



Importance of DNA analysis for identification and confirmation of human remains, following a forensic autopsy

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ABSTRACT

Various forensic techniques are used to identify a human corpse, depending on the circumstances and the state of remains. Unfortunately, the standard forensic identification methods were not sufficient in 30–35% of all victims, therefore DNA identification was necessary. We have found that a forensic autopsy does not always give reliable answers to important questions, for example: What is the amount of time lapsed between the moment of death and the skeletal remains discovery? and What is the age of the deceased? In fact, it could often cause an erroneous identification strategy choice. This paper describes the forensic application of current DNA technology to solve a missing person's case. The disappearance of a 57 year old male was reported in a town in the north of Serbia in August 2017. In January 2018, in that geographical area parts of skeletal remains, remains of clothes and a watch were found and sent to the Institute for Forensic Medicine. The age of the person was estimated to be between 75–80 years, and it was estimated that the remains had been buried for more than 10 years. This report confused the police, because they did not have a missing person of the above description on record. However, the DNA analysis of a bone sample has shown that the remains belong to the 57 year old missing person who disappeared 5 months prior and for whom the son was the reference sample donor. Complete DNA profiles obtained using AmpF ℓ STR[®] Identifiler[®] Plus, AmpF ℓ STR[®] NGM[™], GlobalFiler[™], Investigator[®] 24plex QS kit, AmpF ℓ STR[®] Yfiler[®] and AmpF ℓ STR[®] Yfiler[®] Plus PCR Amplification kits were a match with the reference sample of the victim's son (probability 99.9995147%). This work has shown that for identification of skeletal remains and solving missing persons cases, the forensic application of the latest DNA technology is of utmost importance.

1. Introduction

The identification of human remains belonging to missing persons is one of the main challenges for forensic genetics. When other human identification forensic techniques (e.g. dactyloscopy, anthropology, odontology and medico-legal examinations) provide limited information, or to support further or refute potential associations, DNA typing can be extremely valuable. DNA, in theory, can be recovered essentially from any tissue (e.g. soft, degraded tissues, bones, teeth and hairs) [1–4]. In addition, because close relatives on average share more genetic variants than unrelated individuals, relatives can provide reference samples to effect identifications of unknown individuals or their remains or, at least, to develop potential investigative leads for law

enforcement.

This paper represents our approach and the results obtained during identification of unknown skeletal remains. We describe here our work on the identification of a person whose remains were found in a town in the north of Serbia in January 2018. The disappearance of the 57 year old male was reported in the same geographical area in August 2017. The problem that forensic scientists can face when working with human identification are erroneous approximations following the autopsy with regards to the time lapsed between the moment of death, the skeletal remains discovery and the age of the deceased. These inaccuracies can have potentially devastating effects for the skeletal remains identification process, because the reference sample could be missed. Although the age of the person, in our case, was estimated to be between 75–80

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years, and it was estimated that the remains had been buried for more than 10 years, we have still decided to compare DNA profile from the bone sample and that of the son of the missing person, the only missing person in that area. The DNA analyst was successful in making, with great confidence, associations between skeletal remains and reference samples.

2. Material and methods

2.1. Samples and DNA extraction

We analysed the bone sample of the missing person found buried in a town in the north of Serbia in January 2018. The bone sample for DNA analysis was collected, labelled, and photo-documented. For genetic investigations, a 5–10 cm fragment was taken from the femur. First, the bone was cleaned from the remnant soft tissue and all soil traces. The cut bone fragments were washed and air dried [4]. The resulting sample was pulverized into fine powder in mill MM 301 (Retsch).

Extraction of DNA using PrepFiler® BTA Forensic DNA Extraction Kit (Applied Biosystems) was performed using 50 mg of powdered bone according to Zgonjanin et al. [5]. Modifications of extraction method have been introduced in order to improve the laboratory success rate with identification of these skeletal remains [5,6].

Family reference samples accompanying the skeletal remains are usually obtained from buccal swabs or dried blood samples using the QIAamp DNA Micro Kit (QIAGEN).

2.2. PCR amplification and typing

DNA was quantified with an ABI Prism® 7000 Sequence Detection System (Applied Biosystems) using Quantifiler™ Human DNA Quantification kit. Amplifications were performed on the GeneAmp PCR System 9700 Gold Plate (Applied Biosystems) using the AmpFLSTR® Identifier® Plus kit (Applied Biosystems), AmpFLSTR® NGM™ (Applied Biosystems), GlobalFiler™ PCR Amplification kit (Thermo Fisher Scientific), Investigator® 24plex QS kit (Qiagen), AmpFLSTR® Yfiler® (Applied Biosystems) and AmpFLSTR® Yfiler® Plus PCR Amplification Kits (Applied Biosystems) following the manufacturers' protocols. Amplified products are separated and detected on ABI 3500 Genetic Analyzer (Applied Biosystems).

3. Results

3.1. Case report

We detected 1.089 ng DNA/μL of isolate from bone sample. The victim was identified by comparison to his son; we compared autosomal STRs and Y-STR haplotypes (probability 99.9995147%). Full STR profiles were obtained from the femur using AmpFLSTR® Identifier® Plus, AmpFLSTR® NGM™, GlobalFiler™, Investigator® 24plex QS kit, AmpFLSTR® Yfiler® and AmpFLSTR® Yfiler® Plus PCR Amplification kits. Concordant results were obtained for the STR loci between 16 marker STR kit which was used previously and 6-dye multiplex kits with 24 markers and 27 Y-STR loci kit which is at our disposal today. Similar to the experience of Irwin et al. [7], our experience shows that a combination of a higher number of genetic markers provides extremely high LR that supports the hypothesis that individuals' bones are related to the family references, rather than unrelated individuals.

3.2. Autopsy-biological profile

Following the autopsy a forensic specialist issued an opinion that the skeletal samples in their anthropological and anatomical characteristics were largely human remains (8 bones), and partially they came from animals (3 bones). All human bones were part of a same

skeletal system of a male person. The only skull bone remains present were Zygomatic arch bones and the left cheekbone. Chest, shoulder girdle, pelvic girdle bones as well as vertebral column vertebrae were missing. Only the right upper arm bone from the upper extremity bones was available. From the lower extremity bones, both femur bones and both tibia bones were available.

4. Discussion

Each bone that is received at our laboratory is unique in terms of how degraded the bone may be, the length of time each bone was exposed to environmental conditions, and the quantity of DNA that is available [5,6,8].

The skeletal remains that are found usually end up at medical examiner, coroner, or law enforcement agencies. Without collection or submission of bone samples, the potential to identify an individual is eliminated. Quite often skeletal remains will remain unidentified, they are cremated without the retention of the biological sample, or buried without any family reference sample for comparison. In this case despite the initial age estimate of the person to be between 75–80 years, and the estimate that the remains had been buried for more than 10 years, our results after the DNA analysis have proven that the skeletal remains belonged to the 57 years old person who disappeared only 5 months prior to the analysis.

The results showed that nuclear DNA testing plays a vital role in the identification of skeletal remains.

5. Conclusion

Our experience has shown that many scientific disciplines can be applied to the identification process and some fields are developing techniques especially for forensic purposes. As a result, forensic scientists must be especially careful to ensure that they are clear about the abilities and limitations of their work and do not allow misjudgements to persist.

In cases where wrong partial estimate or time lapsed between the moment of death and the skeletal remains discovery, process of human identification has relied on the DNA typing to acquire additional genetic information from bone samples of skeletal remains.

The results suggest that the identification of skeletal remains should rely on the DNA analysis because it leads to the identification of more missing individuals. Lastly, DNA analysis can be a powerful exculpatory tool eliminating wrongly associated individuals, reducing candidates and redirecting resources to more viable avenues of investigation.

Declaration of Competing Interest

None.

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