IDENTIFICATION OF HUMAN REMAINS USING DNA AMPLIFICATION (PCR).

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INTRODUCTION

Positive identification of human remains has always been one of the main goals of Forensic Science. Fingerprints, dental or skeletal data were considered useful tools for this purpose until now (Farinelli 1993; Sopher 1993). However, the application of molecular biology methods in forensic laboratories has achieved new perspectives in the direct identification by resolving cases with critical amount of sample for analysis (Hagelberg 1991; Sullivan 1992). This can be made through the study of inherited characters in parents or relatives and direct matching with human remains samples. In this comunication we show the results in two cases received in the Servicio de Analitica de la Comisaría General de Policía Científica, Madrid (Spain). In one of them, human remains were buried over four years, in the other they suffered the effect of high temperatures because of a fire. These two cases were selected because they express critical factors in the necroidentification.

CASE REPORT

Case 1: on 11th August 1994, Police sent to our laboratory a purse containing several bones and a photograph of a possible person. These remains were found buried in lime soil in 1992 and they were studied in others laboratories but no clear results were obtained. After morphologycal studies (craneal and dental measurement, cephalic indexes, apical radiographies in the maxillaries and image superpositions) we asked for 5 mL. of fresh blood from the putative parents to compare with DNA extracted the from human remains.

Case 2: Police of Almería (Spain) sent us both maxillaries of a person who comitted suicide by setting fire to his home. As the former case, after mophologycal studies, fresh blood was obtained from the putative parents.

MATERIALS AND METHODS

DNA extraction and quantification

Extraction from fresh blood samples was made by standard methods (Phenol/Chloroform) (Sambrook 1989). In the first case, a femur bone and several teeth were selected. A layer of approximately 1 mm. was removed from the surface of the bone sample in order to reduce contamination from previous handling. The sample was ground to a fine powder and washed by adding 0.5 M EDTA pH 8.0 to remove salts (Manfred 1991). A lysis buffer was added to the tubes, and the extraction was made with the adition of phenol/chloroform. After this organic extraction, the aqueous layers were dialyzed and concentrated to approximately 40 µL. by using Centricon-100 microconcentrators (Amicon, USA). In case of teeth samples, after mechanical opening and recovery of pulp tissue, they were trated the same than the bone, except decalcification. In case N. 2 we only had upper and lower maxillaries. Subsequently, just dental pulps could be used for DNA extraction

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like in the former case. DNA quantification was made through agarose gel electrophoresis and "Quantiblot" (Perkin-Elmer, USA). RFLP's polymorphism was not tried because of the scarce amount DNA extracted from the human remains.

Amplification and typing

A total of 11 loci were amplified by PCR, using 10 ng. of DNA for each amplification. Bovine serum albumine (BSA, 160 mgr./mL.) was added to the samples extracted to facilitate amplification (Hochmeister 1991). Comercial Kits were used for D1S80, HLA DQA1, Polymarker (Perkin-Elmer, USA), HUMTH01, HUMFES/FPS, HUMvWFA31 and HUMF13A01 (Promega, USA) under conditions recommended by the manufacturer. Amplification products were analysed by reverse dot-blot for HLA DQA1 and Polymarker, Gene Amp detection gel (Perkin-Elmer, USA) for D1S80 and 6% denaturing polyacrilamide gel electrophoresis (PAGE) for the others markers. PAGE separations and D1S80 were silver stained.

RESULTS

In the first case, a positive identification resulted from morphologycal and molecular methods. The shape of upper central incisors of the skull overlaps with those in the photograph of the alleged person. In Table 1 genetic profiles of the human remains together with those of his alleged parents are shown. No exclusion can be seen in the markers and no result for HUMF13A01 locus could be obtained for sure. In the second case, a positive identification did not results through morphologycal methods. The possible race and sex, and the approximate age of the individual were made. In Table 2, genetic profiles of human remains and alleged parents can be seen. As in the former case, no exclusion was observed. These results were analyzed by calculation of Likelyhood Ratio (LR) or "Correspondence index" between the putative parents and doubtful remains: LR = X/Y, where **X** is the probability of the human remains if the couple is the progenitors and where **Y** is the probability of the human remains if the couple isn't the progenitors. In the first case, the LR was 68.721 and 3.146.873 in the second. If we assume an "a priori" probability of 0.5, the alleles obtaines from the human remains showed a match with the putative parents with a probability of 99.998545% in the first case and 99.99996% in the second one.

DISCUSSION

In our first case, human remains were found in 1992. Our laboratory analyzed this remains in 1994, and this demonstrates that in spite of delayed time and the conditions of the burial, the positive identification by PCR is possible.

Table 1. Genetic profiles in case 1.

LOCI	HUMAN REMAINS	PROGENITOR 1	PROGENITOR 2
DQA1	2-4	3-4	2-2
D1S80	18-31	18-31	18-24
LDLR	A-A	A-A	A-B
GYPA	B-B	A-B	B-B
HBGG	A-A	A-A	A-A
D7S8	A-A	A-B	A-A
GC	A-C	A-B	C-C
TH01	9-9.3	9-9.3	8-9.3
FES	11-13	11-13	11-12
vWF	17-18	17-18	16-17

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In second case, the remains suffered the effect of high temperatures and equally positive results were obtained. In general, the traditional identification methods via fingerprint, dental records, clinical or personal reports are commonly considered as sufficient for identification of bodies when all available characters match to confirm an assumption (Farinelli 1993; Sopher 1993). But this comparison is not always possible because of the absence of antemortem data. In such cases, only DNA polymorphism test can provide a useful mean of identification (Hagelberg 1993; Gill 1994). PCR has the advantage of needing little amounts of sample and the results are positive even when degraded DNA is extracted from samples.

Table 2. Genetic profiles in case 2.

L O C I	HUMAN REMAINS	PROGENITOR 1	PROGENITOR 2
DQA1	1.2-4	1.2-4	4-4
D1S80	30-31	18-31	20-30
LDLR	B-B	B-B	B-B
GYPA	A-B	A-A	A-B
HBGG	A-B	A-A	A-B
D7S8	A-A	A-B	A-A
GC	C-C	C-C	B-C
TH01	9-9.3	8-9.3	9-9.3
FES	10-11	11-11	10-10
vWF	14-18	14-18	16-18
F13	5-7	7-7	5-6

REFERENCES

Farinelli M, (1993) In: Spitz W.V. (ed.) Medicolegal investigation of death, 3rd edn. Charles C. Thomas, Springfield, III, pp. 71-117.

Gill P, Ivanov P L, Kimphon C, Piercy R, Benson N, Tully G, Evett I, Hagelberg E, Sullivan K, (1994) Identification of the remains of Romanov family by DNA analysis. Nature Genetics 6: 130-135.

Hagelberg E, Gray I C, Jeffreys A J, (1991) Identification of the skeletal remains of a murder victim by DNA analysis. Nature 352: 427-429.

Hochmeister M N, Budowle B, Jung J, Boer U V, Comey C T, Dimhofer R, (1991) PCR-based typing of DNA extracted from cigarette butts. Int. J. Leg. Med., 104: 229-233.

Hochmeister M N, Budowle B, Borer U V, Eggmann U, Comey C T, Dimhofer R,(1991) Typing of Deoxyribonucleic acid (DNA)extracted from compact bone from human remains. J. For. Sci. 36, 6: 1649-1661.

Sambrook J., Fritsch E.F., Maniatis T. (1989) Molecular clonning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y.

Sopher I.M. (1993). In: Spitz W.V. (ed.) Medicolegal investigation of death, 3rd edn. Charles C. Thomas, Springfield, III, pp. 118-136.

Sullivan K.M., Hopgood R., Gill P. (1992) Identification of human remains by amplification and automated sequencing of mitochondrial DNA. Int. J. Leg. Med. 105: 83-86.

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