) loci routinely used for forensic science analysis.

The polymerase chain reaction (PCR) may offer a viable alternative for the analysis of DNA and, especially, degraded DNA [*4*]. With PCR, virtually any defined short DNA sequence can potentially be analyzed easily and rapidly. Several VNTR loci (for example, D1S80, D17S5, Apo B, and COL2A1) [*5–9*], as well as the HLA-DQ alpha locus [*10*] are of a size amenable to amplification by PCR (generally 100 to 1000 base pairs are used for the VNTR loci and 239/242 base pairs for HLA-DQ alpha locus). Therefore, it was necessary to study the feasibility of typing these genetic markers from DNA extracted from compact bone.

This paper describes a method for the successful extraction of DNA from compact bone tissue, which can then be typed potentially by RFLP analysis or PCR-based procedures or both. This method was applied to analysis of DNA from compact bone tissue taken from putrified samples and decomposed bodies.

Materials and Methods

Biological Samples

Femur bone samples (20 cm in length), muscle tissue, and blood samples were obtained from one female and four male cadavers within 12 h after death. The samples were maintained under one of the following conditions: (*a*) frozen at −70°C (control samples), (*b*) exposed to ambient outdoor conditions during the summer months (rain, sunlight, an average temperature of 25°C), (*c*) wrapped in plastic and stored under the same conditions as the exposed tissue, (*d*) immersed in water taken from the Rhine River, in Switzerland, and maintained at a temperature of 25°C, and (*e*) buried in soil at a depth of 50 cm.

Femur bone samples (20 cm in length) and soft tissues were obtained at autopsy from ten severely decomposed bodies, recovered 1 to 16 weeks after death. Only cadavers showing advanced putrification, insect infestation, or partial skeletonization were used as tissue sources.

Whole blood samples were drawn into ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes by venipuncture from parents or offspring of five of the decomposed bodies. The identities of these bodies had been established previously using dental records or personal belongings.

Compact bone was obtained from a body that had been immersed in the Rhine River for approximately 18 months and from a mummified leg recovered in 1979 and stored at room temperature until analysis (11 years).

DNA Extraction

DNA Extraction from Compact Bone—After all traces of soft tissue and bone marrow had been removed using razor blades and sandpaper, the bone was crushed into small fragments and stored in sterile polypropylene tubes at −70°C until analyzed. Fifteen grams of compact bone were ground to a fine powder in a metal blender filled halfway with liquid nitrogen. The powder was transferred into three sterile 50-mL polypropylene tubes (5 g of powder in each tube) and decalcified in 40 mL of 0.5*M* EDTA, at pH 7.5. The tubes were agitated on a rotator at 4°C for 24 h, and then centrifuged at 2000 *g* for 15 min. Table 1 provides a flow chart of the protocol for the extraction procedure.

The supernatant was discarded, an additional 40 mL of decalcification solution was added to each tube, and the process was repeated. Generally, decalcification took three to five days. The decalcification process was monitored by addition of a saturated solution of ammonium oxalate, at pH 3.0, to the decanted supernatant. If the solution remained clear after the addition of ammonium oxalate, the decalcification process was stopped. To remove accumulated ions after decalcification, the pellet was washed in 40 mL of sterile deionized water, and the tubes were agitated for several minutes. Then, the samples were centrifuged at 2000 *g* for 15 min and the supernatant was discarded. This washing process was repeated three times.

Two mL of prewarmed (56°C) extraction buffer containing 0.01*M* tris(hydroxymethyl)aminomethane (Tris), 0.01*M* disodium (Na_2) EDTA·$2H_2O$, 0.1*M* sodium chloride

TABLE 1—*Flow chart of the protocol for extraction of DNA from compact bone tissue.*

Bone specimen
⇩
Complete removal of bone marrow and soft tissues
Crushing and powdering of 3 portions each of 5 g of bone
Decalcification in 0.5*M* EDTA, at pH 7.5, for 3 to 5 days
⇩
Washing 3 times in deionized sterile water
⇩
Addition of 2 mL of extraction buffer and proteinase K
⇩
Phenol/chloroform/isoamyl alcohol extraction 3 times
n-Butanol extraction
⇩
Concentration and purification in Centricon device
⇩
Slot blot quantification of human DNA ⇩ yield gel
⇩ ⇩
PCR amplification and typing RFLP analysis

(NaCl), 0.039*M* dithiothreitol (DTT), and 100 μL of proteinase K (20 mg/mL) were added to each pellet, and the tubes were incubated at 56°C for 2 h with intermediate shaking. The extraction was allowed to continue for an additional 10 h without agitation. Subsequently, 100 μL of proteinase K (20 mg/mL) was added, and the incubation at 56°C with intermediate shaking continued for an additional 3 h. The solutions were then extracted three times with equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1). (The aqueous phases from the three tubes were combined into a single tube after the first phenol extraction). Then, one extraction with water-saturated *n*-butanol was carried out, and the aqueous phase was concentrated using a Centricon™ 30 microconcentrator tube.[3] Afterwards, the retentate was washed three times with 2 mL of TE buffer (consisting of 0.01*M* Tris and 0.001*M* $Na_2EDTA \cdot 2H_2O$), at pH 7.5. The recovered DNA was stored at 4°C.

DNA Extraction from Blood and Soft Tissues—Bloodstains were made on cotton cloth from whole blood and the DNA was extracted as described previously [*10*].

Putrified soft tissues (400 mg each) were sliced into small pieces using sterile razor blades, and the DNA was extracted in a manner similar to that used for bloodstains, except that three phenol/chloroform/isoamyl alcohol (25:24:1) extractions, followed by one extraction with water-saturated *n*-butanol, were used. The aqueous phase was concentrated using a Centricon 30 microconcentrator, and subsequently, the retentate was washed three times with 2 mL of TE buffer. The recovered DNA was stored at 4°C.

Quantification of Human DNA

The quantity of human DNA in the samples was determined using the method of Waye et al. [*11*]. Briefly, 1 μL of the purified DNA extract was immobilized on a nylon membrane (Zeta Probe®, Bio-Rad Laboratories, Richmond, California) using a slot blot vacuum apparatus (Bio-Rad Laboratories, Richmond, California). Subsequently, the membrane was subjected to hybridization to p17H8 (D17Z1; Oncor, Gaithersburg, Maryland). If the slot blot results indicated that the amount of hybridizable human DNA was sufficient for quantitation with ethidium bromide, 5 to 10-μL aliquots of the samples also were subjected to yield gel analysis (agarose gel/ethidium bromide analysis).

Analytical/Typing Methods

RFLP Typing—RFLP analysis was performed as described previously [*10*]. The loci analyzed were D2S44, D10S28, D17S79, and D4S139.

Amplification and Typing of VNTR Loci (Amplified Fragment Length Polymorphisms)—Amplification of D1S80, D17S5, apolipoprotein B, and COL2A1 was achieved using the methods described by Budowle et al. [*5*] and Kasai et al. [*6*], Horn et al. [*7*], Boerwinkle et al. [*8*], and Wu et al. [*9*], respectively. Amounts of 20 to 100 ng of DNA were amplified. The control samples contained 20 to 100 ng of human DNA or no DNA. Amplification was performed in a Perkin-Elmer/Cetus Instruments thermal cycler. Inhibition of PCR, which was observed only for the DNA derived from the 11-year-old bone was overcome by the addition of bovine serum albumin (160 μg/mL) to the PCR [*12*].

Electrophoretic analysis of the PCR products was performed using a slightly modified procedure described by Budowle et al. [*5*]. Ten microlitres of loading buffer (0.07*M* Tris-sulfate, 40% glycerol, 0.01% bromophenol blue) were added to 20 μL of each amplifi-

[3]The names of commercial manufacturers are provided for identification only and their inclusion does not imply endorsement by the Federal Bureau of Investigation.

cation product. The samples were then applied to the cathodal end of a vertical polyacrylamide gel (5%, T, 3% C, 0.75 mm thick). The gels were cross-linked with piperazine diacrylamide (Bio-Rad Laboratories, Richmond, California). All the gels contained 7.1% glycerol and 0.035*M* Tris sulfate buffer, at pH 9.0. The gel separation distance was 20 to 25 cm. The electrode buffer was 0.14*M* Tris borate, at pH 9.0. The electrophoretic running conditions and subsequent silver staining of the separated DNA fragments was conducted according to previously described protocols [*5*].

Amplification and Typing of HLA-DQ Alpha—Amplification by PCR of 20 to 100 ng of human DNA was performed using the AmpliType™ HLA DQ alpha forensic DNA amplification and typing kit (Cetus Corp., Emeryville, California), according to the recommended protocol. Positive and negative control reactions were used. Typing of the HLA-DQ alpha locus was performed using a reverse dot blot format and allele-specific oligonucleotide probes [*13*], following the recommended protocol.

Results

Storage Study

High-molecular-weight DNA was extracted from all five control bone samples. The quantity of DNA determined by slot blot analysis that was recovered from the control samples ranged from 3.3 to 16.0 μg of human DNA per gram of powdered bone (Table 2). DNA also could be extracted from those bone samples exposed to various storage conditions. However, putrefaction appears to have an effect on the quality and quantity of DNA recovered from bone. Generally, all samples from the storage study, in which sufficient DNA was recovered for yield gel analysis, demonstrated significant loss and degradation of DNA. Generally, the largest amounts of DNA could be obtained from bones exposed to outdoor environments, followed by remains wrapped in a plastic

TABLE 2—*Yield of DNA from compact bone and the ability to type extracted DNA by RFLP and PCR-based methods.*[a]

	No.[b]	Human DNA/ g Bone[c]	Average DNA/ g Bone[c]	RFLP	PCR
Storage Study					
Control bone samples (−70°C)	5	3.3–16.0 μg	12.6 μg	+	+
Outdoor conditions (3 months)	5	70 ng–1.7 μg[3]	0.9 μg	+/−[d]	+
Outdoor, wrapped in plastic (3 months)	5	70 ng–280 ng	0.2 μg	−	+
Immersed in water (3 months)	5	30 ng–400 ng	0.1 μg	−	+
Buried in soil (3 months)	5	25 ng–100 ng	0.05 μg	−	+
Decomposed Bodies/Human Remains					
Insect infestation/partial skeletonization	10	25 ng–3.3 μg	1.3 μg	+/−[e]	+
Body immersed in water (18 months)	1	50 ng	50 ng	−	+
Mummified leg (11 years)	1	500 ng	500 ng	−	+

[a] + = typeable; − = not typeable.

[b] Each analysis was carried out in duplicate to demonstrate reproducibility (for example, the five samples of each portion of the storage study represent a total of ten analyses).

[c] Determined by slot blot analysis.

[d] High-molecular-weight DNA and the ability to obtain RFLP profiles were observed only in one case.

[e] DNA from bone from three of ten decomposed bodies was typeable by RFLP analysis.

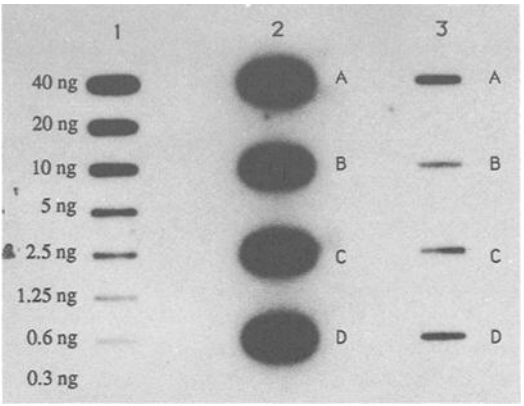

FIG. 1—*Effects of putrefaction processes on the DNA recovery from compact bone. Slot blot quantification of DNA using p17H8 (D17Z1). One-microlitre DNA samples, representing 0.25 to 0.5% of the extract were used for quantification. DNA extracted from the same samples and same amounts of compact femur bone from four different bodies (A, B, C, and D) is shown. Compared with the control bone samples (Lane 2), the overall amount of DNA from bone from body parts which had been buried in soil for three months (Lane 3) decreased significantly. Lane 1 shows the serially diluted human genomic DNA standard (K562 cell line, Promega).*

bag, then by bones immersed in water, and, lastly, by bones buried in soil (Fig. 1 and Table 2). The yield of DNA from these samples was less than that from the control bone samples, ranging from 25 ng to 1.7 μg of human DNA per gram of powdered bone (Table 2).

Decomposed Bodies/Human Remains

The quality of DNA recovered from the compact bone of severely decomposed remains of ten individuals was not correlated with the time of death. Three samples had some high-molecular-weight DNA and seven contained little or no high-molecular-weight DNA. The different conditions the bodies were exposed to were most likely related to the quality of the DNA. Still, the yield of DNA typically ranged from 25 ng to 3.3 μg of human DNA per gram of powdered bone (Table 2). There was no high-molecular-weight DNA recovered from the 18-month-old bone or bone from the 11-year-old mummified leg; however, as determined by slot blot analysis, 50 ng of human DNA per gram of bone and 500 ng of human DNA per gram of bone were recovered, respectively, from these sources. Yield gel analysis demonstrated that the size of the DNA recovered from the mummified leg was less than 1000 base pairs in length.

RFLP Typing

None of the soft tissue samples (muscle tissue) from the storage study (3 to 6 months old) or from severely decomposed bodies (12 to 16 weeks old) provided high-molecular-weight DNA. In fact, the DNA was so degraded and of so little quantity, that it was insufficient for RFLP or PCR typing.

RFLP typing was successful on some of the DNA samples from compact bone. Only DNA from the control bone samples, from one 3-month-old sample from the storage study, and from three samples of the decomposed bodies had sufficient high-molecular-

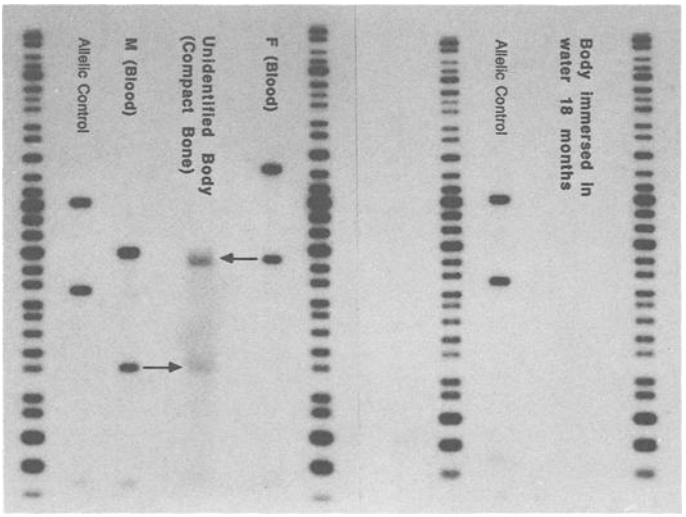

FIG. 2—*RFLP patterns obtained from compact bone from a decomposed body (Body A,* left). *In this case all DNA extracted from 15 g of bone had to be used to generate a fairly weak profile (Lane 4). Mendelian inheritance of alleles from the mother (M, Lane 3) and father (F, Lane 5) is observed. DNA from bone from a body immersed in water for 18 months yielded no RFLP profile (Body B,* right). *Ladders: 23.0 kb ladder (Lifecodes); allelic control: K562 cell line (Lifecodes); DNA probe: D2S44 (Promega). The RFLP procedure was run according to the method described by Budowle and Baechtel* [10].

weight DNA for RFLP analysis for four VNTR loci—D2S44, D17S79, D10S28, and D4S139 (Table 2). However, the entire DNA extract from 15 g of femur bone from each of the three decomposed bodies had to be used to obtain a fairly weak signal (Fig. 2). When using DNA probes for D2S44, D17S79, D10S28, and D4S139 for the three cases in which biological relatives were available, the alleles demonstrated mendelian inheritance (Fig. 2). Therefore, the three family studies suggest that the RFLP results from DNA from bone are reliable.

Amplification and Typing of VNTR Loci and HLA-DQ Alpha

Since, in most cases, the DNA was not suitable for RFLP analysis, typing of the DNA was attempted using PCR-based methods. The soft tissues from three decomposed bodies (12 to 16 weeks old) again proved useless as a source of DNA for typing, even with PCR-based methods (Fig. 3). The DNA was too degraded for analysis. Therefore, the remainder of this study focused on the feasibility of typing DNA extracted from compact bone by PCR-based methods.

All the DNA bone samples were subjected to PCR and typed for D1S80, D17S5, Apo B, COL2A1, and HLA-DQ alpha. Each sample was extracted and analyzed in duplicate to demonstrate reproducibility. PCR was successful initially on all samples, except for the 11-year-old bone. After the addition of bovine serum albumin, which may bind a soluble inhibitory factor for PCR that copurifies with DNA [*14*] to the PCR (at a final concentration of 160 μg/mL) [*12*], amplification was possible.

For the storage study, the types of the five different loci, determined at a three-month interval were consistent with those of the control bone samples (stored at −70°C). Of

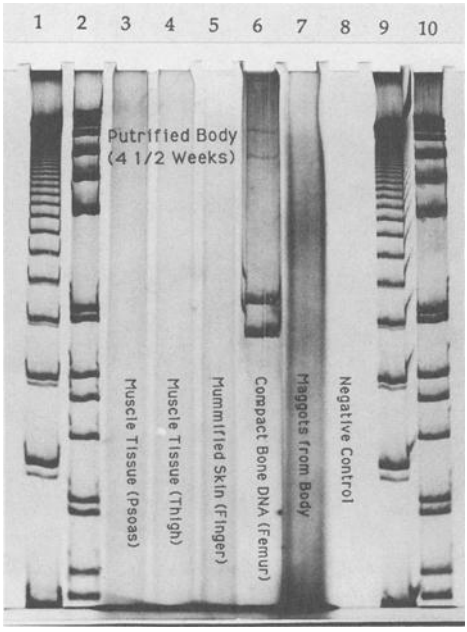

FIG. 3—*A silver-stained D1S80 AMP-FLP profile. Amplification by PCR was possible only with DNA extracted from compact bone. Lanes 1 and 9 are the 1-kb ladder; Lanes 2 and 10 are the 123-bp ladder from BRL (Bethesda Research Laboratories, Gaithersburg, Maryland), respectively.*

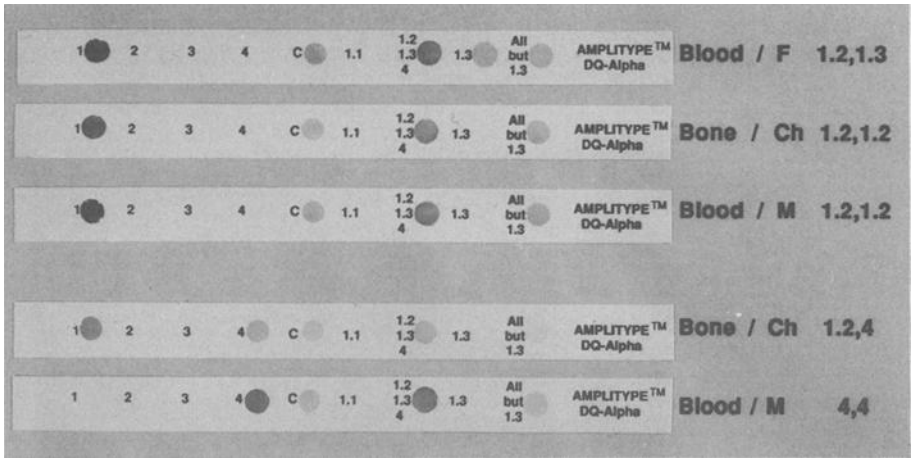

FIG. 4—*Typing of HLA-DQ alpha locus after PCR using reverse dot blot strips. Upper three strips: transmission of 1.2 allele from father (F) and 1.2 allele from mother (M) to child (Ch), who is a 1.2,1.2. Lower two strips: transmission of 4 allele from mother (M) to child (Ch), who is a 1.2,4; no father was available in this case. DNA was extracted from compact bone from the bodies, which were in an advanced stage of decomposition.*

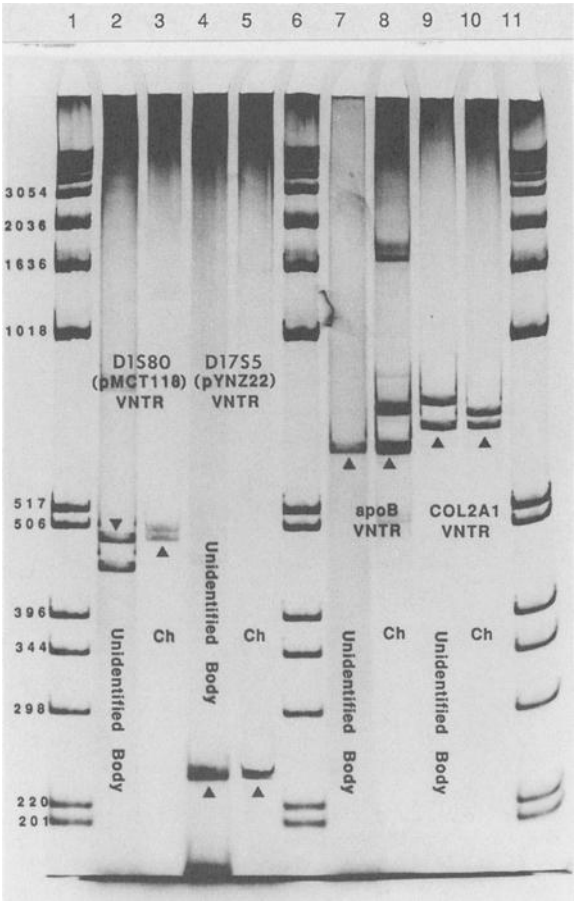

FIG. 5—*Silver-stained amplified-fragment length polymorphism (AMP-FLP) profiles of four VNTR loci comparing DNA from bone of a decomposed body and whole blood from a living relative. The arrows indicate the transmitted alleles from the deceased father (Lanes 2, 4, 7, and 9) to the child (Ch, Lanes 3, 5, 8, and 10). The term "unidentified body" was used only for labeling purposes for the PCR tests; the identity of the deceased father had been determined previously by traditional forensic science means. Additional extra bands can be seen at times (for instance, Lane 8). These bands are probably due to mispriming events or to heteroduplex formation. As long as these bands are outside the area where allele bands reside on the gel, they should not affect the profile interpretation. Lanes 1, 6, and 11 are the 1-kb ladder from BRL (Bethesda Research Laboratories, Gaithersburg, Maryland).*

the ten severely decomposed bodies, five of them had their identities determined by traditional forensic science means. Therefore, by using PCR-based typing methods, family studies could be performed on these samples for five loci. All the samples demonstrated mendelian inheritance (Figs. 5 and 6). Population frequency data for the amplified fragment length polymorphism (AMP-FLP) loci currently are scant. Therefore, statistical weight to the associations was not provided. However, it is known that these four VNTR loci have anywhere from 6 to 30 possible alleles [*5–9*]. Although a value is not placed on the family association, the evidence is strong and the mendelian inheritance of the alleles provides support for the reliability of the PCR for typing DNA derived from compact bone from severely decomposed bodies.

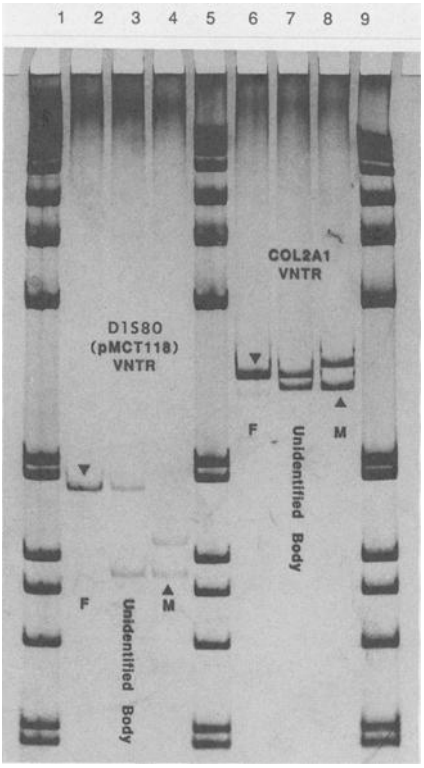

FIG. 6—*Silver-stained AMP-FLP profiles of two VNTR loci comparing DNA from bone of a decomposed body and whole blood from parents. The arrows indicate the transmitted alleles from the father and mother (F and M, Lanes 2, 4, 6, and 8) to the deceased child (Lanes 3 and 7). The term "unidentified body" was used only for labeling purposes for the PCR tests; the identity of the deceased child was determined previously by traditional forensic science means. Lanes 1, 5, and 9 are the 1-kb ladder from BRL (Bethesda Research Laboratories, Gaithersburg, Maryland).*

Discussion

Identification of human remains can be achieved at times by matching dentition with premortem dental X-rays, recognizing individualizing marks or other special features, or by fingerprint comparisons. In cases in which the remains are severely decomposed or solely skeletal, and no identification can be made by traditional means, DNA typing could be a powerful tool for identify purposes.

Regardless of the cause and manner of death, the body will undergo a series of well-defined decompositional changes [*15*]. However, the rate of degradation of human remains will vary greatly with environmental insults (climate, season, bacterial microenvironment, and animal scavengers). After a relatively short period of time, soft tissues may be lost through bacterial lysis and scavenger activity, while bone tissue is far more stable.

After a three-month storage period or when severe decomposition was observed, it was not possible to recover DNA from soft tissues suitable for either RFLP or PCR typing. Since PCR results were obtained from compact bone from those samples, it appears that DNA contained in bone is more stable than DNA in soft tissues. Most of

the DNA in compact bone is located in the osteocytes, which are the remnants of osteoblasts which have secreted bone around themselves and have become more or less isolated from the extracellular matrix. According to Frost [*16,17*] and Martin et al. [*18*], there are 20 000 to 26 000 osteocytes per cubic millimetre of calcified bone matrix. Therefore, compact bone tissue should contain sufficient DNA for analysis. In addition, the bone matrix may afford DNA some protection from degradation. Mitochondrial DNA is maintained in an environment, even in the postmortem state, that generates free radicals [*19*]. Therefore, mitochondrial DNA might be expected to be degraded significantly. However, Hagelberg et al. [*12*] have reported amplification of 600 base-pair long fragments from mitochondrial DNA recovered from ancient bone. Since the osteocytes are embedded in a calcified matrix, access to the DNA in the osteocytes during the extraction process may be difficult. To augment the yield of DNA, it seemed necessary to remove calcium ions during the extraction procedure. We employed a decalcification step described by Hagelberg et al. [*12*]. While fresh bone samples yield large amounts of DNA whether or not a decalcification procedure is used, there was consistently a higher yield of DNA from bone samples 3 months to 11 years old when decalcification was employed (data not shown). A comparison was made with the nondecalcification procedure described by Perry et al. [*3*]. In contrast to results for ancient bone material [*12*], precipitation of accumulated ions during the extraction process can prevent recovery of DNA from bone tissue of recent origin because of its high mineral content. Therefore, in our procedure, after the decalcification process, three water washes were employed to remove accumulated ions. Given the number of osteocytes in bone, an effective extraction procedure, and maintenance of the samples under proper storage conditions, microgram quantities of DNA can potentially be extracted from a gram of bone. However, even under conditions of putrefaction, sufficient quantities of human DNA can at times be recovered from bone for DNA analysis.

Typing of DNA from bone was more successful using PCR-based tests than RFLP typing. This observation was expected, since PCR-based tests do not require high-molecular-weight DNA, in comparison with RFLP typing (generally 100 to 1000 base pairs are required for the VNTR loci and 239/242 base pairs for the HLA-DQ alpha locus versus 10 000 to 20 000 base pairs, respectively). All samples were typeable for all five loci—D1S80, D17S5, Apo B, COL2A1, and HLA-DQ alpha. The storage study and the mendelian inheritance of the alleles for families suggest that the PCR results are reliable. Of course, the PCR results for the other five severely decomposed bodies, the 18-month-old bone, and the 11-year-old mummified leg cannot be confirmed. But, the quantity of DNA that was extracted (25 ng to 3.3 μg) was at such a level as to rule out the potential of contamination from other human sources prior to these specimens' entrance into the laboratory, which would have affected the PCR results. There was no evidence of contamination within the laboratory (positive and negative controls were typed properly), and there is no evidence to date that DNA other than that from higher primates can be amplified by PCR as described for the five loci analyzed.

Slot blot analysis and, wherever possible, yield gels were used to evaluate the quantity and quality of DNA. Each approach has its advantages and limitations. The slot blot method uses the probe p17H8 (D17Z1), which is primate specific [*11*], to estimate the quantity of hybridizable human DNA. It cannot determine the length or size of the DNA fragments extracted from the tissues. The yield gel approach can evaluate to a degree whether or not high-molecular-weight DNA is in the sample. But, it cannot differentiate between human and bacterial or fungal DNA. The yield gel assay was not as easily interpreted, when analyzing DNA from bloodstains and semen stains, because of significant degradation and bacterial contamination in the putrified samples. Therefore, the results from the slot blot analysis were relied upon for quantitation prior to RFLP typing or amplification by PCR.

An ancilliary benefit of the slot blot approach and its detection of human DNA is that, when a positive result is obtained, it confirms that the remains are of higher primate origin. This can aid in differentiating between human and nonhuman skeletal remains. We presently are investigating whether or not the slot blot method for quantifying human DNA should be used routinely prior to PCR typing.

Currently, the RFLP technique is accepted as a reliable means of typing DNA from evidentiary materials. Therefore, when possible, we have advocated typing the DNA by RFLP analysis. Except for HLA-DQ alpha [*20*] validation, data are scant for PCR typing of such materials. This study provides additional data, supporting the potential reliability of PCR typing of evidentiary material. We are performing additional validation studies involving PCR typing and are investigating new marker systems, their population data, and a potential PCR-based sex determination procedure.

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