

The bone collector: when reality overcomes fantasy

Luigi Cipolloni¹
 Silvia Zoppis¹
 Manuela Rosini¹
 Micaela Lucci²
 Alfredo Coppa²
 Carla Vecchiotti¹

¹ Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, "Sapienza" University of Rome, Rome, Italy

² Department of Environmental Biology, "Sapienza" University of Rome, Rome, Italy

Corresponding Author:

Silvia Zoppis
 Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Laboratory of Forensic Genetics, "Sapienza" University of Rome
 Viale Regina Elena 336
 00161 Rome, Italy
 E-mail: silvia.zoppis@uniroma1.it

Abstract

Introduction: the Authors present a case occurred in the suburbs of Rome, concerning the discovery of a skeleton by the fire fighters after a fire broke out in a grassy field. Beside it, a bag containing a bunch of keys and an identity card was found, fortunately not destroyed by the fire. These items belonged to an elderly man who disappeared in that same area four years before.

Materials and Methods: genetic tests were performed on a left femoral bone sample in order to confirm the presumed identity of the skeleton and instead provided a genetic profile that was not compatible with the sons of the missing man. Thus other samples from different bones were examined, resulting in different genetic profiles and none of them compatible with the sons of the missing man.

Results: the Prosecutor asked for an anthropological expertise, which confirmed morphologically that the skeleton was composed by bones belonged to different individuals and could provide an approximate age of these individuals at the time of death. The Prosecutor also asked for the time of death of these individuals and, to this purpose, specific investigations on the bone remains were carried out based on the measure of the isotopic ratio of ¹⁴C in lipids and collagen by AMS (Accelerator Mass Spectrometry).

Discussion: this case represents an interesting example of the application of a multidisciplinary approach in challenging cases of identification of human remains and demonstrates that a case that may apparently seem "simple" may instead reveal great methodological and interpretative challenges.

KEY WORDS: bone remains, forensic, identification, multidisciplinary approach.

Introduction

All who work in the forensic field know that the more critic the biological samples to be analyzed are (charred remains and /or in an advanced state of decomposition, fragments of tissues, bones, etc.), the more complex personal identification is. If on the one hand the analysis of biological traces such as blood, semen, saliva, urine or hairs, implies no particular operational problem from the technical point of view, thus personal identification can be easily reached by comparing the genetic profile obtained from the case-work sample with that of the reference sample (according to the specific case), on the other hand the issues related to the identification of a corpse or human remains can be extremely challenging for the forensic geneticists (1, 2).

The Authors present a case occurred in the suburbs of Rome, concerning the discovery of a skeleton by the fire fighters after a fire broke out in a grassy field. The skeleton was almost complete and its right side was charred because of the fire. Beside it, a bag containing a bunch of keys and an identity card was found, fortunately not destroyed by the fire. These items belonged to an elderly man who disappeared in that same area four years before, thus genetic analyses on bone samples were ordered by the Prosecutor in order to confirm the identity of the skeleton.

Materials and methods

The analyses were conducted on the following bone remains: right and left clavicle; right humerus; right femur; right tibia and left fibula (Fig. 1).

DNA extraction

After removal of the bone surface layer by scalpel blade and cleaning with bleach, an electric drill was



Figure 1 - The skeleton recovered was mostly charred on its left side, thus the right and left clavicle, right humerus, right femur, right tibia and left fibula were chosen to be analyzed among all the bone remains because of their better conditions.

used to sample the inner portion of the bones (0.5-1g). Genomic DNA from the bone samples (right and left clavicle, right humerus, right femur, right tibia, left fibula) was extracted using the commercial kit named GENE CLEAN for Ancient DNA (BIO 101, La Jolla, CA, USA). This kit is based on the guanidinium isothiocyanate extraction method and the purification through silicon oxide suspension (glass milk). Extraction was carried out following the kit protocol.

DNA quantification by quantitative PCR or “Real Time PCR” (qPCR)

For the quantification of the DNA extracts, the technique called quantitative PCR or “Real Time PCR” was used with the Applied Biosystems 7500 Real Time PCR instrument. The Quantifiler[®] Duo DNA Quantification Kit (Applied Biosystems by Life Technologies, Foster City, CA, USA) was used and the quantification range for the analyzed samples was 0.03-0.06 ng/ μ l.

STRs amplification and typing

STR analysis was performed using the Promega PowerPlex[®]16 System amplification kit (Promega Corporation, Madison, WI, USA) with a 25 μ L reaction volume and 0.5-1 ng of template DNA. The analysis was performed following the manufacturer’s protocol. Reaction products were then analyzed using an ABI PRISM[®] 310 Genetic Analyzer (Applied Biosystems

BY Life Technologies, Foster City, CA, USA) and the Internal Lane Standard (ILS) 600 size standard. To exclude possible contamination during the amplification procedures, in addition to an extraction negative control, an amplification negative control was always prepared and analyzed, including all the reagents used for PCR without template DNA. The electrophoretic run parameters were those indicated by the User Manual of the specific PCR kit and the electropherograms obtained were analyzed with the GeneMapper ID v3.2.1 Software (3-5).

Results

The genetic tests were first performed on a right femoral bone sample in order to confirm the presumed identity of the skeleton; they instead provided a genetic profile that was not compatible with the sons of the missing man.

Thus, other samples were taken from different bones (right and left clavicle, right humerus, right femur, right tibia, left fibula) and examined resulting in four different genetic profiles, corresponding to two women and two men, and none of them was compatible with the sons of the missing man. The results obtained are shown in Table 1:

- right clavicle: male (DNA profile not determined)
- left clavicle: male (DNA profile not determined)
- right humerus: male M1 (incomplete DNA profile)
- right femur: male M2 (incomplete DNA profile)
- right tibia: female F1 (complete DNA profile)
- left fibula: female F2 (complete DNA profile).

Table 1 - Results of the genetic analyses performed on the bone samples.

DNA markers	Right humerus: M1	Right femur: M2	Right tibia: F1	Left fibula: F2	Right clavicle: male (DNA profile not determined)	Left clavicle: male (DNA profile not determined)
D3S1358	15-16	15-18	16-17	15-16	-----	-----
TH01	9.3-9.3	6-9.3	6-6	6-9	-----	-----
D21S11	28-30	30-31.2	31-31.2	31.2-32.2	-----	-----
D18S51	14-19	10-12	14-15	14-14	-----	-----
Penta E	----	----	5-13	11-12	-----	-----
D5S818	----	9-10	12-13	10-13	-----	-----
D13S317	11-12	9-12	9-12	11-11	-----	-----
D7S820	11-11	10-10	12-12	10-11	-----	-----
D16S539	12-12	9-13	12-13	11-12	-----	-----
CSF1P0	----	10-11	10-12	12-12	-----	-----
Penta D	9-10	9-12	12-14	10-13	-----	-----
VWA	18-19	17-17	14-19	15-18	-----	-----
D8S1179	10-13	14-16	12-13	11-14	-----	-----
TPOX	8-8	10-11	9-11	11-11	-----	-----
FGA	21-23	18-21	22-23	21-21	-----	-----
Amelogenin	XY	XY	XX	XX	XY	XY

Discussion

After being informed of the results emerged from the genetic analyses (showing four different genetic profiles), the Prosecutor ordered to perform further studies on the same bone remains in order to assess both the approximate age of these individuals at the time of death and the presumptive time of death. An *anthropological expertise* was necessary for both the morphological confirmation that the bone remains belonged to different individuals and the age assessment for these individuals at the time of death (6).

The skull could be morphologically attributed to a female and had slight traces of burning on the left parietal bone. As the mandible articulated well with the skull, it could be inferred that both specimens belong to the same individual. Given some skull parameters, such as intracranial and external cranial sutures and tooth wear, we deduced an age at time of death of about 25-30 years old. There were 19 vertebrae, probably belonged to the same individual and certainly to the skull because the atlas articulated perfectly with it. Both the 3rd and 6th cervical vertebrae were missing. The lumbar vertebrae were very fragmented due to the fire that must had been particularly intense in this portion, so that the vertebral bodies were completely destroyed. The morphological examination of the bone remains showed that none of them was observed twice and even the smaller charred fragments were identified and allocated to specific segments of a bone. The skeleton looked almost complete: only the

sternum, the two flaps and nearly all the hand and foot bones were missing. For some parts it was possible to restore the natural connections and consequently the remains could be attributed to specific individuals. The skull, the mandible and the column up to the dorsal vertebrae belonged to a single individual. The fire destroyed consistently the lumbar vertebrae thus not allowing to determine with certainty whether these vertebrae and the underlying basin (the sacrum and hips) corresponded to the same individual. The upper limbs seemed to belong to males of different body size and this was in contrast with the morphology of the skull and the column that were related to a female. As for the lower limbs, some bones appeared to be feminine and some other masculine. The bones of the basin belonged with certainty to the same individual at least as for the sacrum and the right hip, which perfectly articulated together, but had a difference in morphology as the sacrum looked masculine while the hip bones were feminine.

With regard to the assignment of sex and age at time of death, the differences in morphology suggested that the remains did not belong to a unique individual, although none of the bone fragments, even the smaller, was observed twice. The hygienic-sanitary situation of the oral cavity suggested that the individual did not have any kind of dental care. The absence of the sternum, ball joints and hand and foot bones indicated that the corpse/s had been moved in the place where the skeleton was finally found and the analysis of the effects of fire on the findings suggested that the bones

were somehow placed in a sort of anatomical connection.

The general impression was that the skeleton had been reconstructed from stripped bones belonged to multiple individuals: it is highly probable that at the time of the transportation of these remains in the place of their discovery some parts were still connected by the presence of dry soft tissue residues; the evidence of traces of animal bites (probably rodents) on the bones supports this hypothesis.

The parameters used for age assessment for these individuals at the time of death were the degree of synostosis of the external cranial sutures, the degree of tooth wear (which, however, can vary considerably from a population to another), the morphology of the symphysis pubis (the articular surface has ridges and furrows that tend to smoothen with age) and the bone tissue structure (cortical thickness decreases with age; the spongy bone of the proximal humerus and femur is more compact than in the young than in older adults). Here is reported the final age assessment for the bones that could be morphologically analyzed:

- skull-mandible: 45-55 years old
- male M1 (right humerus): 40-50 years old
- male M2 (right femur): 25-40 years old
- female F1 (right tibia): 20-35 years old.

The prosecutor also required a *physical-chemical expertise* for the determination of the time of death for each individual. Specific analyses on the bone samples were performed in order to measure the isotopic ratio of ^{14}C in lipids and collagen by AMS (Accelerator Mass Spectrometry) (7, 8). Regarding these analyses, we refer to the article by Marzaioli et al. (9) whose results confirmed the belonging of the bone remains to individuals of different age at death and time of death, thus agreeing with the results of the genetic and anthropological expertises.

Four DNA profiles have been identified but not all the bones available have been genetically examined because the left side of the skeleton was charred and no DNA profile could be obtained from most of these samples. Thus it is highly probable that the genetic profiles, and therefore the number of individuals involved in the case, may be more. We consider that the most disturbing detail of the recovery of the recom-

posed skeleton is the macabre collection of single anatomical body parts from different individuals deceased at different times and maybe buried in different places. To date, these corpses have not been recovered yet, in spite of the careful investigations of the Prosecutor.

This case represents an interesting example of the application of a multidisciplinary approach in challenging cases of identification of human remains and demonstrates that a case that may apparently seem “simple” may instead reveal great methodological and interpretative challenges, making it imperative to use a multidisciplinary approach with methods that require specific professional expertise in various specialties (e.g. pathology, genetics, anthropology, physics, chemistry).

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