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Determination of DNA yield rates in six different skeletal elements in ancient bones

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ABSTRACT

Current sampling strategy for laboratories typing bones for human identification include samples obtained from femur, tooth and temporal bone. Latest studies suggest that the small bones of the hands and feet were very similar or even better in DNA yield. These bones can be easily sampled with a disposable scalpel and thus reduce potential DNA contamination. The aim of our study was to determine the suitability of metatarsals, metacarpals and phalanges for genetic identification. 48 bone samples from 8 different skeletons (six from 18th century and two from 3rd century) were obtained from 5 archaeological sites in Slovenia. In each skeleton, 6 different skeletal elements were sampled (temporal bone, molar, femur, metacarpal bone, metatarsal bone and proximal phalanx of the hand), and strict precautions followed to prevent contamination. Half of gram of bone powder was decalcified using full demineralization extraction method. The DNA was purified in a Biorobot EZ1 (Qiagen), DNA content determined with the PowerQuant kit (Promega), and autosomal STR typing performed with the Investigator ESSplex Plus kit (Qiagen). Up to 8.75 ng DNA/g of powder was obtained from samples analyzed. The highest yields were detected in temporal bone and the lowest in femur. The success rate of STR typing was evaluated according to the number of successfully typed loci and a strong correlation between the success rate of STR typing and the amount of extracted DNA was confirmed. For all eight skeletons full consensus genetic profiles were determined from skeletal elements analyzed. Our findings suggest it would be suitable to include metatarsal and metacarpal bones in sampling strategy for human identification although further research is needed to substantiate the findings of this study.

1. Introduction

In forensic-genetic investigations of skeletal remains, researchers are confronted with the decision of selecting the skeletal elements that will be used for genetic testing. In the past, several studies were conducted to determine which skeletal elements yielded the best results. One of them is a retrospective study done by Miloš et al. in 2007, in which the highest success rates were observed with samples from dense cortical bone of weight-bearing leg bones (femur) and tooth [1]. Misner et al. compared DNA yields between femur, pelvis and rib samples resulting in femur samples performing the best [2]. Therefore, the recommendations for genetic identification of skeletal remains include long cortical bone samples of the leg (femur, occasionally tibia) and tooth. These recommendations are supported by the ISFG, Interpol, American National Association of Medical Examiners and the American National Institute of Justice. The same recommendations have been applied in most the studies of ancient DNA (aDNA), which is defined as any DNA that is older than 70 years post mortem interval (PMI) [3]. Alongside femur and tooth the temporal bone of the skull has been shown to be very efficient in genetic studies of aDNA [4,5]. The recommended set of bones is not always available for sampling and subsequent genetic testing. In the World Trade Center (WTC) disaster victim identification study they extracted DNA from smaller bones including patellae, metatarsals, and foot phalanges which yielded rates comparable to both femur and tibia [6]. In another study, Mundorff and Davoren systematically researched a larger amount of different skeletal elements in the same skeletons. At increasing PMI, small trabecular bones continued to yield more DNA and more successful STR typing than cortical bones [7]. In the analysed literature we could not find studies done on aDNA for which small bones of the hands and feet were used as samples for genetic research. Therefore, the aim of our study was to determine whether obtaining sufficient amounts of DNA and

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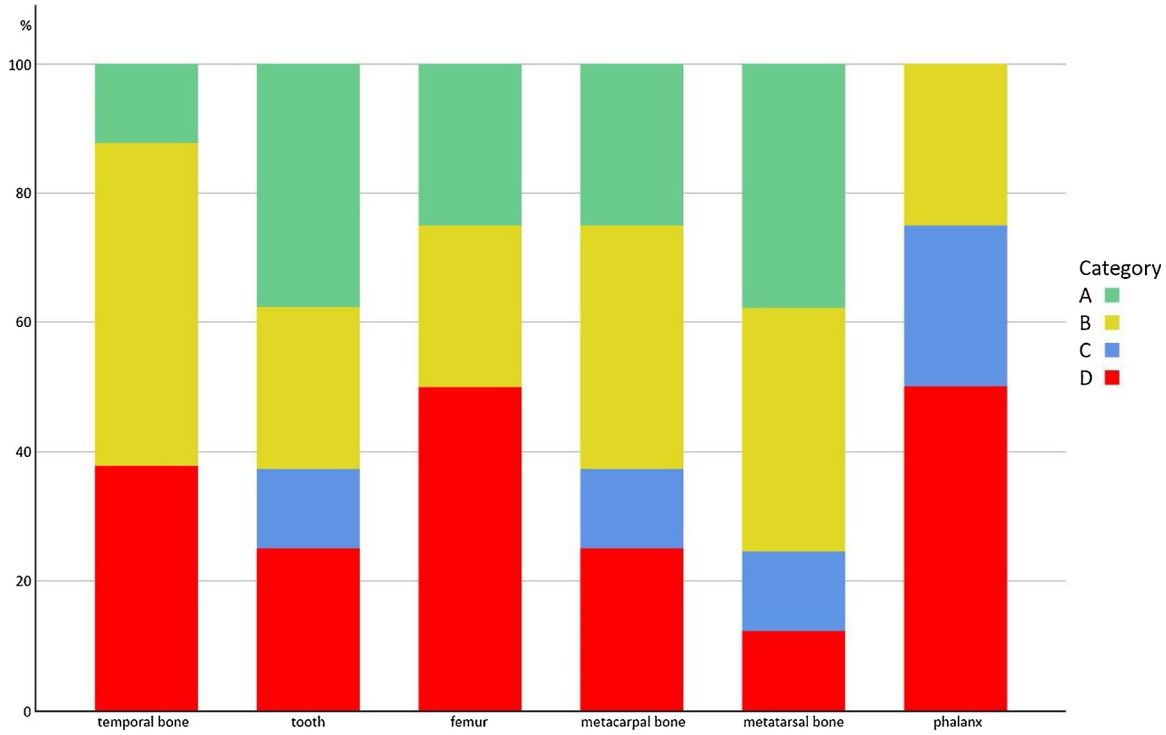

Fig. 1. Autosomal STR typing of DNA by categories (n = 48 samples). Category A (successful multiplication of all 16 analyzed loci), Category B (successful amplification from 11 to 15 loci out of 16 total analyzed), Category C (successful amplification from 8 to 10 loci out of 16 total analyzed), Category D (successful multiplication in less as 8 loci out of 16 total analyzed).

profiling its autosomal STR markers could be successful in small bones of the hands (metacarpal bones and phalanges) and feet (metatarsal bones) of ancient skeletal remains in comparison to skeletal elements proposed in current recommendations (femur, temporal bone, tooth).

2. Materials and methods

We included 48 bone samples from 8 different skeletons (6 from 18th century and 2 from 3rd century) obtained from 5 archaeological sites in Slovenia. In each of the skeletons, 6 different skeletal elements were selected (temporal bone, tooth – one of the molars, femur, one of the metacarpal bones, one of the metatarsal bones and proximal phalanx of the hand). Buccal swabs on sterile cotton swabs were collected from the persons involved in the elimination databases for archaeological sites. The bone and tooth samples were cleaned, the surface layer was removed by grinding and then crushed into fine powder. Total demineralisation of 0.5 g bone/tooth powder was achieved using 10 ml of 0.5 M ethylene diamine tetra acetic acid (EDTA), pH 8.0 (Promega). The DNA was purified in a Biorobot EZ1 (Qiagen), DNA content determined with the PowerQuant kit (Promega), and autosomal STR typing performed with the Investigator ESSplex Plus kit (Qiagen). A non-parametric Friedman ANOVA test was used to check the aim of our study. We considered the significance limit $p = 0.05$. To determine the success of STR profiling samples were divided into 4 groups according to the number of successfully genotyped loci (A = 16 of out 16 loci, B = 11 to 15 loci, C = 8 to 10 loci and D = 0 to 7 loci) [8]. To determine the correlation between the quantity of extracted DNA and the success of the autosomal STR typing a non-parametric test, Spearman's rho, was used. The statistical analysis was carried out with the computer program SPSS Statistics for Windows, version 25.0 (Statistical Package for the Social Sciences Inc., Illinois, USA).

3. Results and discussion

Up to 8.75 ng DNA/g of powder was obtained from samples analyzed. The highest yields were detected in temporal bone and the lowest in femur. There were no statistically significant differences in the quantity of isolated DNA between the analysed skeletal elements ($p = 0.071$).

The success rate of STR typing was evaluated according to the number of successfully typed loci. In each of the 8 skeletons we obtained a full genetic profile (category A) or at least a partial genetic profile (category B and C) from at least 1 of the DNA extracts. Sixteen DNA extracts (33.3%) were not appropriate for genetic identification. From 11 out of 48 DNA extracts (22.9%) we obtained a full 16-locus STR profile (Fig. 1). In 6 out of 8 skeletons (skeletons 1, 3, 4, 5, 6, 7) we obtained at least one full profile (category A) from at least one DNA extract. From 16 DNA extracts (33.3%) we obtained a partial genetic profile containing from 15 to 11 STR loci. From 5 DNA extracts (10.4%) we obtained a partial genetic profile containing from 10 to 8 STR loci. There was a statistically significant difference in the typing performance between the different skeletal elements ($p = 0.008$).

A strong correlation between the success rate of STR typing and the amount of extracted DNA was confirmed (Spearman's rho ≥ 0.8). For all eight skeletons full consensus genetic profiles were determined from skeletal elements analyzed.

4. Conclusions

Our findings suggest it would be suitable to include metatarsal and metacarpal bones in sampling strategy for human identification although further research is needed to substantiate the findings of this study.

Declaration of Competing Interest

Authors declare that they have no conflict of interest.

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