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CASE REPORT

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Individual identification of flood victims by DNA polymorphisms and autopsy findings

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Abstract On September 23rd 1993 Genova was flooded by heavy rainstorms and 4 people disappeared, including an elderly couple. Four days later a partially skeletonized body was found floating near the coast. No visual identification was possible. Autopsy findings were consistent with the medical history of a possible victim. DNA was extracted from a muscle sample and compared to paraffin embedded prostatic gland fragment taken by surgery. A positive identification could be made. On October 11th the body of a decomposed and partially skeletonized female was found. The visual identification was also uncertain and no clinical records were available. A blood sample from the son was obtained for maternal identification by the polymerase chain reaction.

Key words Identification · Human remains · PCR · DNA polymorphisms

Zusammenfassung Am 23. September 1993 wurde Genua infolge heftiger Regenstürme überflutet, wobei vier Personen, darunter ein älteres Ehepaar, vermisst wurden. Vier Tage später wurde ein teilweise skelettierter Leichnam in Küstennähe angetrieben. Eine visuelle Identifizierung war nicht möglich. Die Autopsiebefunde stimmten mit der Krankengeschichte eines der möglichen Flutopfer überein. Daraufhin wurde aus einer Muskelprobe DNA extrahiert und mit einem Stück Paraffin-eingebetteten Prostatagewebes, das anlässlich einer Operation entnommen wurde, verglichen. Hierdurch konnte eine positive Identifizierung erzielt werden. Am 11. Oktober wurde der verweste und teilweise skelettierte Leichnam einer Frau aufgefunden. Die visuelle Identifikation war ebenfalls unsicher und Krankenakten standen nicht zur Verfügung. Zur maternalen Identifikation mittels Polymerase-Kettenreaktion wurde eine Blutprobe des Sohnes herangezogen.

Schlüsselwörter Identifikation · Menschliche Überreste · PCR · DNA-Polymorphismen

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Introduction

Positive identification of decomposed bodies or human remains has always been one of the goals of Forensic Science. Dental or skeletal data and other individual findings are considered useful tools for this purpose (Farinelli Fierro 1993; Sopher 1993), but they are not informative when the circumstances do not allow a direct match for identification.

The application of molecular biology methods in forensic laboratories has opened up new frontiers in this field, either by the study of inherited characters in parents and relatives (Hagelberg et al. 1991; Sullivan et al. 1992) or by direct matching with a previously drawn sample. Even data on the sex of the victim can be considered in the match (Mannucci et al. 1993; Gill et al. 1994; Casarino et al. 1995).

In certain circumstances, both strategies can be utilized to obtain an identification. These conditions allow a clearer proof of parenthood or a familial relationship, especially when more than one person in a family has to be identified at the same time.

In the present case such a situation is described. Two bodies were identified as being the parents of a putative son by directly matching the male sample with a block of paraffin embedded prostatic gland tissue and analysis of genetic inheritance for confirmation of paternity and detection of maternity, for the female body.

Case report

On 23rd September 1993, 4 people were reported missing after a flood in Genoa, including an elderly couple who disappeared from their house. Four days later a body was found floating near the coastline. At the external examination, the body appeared as a macerated, partially skeletonized white male. Multiple blunt trauma and animal activity caused the destruction of maxillary

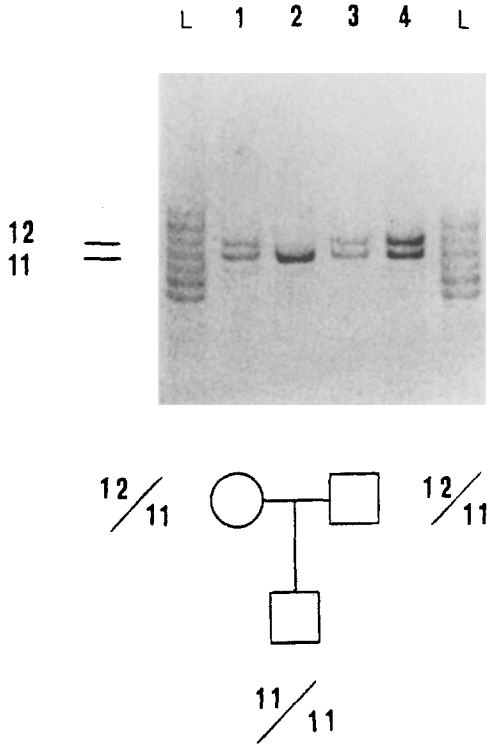

Fig. 1 12% native PAGE for HUMFES/FPS alleles. L: allelic ladder; 1: muscle sample from the female body, putative mother; 2: sibling of the missing elderly couple; 3: sample from the male body, putative father; 4: paraffin embedded prostatic tissue from the male of the elderly couple

alveoli and the loss of the lower jaw, the disarticulation at the shoulder joint of the right arm, the partial skeletonization of the left arm, fracture and amputation of both legs. The autopsy showed a partial gastrectomy and a small nodular prostate gland.

These data were consistent with the medical history of the male of the elderly couple, who had received surgery in the past (partial gastrectomy due to gastric ulcer and transurethral prostatectomy due to adenoma of the prostate gland), but a positive identification was not possible by means of morphological data alone.

Paraffin embedded tissue from the transurethral prostatectomy was supplied by the hospital where the surgery was performed and our laboratory was commissioned to carry out DNA investigations.

On October 10th 1993, another decomposed body of a white elderly female was found under a layer of debris at the outlet of the river on which the house of the couple had stood. At the external examination, a surgical thyroidectomy scar was noted. The autopsy showed the absence of the thyroid gland. No clinical data were available and the son could not make a positive identification.

As there were indications that the body could be the female of the elderly couple, a blood sample was obtained from the putative son to carry out a reverse parentage test, combining data from the DNA profiles of the 2 bodies, the paraffin embedded prostate gland and the putative sibling.

DNA from ileopsoas muscles, sampled from the bodies at autopsy, and fresh blood from the sibling were extracted by the phenol/chloroform method (Sambrook et al. 1989). Extraction from the paraffin embedded tissue was performed following procedures described by Goelz et al. (1985).

A total of 11 loci were amplified via the polymerase chain reaction (PCR), using 10 ng of DNA for each amplification. Bovine serum albumin (BSA) was added to the sample extracted from paraffin embedded tissue to facilitate amplification (Hochmeister et al. 1991). Commercial kits were used for D1S80, HLA-DQA1 and Polymarker with conditions recommended by the manufacturer (Perkin Elmer, Norwalk, U.S.A.). Published sets of primers and conditions were used for ApoB, HUMTH01, HUMCD4 and HUMFES/FPS loci amplification (Boerwinkle et al. 1989; Polymeropoulos et al. 1991a; Edwards et al. 1991; Polymeropoulos et al. 1991b).

Table 1 DNA markers for individual identification of the male body by direct comparison with the paraffin embedded gland tissue and maternity indication of the female body. PE = Paraffin embedded prostatic gland, AM = alleged mother. Random genotype sharing in male body-paraffin embedded tissue identity 1.46×10^{-8} Random allelic sharing in maternity identification 1.027×10^{-4}

System	Male body	PE	Sibling	AM
D1S80	T24/T24	T24/T24	T24/T24	T24/T24
ApoB	37/37	37/37	37/37	37/37
HLA-DQA1	1.2/3	1.2/3	1.2	–/–
HUMTHO1	9.3/9.3	9.3/9.3	9.3/9.3	9.3/9
HUMCD4	10/5	10/5	10/5	10/6
HUMFES/FPS	12/11	12/11	11/11	12/11
GC *	A/C	A/C	A/A	A/B
GYPA *	B/B	B/B	B/B	A/B
D7S8 *	A/B	A/B	A/B	B/B
HBGC *	A/B	A/B	B/B	B/B
LDLR *	A/B	A/B	A/B	A/B

*Amplitype PM (Perkin Elmer, Norwalk, USA)

Amplification products were analysed by reverse dot-blot for Polymarker and HLA-DQA1, GeneAmp detection gel (Perkin Elmer, Norwalk, U.S.A.) for D1S80, 8% denaturing polyacrylamide gel electrophoresis (PAGE) for HUMTH01, while native PAGE was used for HUMCD4 and HUMFES/FPS (8% and 12% respectively) (Fig. 1). Ethidium bromide stained 2% agarose gels were used for ApoB. PAGE separations and D1S80 were silver stained (Bassam et al. 1991).

Table 1 shows the genotype profiles of the samples tested. The alleles obtained from the body of the male showed a match with the paraffin embedded gland tissue and a probability corresponding to 1.46×10^{-8}. No exclusions of paternity were found with the results of the putative sibling.

Genetic profiles of the identified male, the sibling and the female body were compared to achieve information about the maternal identity. No exclusions of maternity were found and casual allelic sharing for maternal identification was calculated as 1.027×10^{-4}.

Discussion

The importance of fingerprints, dental records, clinical or personal reports (e.g. radiographs, surgery, tattoos, etc.) is well known and standard protocols are defined for individual identification (Farinelli Fierro 1993; Sopher 1993), especially in cases of decomposed bodies or human remains. These data are commonly considered as sufficient, especially in mass disasters, for identification of bodies when all available characters match to confirm an assumption. But a direct comparison with bone measurements, clinical reports or other personal data is not always possible and, in our experience, dental records are frequently not available.

In such cases, only DNA polymorphism tests can provide a useful means of identification (Hagelberg et al.

1991; Mannucci et al. 1993; Gill et al. 1994). In fact, it is possible to achieve a positive identification if a direct match with previously drawn samples can be used. In cases where no samples are available, important indications of identity can be obtained by testing for mendelian inherited characters from putative parents, offspring or relatives of the deceased. Moreover, DNA polymorphisms are detectable even when other data cannot be obtained, such as for small bone or other biological tissue fragments. Finally, the use of PCR analysis allows genotypic definitions even when degraded DNA is extracted from samples.

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