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Neither femur nor tooth: Petrous bone for identifying archaeological bone samples via forensic approach

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ABSTRACT

One of the major challenges of molecular biology in anthropological analysis is the identification via DNA typing of bone or teeth samples that can be collected from archaeological site in order to investigate kinship relationships. Due to the difficulties of isolating and analysing DNA from such samples, several efforts have been made to solve these problems, but less work has been conducted to identify the proper type of bone samples for the DNA analysis. Therefore, following the promising results obtained from the DNA analysis of petrous bones by different groups of researchers, for the first time, here we investigated the possibility of using petrous bones as skeletal elements useful for short tandem repeat (STR) typing via capillary electrophoresis technique in ancient bone samples. In order to compare the results from petrous bone, femur and tooth samples, a total of 39 skeletal elements were collected from 13 different individuals excavated from Italian archaeological sites, dating from the sixth to seventh century C.E. The DNA was extracted, quantified, and subsequently amplified using two STR multiplex kits. The presence of a good amount of genetic material, despite high degradation, allowed us to quantify and subsequently identify STR profiles via CE analysis from ancient petrous bones that were complete for four out of thirteen samples and higher than 11 autosomal loci for all samples. Our results indicated that petrous bone is the best skeletal element with regard to DNA conservation and is a valuable element from which it is possible to obtain a complete STR profile also when analysing ancient bones. The STR results showed the possibility to use the petrous bones for identification and matching purposes in cases in which the biological material is poor and highly degraded such as in archaeological studies. Therefore, STR typing could represent a time-saving and cheap chance to verify kinship relationships in archaeological sites and evaluate sex when skeletal material is not suitable for morphometric estimate as in case of infants.

1. Introduction

The ability to recover and analyse DNA from highly degraded skeletal remains represents one of the most significant challenges for molecular anthropologists and forensic experts. The DNA analysis of human remains has become a common practice for identifying human remains and providing genetic information for evolutionary and kinship studies in archaeological contexts. Bones, teeth, and hair are more durable biological material than other remains, and therefore, in many forensic cases and anthropological studies, they represent the unique potential source of genetic material. When working with bone samples, the main potential issue are low amounts of starting molecules, degradation of DNA, and the presence of polymerase chain reaction (PCR) inhibitors [1]. Additionally, contamination with modern DNA can occur by working staff during exhumation, improper handling or storage, and anthropological studies and it can make difficult to gain authentic STR profiles or sequences. Several efforts have been made to optimize DNA extraction from bone specimens for both anthropological (e.g. Refs. [2–4]) and forensic purposes (e.g. Refs. [5–8]), to modify the amplification approach for improved short tandem repeat (STR) typing [9,10] and to prevent the contamination in ancient DNA samples [11] but less work has been done on the identification of the best skeletal element for DNA preservation. Hagelberg et al. [12] have noted that not all skeletal elements may contain enough DNA for successful typing, and, as a result, several forensic researchers have tried to determine which skeletal parts are superior to others in terms of DNA preservation [13–18].

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In 2009 Edson et al. [18] sampled different cranial fragments for mitochondrial DNA analysis and their results highlighted that petrous bone was an optimal region from which to recover mitochondrial DNA. Petrous bone is located at the base of the skull between the sphenoid and occipital bones, composing the endocranial part of the temporal bone that houses the organs of hearing and equilibrium. The petrous bone, as highlighted by the name itself, is the hardest and densest bone part in the mammalian body [19]. Recently Kulstein et al. [20] demonstrated the possibility to produce remarkable and reproducible STR typing results from petrous bone samples collected by criminal investigation departments for forensic identification purposes. Thanks to development of high-throughput sequencing techniques associated to shotgun approach, in 2014 two different studies [21,22] published in *Nature Communications* and *Nature* respectively, indicated that petrous bone provides high endogenous DNA yields in ancient samples. Gamba et al. [21] analysed complete and partial genomes from petrous bones of 13 individuals (all individuals have been directly dated, spanning from the Early Neolithic (~5700 cal BC) to the Iron Age-IR1 (~800 cal BC)), dentine of four individuals, ribs of two individuals, and a metacarpal and metatarsal of two different individuals from archaeological sites in order to compare endogenous yields from petrous bones to those from other skeletal elements within the same individual. Their results indicated that DNA yields from petrous bones exceeded those from teeth and other bones presumably due to the high density of petrous bones associated with reduced bacteria-mediated DNA decay and other post-mortem DNA decay. One year later, these results were confirmed by Pinhasi et al. [23] on specimens from Holocene archaeological contexts across Eurasia dated between 10,000–1800 calibrated years before present (cal. BP). Following the promising results obtained via NGS (Next Generation Sequencing) technique from previous DNA analysis of petrous bones, for the first time, here we investigated the possibility to use this bone as a skeletal element useful for STR typing in ancient samples via PCR/CE (Capillary Electrophoresis) analysis. Therefore, we extracted, quantified, and amplified using recent STR multiplex kits DNA samples from three different ancient skeletal elements: petrous bone, femur (as recommended by the International Society for Forensic Genetics DNA Commission [24]), and teeth (more refractory to contamination by exogenous DNA than bones, as proposed by Pilli et al. [25]).

2. Material and methods

2.1. Samples

A total of 39 skeletal elements were collected from 13 different individuals. The specimens were selected from Italian archaeological sites, dating from the sixth to seventh century C.E. Individuals were buried in earthy graves in structured medieval necropolises. Three different skeletal elements (femur, teeth, and petrous bone) from each individual were examined in this study. Bones were sampled following protocols for ancient DNA analysis [26]. Some petrous bones were already disconnected from the skull. If the skull was intact, the petrous bone has been cut vertically from the lower part of the skull with a plunge-cut saw blade and removed through the foramen magnum. An example of a petrous bone collected for this study is shown in Fig. 1. The isolation of the inner ear portion was conducted as in Pinhasi et al. [23].

2.2. Contamination prevention

In order to obtain reliable results, the genetic analysis was conducted according to the most stringent criteria proposed for ancient DNA studies [27–30], and all steps of the DNA analysis were performed in a dedicated laboratory for ancient DNA studies. Different precautions were taken to avoid contamination of samples with extraneous DNA: 1. all DNA extractions and PCR involving the samples were carried out in a laboratory physically separated from the laboratory in which PCR cycling and post-PCR analyses were performed; 2. disposable masks, gloves and

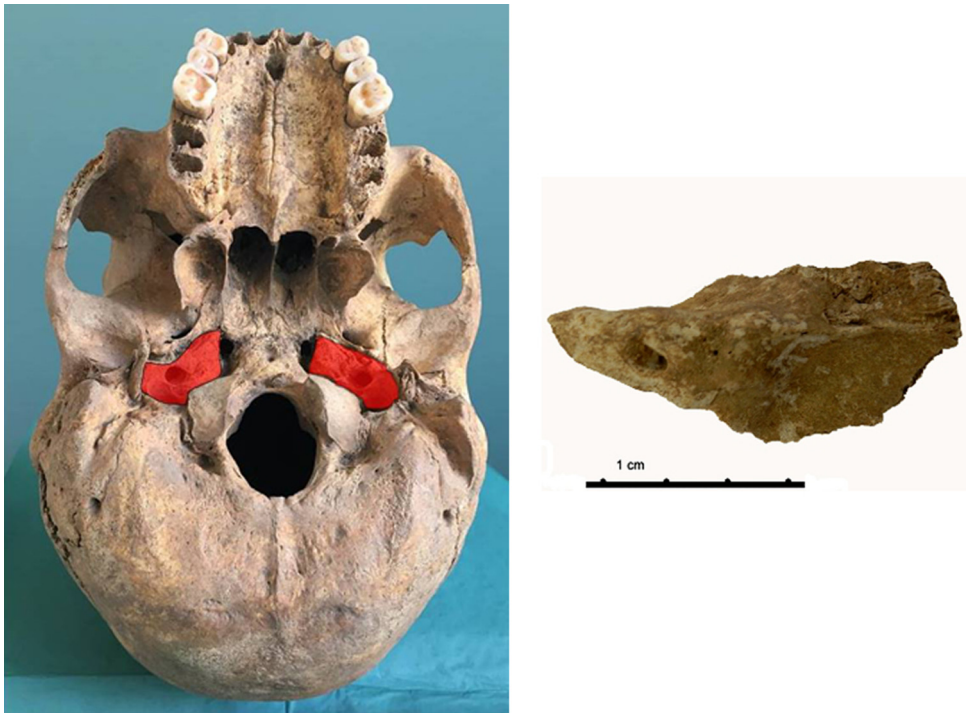

Fig. 1. The location of petrous bones in a skull is colored in red. The skeletal element collected from the skull is represented on the right. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)