Science and Justice 53 (2013) 253–260

Contents lists available at SciVerse ScienceDirect

Science and Justice

journal homepage: www.elsevier.com/locate/scijus

Difficulties of sex determination from forensic bone degraded DNA: A comparison of three methods

Danielle Quincey [a], Georges Carle [a], Véronique Alunni [b], Gérald Quatrehomme [b,*]

[a] UMR CNRS 6235, Université de Nice Sophia Antipolis, Faculté de Médecine, Avenue de Valombrose, 06107 Nice cedex 2, France
[b] Laboratoire de Médecine Légale et d'Anthropologie Médico-Légale, UMR CNRS 6235, Université de Nice Sophia Antipolis, Faculté de Médecine, Avenue de Valombrose, 06107 Nice cedex 2, France

ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form 25 February 2013
Accepted 3 April 2013

Keywords:
Forensic sciences
DNA
Identification
Post mortem DNA alteration
Bone
Anthropology

ABSTRACT

Sex determination is of paramount importance in forensic anthropology. Numerous anthropological methods have been described, including visual assessments and various measurements of bones. Nevertheless, whatever the method used, the percentage of correct classification of a single bone usually varies between 80% and 95%, due to significant intra- and inter-population variations, and sometimes variations coming from secular trends. DNA is increasingly used in a forensic context. But forensic DNA extraction from bone raises several issues, because the samples are very often badly altered and/or in very small quantity. Nuclear DNA is difficult to get from degraded samples, according to low copy number, at least in comparison with mitochondrial DNA. In a forensic context (as in a paeleoanthropological context) DNA sex determination is usually complicated by the weak amount of DNA, the degraded nature of nucleic acids, the presence of enzymatic inhibitors in DNA extracts, the possible faint amplification of Y band and the risk of contamination during either excavation or manipulation of samples.

The aim of this work was to compare three methods of DNA sex determination from bones: procedure #1 using a single PCR amplification, procedure #2 using a double PCR amplification, and procedure #3 adding bleaching for decontamination of the bone, instead of simply rubbing the bone. These processes were applied to samples of bones (49 samples coming from 39 individuals) that were in various states of *post mortem* alteration.

The main results are the following. (i) No DNA could be extracted from three skulls (parietal bones, mastoid process), the compact bone of one rib, and the diaphysis of one femur; (ii) there was a contamination in three skulls; and (iii) the Y band did not appear in two male cases, with one of the three procedures (male tibia, procedure #2) and with procedures #2 and #3 (male femur).

This study emphasises the main issue while working with altered bones: the impossibility to extract DNA in some cases, and, worth of all, the contamination of the sample or the faint amplification of Y band which leads to a wrong sex answer. Multiple and significant precautions have to be taken to avoid such difficulties.

1. Introduction

Sex determination is crucial in forensic anthropology, since it immediately sorts half the general population. Numerous anthropological methods have been described, including visual assessments and various measurements of bones. The main drawbacks of these methods are that the percentages of correct classification (in terms of sex) vary between 80% (the minimum that is usually said to be acceptable) and about 90% (until 95% in favourable cases), from a single bone. From the whole skeleton, it is said that the correct classification is a little better. Nevertheless the probability of correct sex assignment in an actual forensic case is probably close to 90%, whatever the methods that are used. This can be explained by the fact that the visual traits or the measurements show a significant intra- and inter-population variation, and also are altered with the expected modification of the skeletons (secular trends). As an example, it has been demonstrated that the average femur neck is larger in modern populations than in populations from the 19th century [1]. Thus it is of paramount importance to re-evaluate any anthropological method with modern bone collections. Nevertheless, even if one improves anthropological methods for sex determination, the percentage of correct classification will never been 100%, which is very disappointing within a forensic practice.

* Corresponding author. Tel.: +33 492034820; fax: +33 492034827.
E-mail address: gquatreh@unice.fr (G. Quatrehomme).

1355-0306/$ – see front matter
http://dx.doi.org/10.1016/j.scijus.2013.04.003

Therefore DNA sex determination is an alternative, at least when there is some doubt about sex assignation in an actual forensic case. But forensic DNA raises several issues, because the samples are very often badly altered and/or in very small quantity. When the body is decomposed or skeletonised, the only valuable matrix is bone (and sometimes tooth and hair when available) [2]. It is not always easy to extract DNA from bone, but this matrix has been routinely employed in forensic sciences, and also in palaeopathological cases (mummies, fossils). Nuclear DNA is difficult to get from degraded samples, according to low copy number, at least in comparison with mitochondrial DNA [3]. There are a lot of pitfalls, because sex assignment leads to a binary answer (male or female). In a palaeoanthropological context analysis of ancient DNA is complicated by the weak amount of DNA, the degraded nature of ancient nucleic acids, the presence of enzymatic inhibitors in DNA extracts, the possible faint amplification of Y band and the risk of contamination during either excavation or manipulation of samples [4]. In a forensic context, a wrong sex answer is a striking situation, and proceeds from the same causes.

Therefore the aim of this work was to compare three methods of DNA sex determination from bones: the first one using a single PCR amplification, the second one using a double PCR amplification, and the third one using bleaching for decontamination of the bone, instead of simply rubbing the bone. These processes were applied to samples of bones that were in various states of post mortem alteration.

2. Samples (Table 1)

49 bone samples coming from 39 individuals were studied. The sex was either known with certainty as male (M) or female (F), through a positive sex knowledge of the body (when people had given their body to the science) or through a positive ID by scientific comparison and the whole final result of the case (in forensic cases); or unknown but morphologically assessed as male (U/M) or female (U/F). In some cases sex was unknown, and the morphological features were ambiguous or there were only fragments of bones, so that it was impossible to assess the sex of the bone (U/U).

3. Methods

Three procedures (Table 2) were compared in this experiment

3.1. Bone samples

The preservation of the bone was assessed by visual peering, according to the absence or presence of weathering cracks or defects, and the porosity of the bone. More precisely, the scale of classification was as follows: (i) score 1: normal bone surface or minimal flaking ("good to excellent preservation"); (ii) score 2: significant flaking, some fissuration and fragmentation ("middle preservation"); and (iii) score 3: important flaking, fissuration, and fragmentation, along with significant porosity of the bone ("bad to poor preservation"). The alterations of each bone are scored in Table 1.

3.1.1. Bone samples for procedures #1, #2

A total of 43 samples were taken from 33 individuals. The samples came from 9 skulls, 3 tibias, 14 femurs, 1 humerus, 3 ribs, 1 innominate, and 1 vertebra (atlas). The samples came either from forensic bones (each time the identification of the remains was required), or bodies coming from the medical school (within a French law that permits people to "give one's body to the science", and allows for teaching and research). The area of sampling and the known or estimated span between death and sampling are described in Table 1. Sex was either perfectly known (M or F: 28 cases out of 43) or estimated by usual morphological assessment (U/M or U/F: 6 cases out of 43 samples). The 9 left cases represent the cases where sex is unknown, or undetermined between male or female (U/U) (Table 1).

3.1.2. Bone samples for procedure #3

11 femur samples were taken from 11 individuals, among which 5 coming from the samples utilised in procedures #1 and #2, and 6 coming from other cases (Table 1).

3.2. Preparation of bone samples (Table 2)

3.2.1. Procedures #1 and #2

The external surfaces of all samples were removed by mechanical abrasion using a drill to eliminate possible contamination from exogenous DNA; the internal middle zone was then drilled to fine powder before DNA extraction.

3.2.2. Procedure #3

The bone fragment was decontaminated by bleach cleaning (20% (v/v) solution of 2.5% (w/v) sodium hypochlorite for 1 h and overnight air-drying. Then the bones followed the preparation of procedures #1 and #2.

3.3. DNA extraction and purification for all samples (three procedures)

Nucleospin Tissue columns Kit (Macherey-Nagel, Düren, Germany) based on nucleic acid binding to silica membranes was used for DNA isolation. DNA was extracted from about 30 mg of bone powder following the manufacturer's recommendations, with slight modifications of pre-lysis and lysis buffer. 200 µl buffer T1 (SDS solution), 100 µl buffer (0.5M EDTA, 0.1M DTT) and 30 µl proteinase K solution (20 mg/ml) were added to the bone powder and incubated at 56 °C overnight. Then 300 µl of buffer B3 (guanidine hydrochloride solution) was added and incubated at 70° during 10 min.

After centrifugation, supernatant was loaded on the column. Then, the silica membrane was washed twice and the DNA elutions using 50 µl of BE were performed; aliquots were made and stored at −20 °C.

The supernatant was applied to the column. Then, silica membrane was washed twice and final elution steps with 50 µl of BE were performed; aliquots were made and stored at −20 °c.

The amount of DNA was estimated photometrically. The measurement was done at 260 nm.

3.4. PCR amplification of DNA remains after extraction

PCRs were performed using a PTC 100 MJ Research System and were carried in a 25 µl total reaction volume. The reaction mix was prepared according to the manufacturer's protocol (Invitrogen, Paisley, UK): 2.5 µl 10× PCR buffer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 5 µg of BSA (10 mg/ml), 0.4 µM of each primer and 1.5 UI of Platinum Taq polymerase.

3.4.1. Single PCR amplification for procedure #1

Single PCR reactions were performed using the following human gene-specific primers: *HPTR* (house keeping gene) (F5′-atgtaat ccagcaggtcagcaa; R5′-gctcgagatgtgatgaaggagat; GI: 184369), *SRY* (F5′-gcgacccatgaacgccatt; R5′-agtttcgcattctgggattctct; GI:292513), *amelogenin X* (F5′-tgcttcctgatctgactctgga; R5′-acttcctcccgcttggtctt; GI:15028582).

Product lengths were 98 pb, 80 pb and 60 pb, respectively.

Two and 5 µl templates of the DNA extract were tested for each sample.

Amplification cycles consisted of an initial denaturation step at 94 °C for 10 min, 40 cycles of 30 s at 95 °C, 30 s at 61 °C and 45 s at 72 °C followed by a final extension step of 10 min at 72 °C.