

PCR ANALYSIS OF DNA FROM SKELETAL REMAINS IN CRIME INVESTIGATION CASE

Katja DROBNIČ

Forensic Science Laboratory, Ministry of the Interior, Ljubljana, Slovenia

ABSTRACT: The objective of this presentation is to demonstrate the positive identification of skeletal remain of a murder victim by comparison of several short tandem repeat (STR) loci in sample from femur bone of decomposed corpse with the samples from personal articles of the presumptive victim.

In April 1998, a 72-year old woman disappeared from her home. It was believed that she had been murdered. The presumptive victim was found dead after more than one year in a shaft of the suspect garage. For identification purposes, the crime scene investigators already in September 1998 collected the clothes belonging to the above mentioned missing woman and three teeth found in a paper sac marked by dentist. Two hairs found on the collected clothes and the epithelial cells found on the surface of a tooth were subject to DNA analysis. There was no possibility that these samples belonged to other people, because the presumptive victim lived alone and was known as unsociable. For the identification of the corpse, we obtained a portion of femur bone. The co-amplification of ten STR's along with the amelogenin locus on the X and Y chromosome was performed using AmpFISTR SGM Plus kit (Perin Elmer/ABD, CA). STR profiles were the same for the DNA recovered from the hairs and for the DNA recovered from the femoral bone of decomposed body. The combined power of discrimination for the used ten STR loci is greater than 0.99999999 in our population sample.

In conclusion, DNA profiling of personal articles is a very powerful method for the identification of murder victims or missing persons, especially on occasions when relatives are not available.

KEY WORDS: Forensic science; Human identification; Bone tissue; Short tandem repeat (STR); Articles left behind.

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INTRODUCTION

The identification of murder victims is essential both from a humanitarian and a religious point of view as well as for judicial reasons. From a legal point of view, the establishment of the identity of a murder victim is necessary for the beginning of penal proceedings. However, if a body is decomposed to such a degree that the facial structures are destroyed, no finger-

prints exist, dental records are uninformative, X-ray comparisons are useless, it is probable that a positive identification will not be made. Today DNA analysis has become a method of choice for identification purpose. Microsatellite DNA, namely short tandem repeats (STRs) loci show high polymorphism. Since they consist of no more than several hundred bp, there is a strong possibility than they can be amplified by polymerase chain reaction (PCR) even from degraded DNA extracted from old skeletal remains [1, 5]. For this reason, STR polymorphism has received increasing attention as an effective genetic marker for the personal identification of unknown skeletal remains or decomposed corpses. However, there are several difficulties in such tests. To compare the results of DNA typing, it is necessary to have related subjects with which to perform comparative analyses. Such tests are normally performed by comparison with the DNA profiles from people known to be immediate family members of the presumptive victim, such as parents [2]. However, such genotyping does not necessarily provide conclusive proof of identity for several reasons. The mutations of microsatellite loci during spermatogenesis and oogenesis may become a major problem in deciding whether sporadic discordance of profiles between a skeletal remain and its putative parents could be due to mutations or to non-parentage [7]. A great problem could also arise when close relatives of the putative victim could not be found. To avoid such difficulties, we decided to analyse samples from personal belongings of the presumptive victim.

In this criminal case we performed a personal identification of a murder victim by comparison of 10 STR loci in samples from bone tissue of the victim with those in personal articles.

CASE HISTORY

In April 1998, a 72-year old woman disappeared from her home. She was last seen entering in her friend's son's car. It was believed that she had been murdered. The probable reason of her death was financial benefit which a suspect gained by it. An unidentified, badly decomposed corpse was discovered in a shaft of the suspect garage in July 1999 (Figure 1). The body was wrapped in a sheet, a blanket, a carpet and packed into a cardboard box. An autopsy revealed that the cause of the death was compression of the victim's neck. The female nature of the corpse was determined. A putative identification of the body as a missing woman from Ljubljana was established using circumstantial evidence. However, it became important to establish a positive identification using a reliable scientific method.

For identification purpose, the crime scene investigators already in April 1998 collected the clothes belonging to the above mentioned missing woman

and a paper sac marked by a dentist, within which three teeth appurtenant to the missing person were found. All the evidence was found in the missing person's apartment-basement.

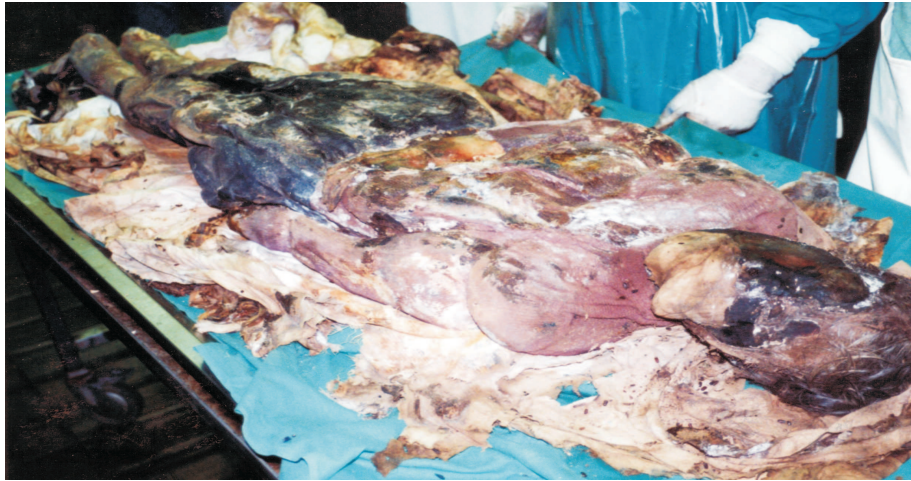


Fig. 1. Photography of unidentified badly decomposed body found in the shaft of the suspect garage.

MATERIAL AND METHODS

Samples

Two hairs found on the collected clothes and the epithelial cells found on the surface of a tooth were subject to DNA analysis. There was no possibility that these samples belonged to other people, because the presumptive victim lived alone and was known as unsociable. For the identification of the corpse, we obtained a portion of the femur bone.

DNA extraction

A femur bone sample approximately 10 cm in length was obtained from the victim. At first the sample was divided in halves to expose the marrow cavity of the bone. Each portion was then sawed to 1 cm long pieces. All traces of soft tissue and bone marrow were removed from the surface of the bone pieces using razor blades and sandpaper. Each of them were then rinsed briefly with 5% bleach solution followed by three washed with distilled water. Then, the bone pieces were crushed into smaller fragments and stored in sterile polypropylene tubes at -20°C until analysed. Two portions

of 5 grams of compact bone were ground to a fine powder in a metal blender filled halfway with liquid nitrogen. Bone extraction was completed following the protocol described by Hochmeister [5]. A sample was recovered in 100 μ ml of TE⁻⁴ (Maniatis). The recovered DNA was stored at 4°C.

Each of two grey hairs were cut with scissors 1 cm from the root portion and rinsed in distilled water followed by absolute ethanol. After drying, DNA was extracted from both hairs using a protocol derived from the rapid Chelex[®] extraction procedure [6]. One tooth was incubated in distilled water at room temperature with intermediate shaking for 1 hour. The solution was then centrifuged at 12 000 rpm for 5 min. As much as possible of the supernatant was discarded. DNA was extracted from the pellet containing human cells using the Chelex procedure. All DNA extracts were quantitated using the Quantiblot[®] Human DNA Quantitation Kit (Perkin Elmer, Norwalk, CT).

STR typing

The coamplification of D3S1358, VWA, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, THO1 and FGA loci was performed using AmpFlSTR SGM Plus kit (Perkin Elmer/ABD, Foster City, CA). Reactions for PCR were prepared according to the manufacturer's recommendations except that the PCR volume was 25 μ l using 2 ng of sample DNA. A Perkin Elmer GeneAmp PCR System 9600 thermal cycler was used for PCR. The STR loci were separated and detected by PE Applied Biosystem PRISM 377 DNA Sequencer. The data were collected and analysed by the geneScan computer software.

Statistical tests

In order to estimate the significance of the observed match at the STR loci, the probability of random match and power of discrimination were calculated as described [3] using our microsatellite allele frequencies [4]. The combined power of discrimination for the ten STR loci is greater than 0.99999999 in our population sample.

RESULTS AND DISCUSSION

A portion of femoral bone was used for DNA analysis because of its good condition compared to other bones. The outer layers were removed and pieces of about 5 g were pulverised and subjected to DNA extraction. DNA was extracted twice along with the blank extraction required to check for the presence of contemporary DNA contamination. Both extraction were suc-

cessful and the quantity of the purified DNA determined by slot-blot analysis was approximately 50 ng and 25 ng of human DNA per gram of bone.

The sample extracted from the hairs contained approximately 7.0 ng of human DNA per μl and the sample extracted from the cells on the tooth surface contained 0.6 ng per μl . The quantity of the DNA recovered from the tooth was relatively low, so we decided to analyse only the DNA from the hairs and the bone remains. STR profiles produced using the AmpFISTR SGM Plus Amelogenin systems were the same for the DNA recovered from the hairs and for the DNA recovered from the femoral bone. The allele calls for all of the loci tested are shown in Table I. Since one band (X; X) was detected at amelogenin locus, the unknown corpse was female. Not all loci amplified in PCR reaction from the hair sample. Alleles from D18S51 locus did not appear in the profile. The results as shown were credible because only one or two alleles at all amplified loci were detected from each sample. No contamination occurred as precautions were taken, especially during DNA extraction and PCR preparation.

TABLE I. ALLELE CALLS FOR TEN STR LOCI PLUS AMELOGENIN GENDER LOCUS COMPARING DNA FROM THE HAIRS TO DNA FROM THE BONE, INCLUDING FRAGMENT LENGTH IN BASE PAIRS

Locus	DNA from bone	DNA from hairs	Fragment size [bp]
Amelogenin	X, X	X, X	106.08, 106.08
D3S1358	16, 17	16, 17	130.20, 134.32
VWA	17, 17	17, 17	180.66, 180.66
D16S539	11, 12	11, 12	257.16, 261.03
D2S1338	17, 18	17, 18	300.32, 304.27
D8S1179	13, 15	13, 15	148.34, 156.44
D21S11	30.3, 32.2	30.3, 32.2	213.37, 221.48
D18D51	18, 19	*	309.66, 313.70
D19S433	14, 16	14, 16	124.33, 132.36
THO1	9, 9.3	9, 9.3	185.48, 188.60
FGA	19, 19	19, 19	224.12, 224.12

* Allelic drop-out was seen in sample from hair at STR locus D18S51 (longest fragment lengths).

The calculation of a conservative estimate of the frequency of the resulting profile at 9 loci in the Slovene populations results in a value of 1×10^{-12} .

In this study, we performed a personal identification by genotyping several STR loci and comparing them with those of the presumptive victim's personal articles. The present method is a very powerful tool for the identification of murder victims, especially on occasions when medical records and relatives of the victim are not available.

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