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Different skeletal elements as a source of DNA for genetic identification of Second World War victims

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ABSTRACT

To this day process of identification of missing persons from skeletonized human remains with help of forensic genetics proves to be complex and challenging. The success rate of genetic identification in bones strongly depends on a combination of various factors, most importantly environmental factors and post-mortem interval. Furthermore, there are individual-specific factors that affect DNA preservation, such as race, gender, age and type of skeletal elements. The goal of our study was to optimize sampling process through determining which skeletal elements are superior in their preservation of DNA in 70-yearold skeletons belonging to victims of Second World War. We sampled different types of bones and teeth from three such skeletons found in Slovenian hidden mass grave Huda jama, 56 elements from each respective skeleton, together 168 elements. With the help of parameters, such as quantity of DNA, degradation rate and typing success, we tried to find the best types of elements to identify the victims. Prior to powdering bones and teeth, we removed contaminants. We decalcified 0.5 g bone and tooth powder followed by extraction and purification of DNA using Biorobot EZ1 (Qiagen). Quantification of obtained nuclear DNA was carried out using PowerQuant kit (Promega) and autosomal STR typing using ESSplex SE QS kit (Qiagen). Best parameters to assess skeletal elements that are superior in their DNA preservation were quantity of DNA and number of successfully typed STR loci. Metacarpal and metatarsal bones proved to be the best, followed by intermediate cuneiform, first distal foot phalanx, talus, petrous bone and tibia. We also created elimination database for persons involved in exhumation, anthropological and genetic analyses and exclude potential contamination.

1. Introduction

For almost three decades DNA analyses have been go-to methods for assessing kinship, identifying missing persons and other kinds of forensic research. A rapid development of the field has enabled myriad new options to be put to use, but even so such forensic investigations are still among most challenging ones, for more often than not the sole and the best source of DNA are teeth and bones, as their structure permits longer post mortem conservation compared to other tissues. Quantity and fragmentation of DNA widely depend on numerous factors these tissues undergo, most accountable being post mortem interval and properties of the environment in which bones and teeth were located prior to discovery [1]. Moreover, some types of bones and teeth may preserve DNA better than others; current studies recommend cortical bone tissue to be used [2–5], though some newer studies are finding great potential in cancellous bone tissue [6–8]. Thereby, to optimize the effectiveness and costs, numerous studies are presently addressing this by attempting to find the most suitable element to acquire sufficient quantities of well preserved DNA molecules [9,10]. Objective of this research was to determine best types of skeletal elements for DNA analysis of approximately 70-year-old remains of Second World War victims in order to improve sampling process.

2. Materials and methods

Three different skeletons, belonging to the Second World War casualties, were utilized to sample 56 different elements from each respective skeleton, a total of 168 bones and teeth. Preparatory to grinding them into powder, aforementioned bone samples were subjected to mechanical as well as chemical cleaning, whereas teeth were cleaned chemically and irradiated with UV light, all of which aimed to decontaminate bone and teeth surface. Next, 0.5 g of each powdered sample was decalcified using EDTA, followed by DNA extraction and later on purification with use of Biorobot EZ1 (Qiagen). Cleaning, powdering, decalcification and purifying were carried out as described by Zupanič Pajnič [11].

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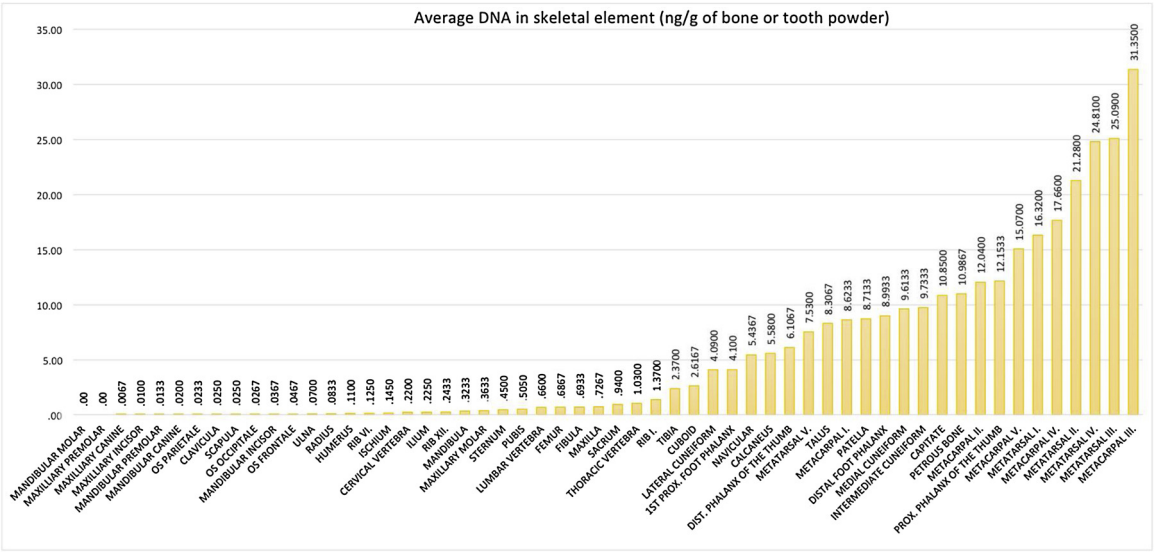

Fig. 1. Average DNA quantities for 56 respective elements ranked from lowest average yielding to highest average yielding skeletal element.

Purified samples of nuclear DNA were quantified using PowerQuant (Promega) kit and autosomal STR typing was performed using ESSplex SE QS (Qiagen) kit.

In order to identify possible contamination, an elimination database containing genetic profiles of all individuals, who participated throughout the processes of exhumation, anthropological investigation and genetic analysis, was created.

Method described by Poetsch et al. [12] was used to determine lower limit of DNA quantification that would be appropriate for STR profiling and still give complete or partial genetic profiles.

3. Results

Among skeletal elements containing on average the most DNA in all three examined skeletons were exclusively upper and lower limb bones, the highest yielding being metatarsal and metacarpal bones and the lowest yielding being teeth (Fig. 1).

Genetic profiles were categorized as complete (17/17 loci successfully amplified), partial (8 or more loci successfully amplified) or unsuitable for interpretation (7 or less loci successfully amplified). Out of 56 different elements a complete profile (17/17) was obtained for 15 elements in all 3 skeletons.

From 15 bones that complete genetic profiles were obtained in all 3 skeletons, 14 (metacarpal and metatarsal bones, intermediate cuneiform, distal phalanx (foot), talus, petrous bone) were also among those containing highest average amounts of DNA with the exception of tibia, of which average DNA yield did not coincide with profiling success, as the quantity was lower (Fig. 1).

Lower limit of DNA quantification that would still be appropriate for STR profiling resulting in obtaining partial or complete genetic profiles was determined at 0,00095 ng/µl.

4. Discussion

On average, lowest yielding DNA samples were teeth, which was surprising, given that DNA should preserve well in them according to other studies [13,14].

To foretell success of DNA profiling, degradation index proved to be a less suitable parameter whilst quantity of DNA in ng per g of bone or tooth powder much largely coincided with obtaining complete genetic profiles.

Bones that proved to be most suitable to be used in process of identification were metacarpal and metatarsal, followed by intermediate cuneiform, distal foot phalanx, talus, petrous bone and tibia, the latter being included among those most suitable for being sampled for genetic identification despite lower average quantity of DNA compared to other recommended skeletal elements.

5. Conclusions

Current sampling guidelines for skeletonized human remains may have to be strongly reconsidered, especially when working with samples originating from similar environment and of about the same age as the ones used in this study.

Declaration of Competing Interest

Authors declare that they have no conflict of interest.

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