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CASE REPORT

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Skeletal Remains Presumed Submerged in Water for Three Years Identified Using PCR-STR Analysis

ABSTRACT: We describe the successful identification of the remains of a saponified body found in a dam by typing of nuclear DNA. Whereas DNA extracted from soft tissues yielded negative PCR results, DNA extracted from the bone by a slightly modified Qiagen procedure allowed the typing of sex (AMG locus) and of 10 additional STR loci. An identity document was found belonging to a man missing for 3 years and comparison of the results to the DNA profiles of his son and wife confirmed the identity. The longest delay reported until now for successful nuclear DNA genotyping after immersion in river water was 18 months. This case demonstrates a delay of up to 3 years.

KEYWORDS: forensic science, identification, drowning, bone genotyping, DNA extraction, STR

Recovery of human DNA from bones of severely decomposed bodies was reported for the identification of unidentified bodies like murder victims (1) or ancient human remains (2). Nevertheless, few studies concern genotyping of bodies immersed in water, the longest reported delay for successful identification being 18 months (3). The main problem for this type of analysis is the recovery of DNA from a highly degraded tissue to ensure that the quantity and quality of the extracted DNA is suitable for amplification reactions. We used in this paper a simple and rapid commercially available silica-based purification method for the extraction of DNA from bones, which allowed successful identification of decomposed human remains after staying 3 years in a dam on the Seine River.

Materials and Methods

Soft Tissue DNA Extraction

DNA was extracted from sternocleidomastoid muscle by a commercial kit (Qiamp tissue kit) according to the manufacturer's recommendations.

Bone DNA Extraction

A fragment (3 cm) of frozen clavicle was crushed to powder and decalcified by incubation for 48 h in 50 mL Tris 10 mM, EDTA 0.5M buffer pH 8.0 (changing the buffer after centrifugation every 12 hours). The decalcified bone was then washed 3 times in Tris 10 mM, EDTA 1 mM pH 8.0. We next used a slight modification of the commercial kit (Qiamp tissue kit). The centrifugation-packed decalcified bone tissue (0.5 ml) was digested overnight at 56°C with 60 μL proteinase K in 540 μL of the ATL buffer provided by the manufacturer. Then, 600 μL AL buffer was added. After 10 min at 70°C, 600 μL of ethanol was added before loading onto a Qiamp column by 750 μL aliquots. The manufacturer's washing and elution steps were strictly followed.

DNA Typing

DNA was amplified using the Profiler Plus® kit (Perkin-Elmer) according to the optimized conditions (34 cycles, 4 μL of the 200 μL total DNA extract per 25 μL PCR reaction) described by Gill et al. (4) The Profiler Plus® kit allows simultaneous amplification of 9 STR loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820) and of the Amelogenin (AMG) locus. Five additional STR loci were examined (TPOX, CSF1PO, F13, THO1 and FES/FPS) in individual PCR reactions using 0,5 μM of the relevant primers (Perkin-Elmer, ABI PRISM® STR Primer sets), 1 IU Taq polymerase (Boehringer Mannheim) and 0.2 mM each dNTP, in 25 μL reaction mix containing 1.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris-HCl pH 8.3. These amplifications were performed in a Perkin Elmer 2400 thermocycler for 35 cycles. Alleles were identified by capillary electrophoresis (ABI 310, Perkin Elmer) by side-to-side comparison to the specific ladder using standard protocols.

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Results

Case Report

Remains of a body were found in a dam on the Seine River in the suburbs of Paris, France. The autopsy showed a saponification process for the few remaining soft tissues dating the death back to more than one year. The limbs and head were missing and therefore identification by fingerprints or dental records was impossible. Nevertheless, an identity document belonging to a man missing for 3 years was found in the cloth items. Because there was no previous biological sample available for this man, we were asked to confirm the identity of the body by a reverse paternity testing (identification of the presumed father by comparison with the genetic profile of his son). The mother (the wife of the deceased man) was included in the test, to discriminate in the genetic profile of the son the paternal and the maternal alleles.

DNA Analysis

A first attempt to amplify the DNA extracted from soft tissues (saponified muscle) by the Qiagen procedure did not show any results either for nuclear DNA or for mitochondrial DNA (up to 40 cycles). In contrast, DNA recovered from the bone allowed sex characterization and successful typing of 10 of the 14 analyzed STR loci. Results are given in Table 1, and Fig. 1 shows the Profiler plus® data. Comparison of the STR profile of the remains with the profiles of his close relatives (wife and son) showed a probability of paternity of 99,9995%.

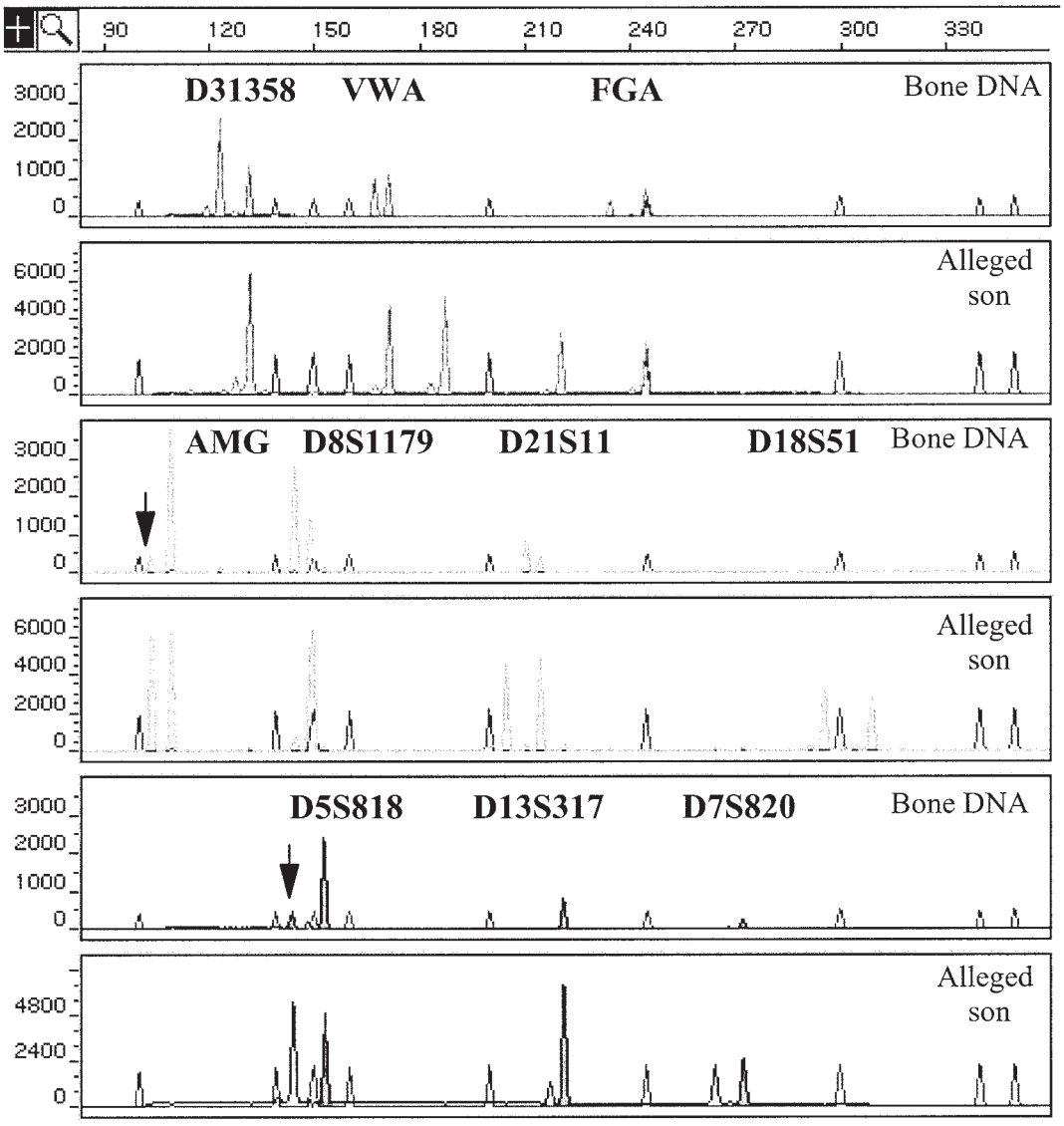

FIG. 1—*Analysis of the DNA extracted from the bone. DNA was amplified with the ProfilerPlus® kit as described in the Materials and Methods section. The vertical scales are RFUs and the horizontal scales are the size of the markers as calculated from the Rox 500 size marker (appearing as empty peaks). Top panel: FAM-labelled STRs (filled peaks, from left to right: D3S1358, vWA and FGA), middle panel : JOE-labelled STRs (filled peaks, from left to right: AMG, D21S11 and D18S51), bottom panel : NED-labelled STRs (filled peaks from left to right: D5S818, D13S317 and D7S820. The filled peaks are the alleles identical in the bone DNA and alleged son. The arrows show the severely imbalanced allele amplifications.*

TABLE 1—*Genotypes of the remains and comparison with the putative relatives. Underlined and in bold, the father alleles transmitted to the son.*

	Unknown	Remains	Son		Wife	
AMG	X	Y	X	Y	X	X
D3S1358	15	**17**	17	17	16	17
VWA	14	**15**	15	19	16	19
FGA	23	**25**	19	25	19	21
D8S1179	13	**14**	14	14	10	14
D21S11	30.2	**31.2**	29	31.2	29	30
D18S51	…	…	15	18	13	18
D5S818	**10**	12	10	12	12	12
D1 3S317	**12**	…	12	12	12	13
D7S820	10	…	8	10	10	10
TPOX	…	…	9	9	8	9
CSF1PO	…	…	11	11	10	11
F13	…	…	6	6	6	7
THO1	8	**9.3**	9.3	9.3	8	9.3
FES/FPS	10	**11**	11	11	11	12

… = failure of amplification or allelic drop out.

Discussion

Our results show that amplification for nuclear DNA failed for the highly degraded soft tissues, although it was successful for DNA extracted from the bone. Usually, bone immersed in water is a very poor material for DNA analysis; however, positive PCR amplifications were reported for bones recovered after up to 18 months immersion in river water (3,5). Literature data outline the major role of the DNA extraction method, where the decalcification and purification steps proved to be critical (3,6). Therefore, we used an extensive decalcification step to remove accumulated ions. The originality of our study concerns the extraction method by aid of silica columns (Qiagen), a simple, rapid and reliable procedure avoiding organic solvent manipulations (phenol, chloroform, see (7,8)) or long-lasting protocols like the preparation of silica suspension (9,10). In fact, Qiagen columns allow extraction and purification of the DNA in a single step. We overcame the limitation caused by the low volume of the column by loading it with digested bone 3 times before the washing procedure. This fact appears critical, since it allows to bind 3 times the maximal volume of the column. Our procedure yielded an eluate suitable for further amplification of nuclear DNA (STR) on an old and very degraded sample. With this procedure, 34 cycles (Profiler Plus® kit) or 35 cycles (other STR) of amplification allowed confirmation of paternity and therefore the identification of the anatomical remains. Interestingly, repeated amplification under identical experimental conditions with the Profiler Plus® kit led to erratic allelic drop out (not shown), with up to 5–6 unamplified alleles. Figure 1 shows an example with D18S51 failure of amplification, severe imbalance in amplification of AMG and D5S818 alleles. For this reason we cannot conclude whether the presence of only one allele for D13S317 or D7S820 loci was caused by homozygosity or allelic dropout. Severe imbalance of allele amplification might be a critical issue for the purpose of identification.

Failure to amplify long size alleles (CSF1PO, TPOX, D18S51 and F13 loci), may probably be explained by individual robustness of different loci but also by the poor quality of the extracted DNA. However, we avoided to increase the number of PCR cycles to amplify the missing alleles as, in our experience, this would lead rather to the appearance of extra non- specific peaks whose sizes may be confusing.

This procedure has been successfully repeated on bones from corpses immersed for a shorter period of time (data not shown).

Conclusions

DNA extracted from a bone of severely degraded human remains using a modified method on silica columns (Qiagen) proved to be suitable for identification by STR. This paper does not give clues to whether the Qiagen extraction is superior in terms of recovery or quality compared to organic extraction procedures, but it is quick, inexpensive and avoids the handling of toxic chemicals. The protocol used here has to be considered because of its simplicity.

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