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MtDNA: a successful identification case in burned remains

N. Cerri*, A. Verzeletti, F. De Ferrari

Institute of Legal Medicine, University of Brescia, Piazzale Spedali Civili 1, 25123 Brescia, Italy

Abstract. Some human carbonized remains were found in a wood near a lake. Investigations and a macroscopic analysis of the remains led suspicions to a killed and burned 20-year-old man. To identify with certitude the remains, nuclear DNA from the remains and from the alleged mother were analysed. Only at vWA locus an exclusion was found. Because of the delicacy of the case (a murder) and since only the alleged mother's DNA was available, a mt-DNA analysis was performed. It was found a good correlation in both HV1 and HV2 regions between the DNA from the remains and the mother's.

Keywords: STR; mtDNA; Identification

1. Introduction

Some human carbonized remains were found in a wood near a lake in Northern Italy (Fig. 1). The remains examination led to the recognitions of some bones and pieces of organs such as the liver and a kidney. According to bones morphology and investigations, the remains were ascribable to a killed and burned 20-year-old man.

To establish with certitude the identity of the found body, a genetic analysis was carried out between the remains' and alleged mother's DNA (the only relative available) regarding both nuclear DNA and the mitochondrial one.

2. Materials and methods

DNA was obtained from the carbonized remains (liver and kidney) and from an oral swab for the alleged mother. DNA was extracted using Chelex®-100 method according to Walsh colleagues.

For nuclear DNA, amplification was carried out applying AmpFlSTR® Profiler Plus™ and SGM Plus™ PCR Amplification Kits (Applied Biosystems) in a Perkin Elmer 2400 thermal cycler, according to manufacturer's recommendations. Products were

* Corresponding author. Tel.: +39-030-3995480; fax: +39-030-3995839.
E-mail address: deferrar@med.unibs.it (N. Cerri).

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Fig. 1. The remains.

loaded on the ABI Prism 310 Genetic Analyser and analysed with GeneScan analysis software V.3.2.1.

For mt-DNA, HV1 and HV2 regions were analysed. HV1 PCR products were generated by using Primer L15971: 5′-TTAACTCCACCATTAGCACC-3′ and Primer H16414: 5′-CACGGAGGATGGTGGTCAAG-3′. HV2 PCR products were generated by using Primer L15: 5′-CACCCTATTAACCACTCACG-3′ and Primer H389: 5′-CTGGTTAGGCTGGTGTTAGG-3′ according to Buscemi et al. [1] PCR amplification was performed in a total volume of 50 μl: 5 μl 10× PCR-Buffer (Applied Biosystem), 3 μl 25 mM $MgCl_2$ (Applied Biosystems), 4 μl 10 mM dNTPs (Applied Biosystems), 0.4 μl 50 μM of both Fw and Rv Primers, 0.5 μl Taq-Gold (Applied Biosystems). The amount of DNA was 1 ng for each reaction. Amplification was performed on a P-E 2400 Thermal Cycler. The conditions were as follows: 94 °C for 30 s, then 94 °C for 1 m, 56 °C for 10 s, 72 °C for 30 s for 38 cycles. The PCR product was purified with a Microcon 100 device (Amicon) and then it was evaluated from a quantitative point of view on a 2% agarose gel.

BigDye Terminator cycle sequencing of PCR products was performed according to manufacturer's protocol. Both strands were sequenced using the Primers F15971 and R16414 for HV1 region; F29 5′-CTCACGGGAGCTCTCCATGC-3′ and R381 5′-GCTGGTGTTAGGGTTCTTTG-3′ were used for HV2 region. PCR sequencing was performed in a total volume of 20 μl: 4 μl BigDye Terminator RR Mix (Applied Biosystems), 1 μl of F or R Primers and 3–5 μl PCR product. PCR conditions were the following: 96 °C for 15 s, 50 °C for 5 s, 60 °C for 2 min for 25 cycles. Analysis was carried

Table 1
STR analysis

Loci	Remains	Alleged mother
D3S1358	18–18	17–18
VWA	16–16	14–14
FGA	21–23	23–23
D8S1179	13–14	13–14
D21S11	30–32.2	30–32.2
D18S51	16–18	14–16
D5S818	9–13	9–11
D13S317	11–12	8–11
D7S820	8–10	8–10
D16S539	13–13	9–13
D2S1338	17–25	17–17
D19S433	13–15.2	13–13
TH01	8–8	8–9.3
Sex	M	F

out by capillary electrophoresis on an ABI Prism 310 (Applied Biosystems); data were analysed by Sequencing Analysis Software 3.4.1 (Applied Biosystems).

3. Results and discussion

STRs analysis gave a successful attribution, but an exclusion at vWA locus was found in the nuclear DNA of the remains (Table 1). The paternity testing supplied a good value (0.9999182), but because of the delicacy of the case (a murder) and since only the alleged mother's DNA was available, a mt-DNA analysis was performed.

Human mt-DNA is a small molecule with circular structure, containing 16,569 bp, entirely sequenced [2], it is well known that it is very resistant to extreme environmental conditions, and as a consequence, it can be used in forensic applications [3,4].

The two samples were analysed for both HV1 and HV2 regions and their sequence was compared with the Anderson's one. A perfect compatibility was found in both HV1 and HV2 regions between the DNA from the remains and the mother's.

This work evidence the importance of mt-DNA in cases where a maternal transmission is involved and STR analysis is limited by an ''incompatibility''. Otherwise, it is clear that another approach could have been done: the vWA sequencing to demonstrate the mutation at this locus.

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