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Forensic DNA typing of human nails at various stages of decomposition

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Abstract. Forensic scientists often face the problem of extracting and typing human DNA from degraded materials such as muscle and bones from decomposed bodies. Bone samples are particularly difficult and time consuming to be analyzed and other body tissues suffer from rapid deterioration. Nails are a well-known source of DNA and their composition makes them less predisposed to decomposition compared to other soft tissues. With the aim of evaluating the usefulness of DNA extracted from aged human nails we analyzed nails taken either from exhumed and partially skeletonised bodies or from nail clippings stored at room temperature for 10–12 years. The adopted DNA extraction procedures yielded enough DNA for reliable PCR results even when no results were obtained either from soft or bone tissues. This study confirms the usefulness of nails as a source of DNA even in cases when PCR failed to amplify DNA extracted from bones.

Keywords: Exhumed body; Nail; Bone; STR

1. Introduction

Bones and teeth are less prone to fast decay compared to soft tissues [1]. However, the recovery of sufficient DNA for identification purposes is often frustrating, even after short time from inhumation [2,3] and it is often necessary to remove PCR inhibitors [4]. These problems may require other tissues to be considered for DNA extraction.

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Despite nails have proven to be suitable for analysis (e.g. [5–7]), their use as a source of DNA in the forensic field is rather uncommon. In this study we evaluated the usefulness of DNA extracted from aged human nails collected from partially skeletonised bodies and from nail clippings stored at room temperature for 10–12 years.

2. Materials and methods

Samples: 4 human bone samples buried for 6, 7, 17 and 48 years respectively (forensic paternity cases); 5 nail samples buried for 2, 7, 8, 10 and 15 years respectively (forensic paternity cases). Only in 1 case nails and bone came from the same individual (7-year-old sample). A medical genetics laboratory entrusted us with the DNA extraction from 10 nail clippings stored at room temperature for 10–12 years which represented the only remains of deceased patients. Prior to DNA extraction all the instruments used had been thoroughly cleaned into a 10% acetic acid solution. The outer diaphyseal surface of femur bones was discarded and the compact bone (5 g/bone) was scraped and pulverised into liquid nitrogen. The protocol suggested by Hochmeister et al. [8] was followed with minor modifications for 3 out of 4 bone samples with DNA concentration using commercial devices. DNA from the remaining bone sample, as well as for 2 bone samples for which the organic protocol gave no or poor PCR results, was extracted using commercial kits (QIAamp DNA Mini and/or Maxi kit, Qiagen, 5 g bone powder for each sample). Each nail clipping and approximately half of one nail from each exhumed body were minced using a sterile scalpel. The fragments were incubated in digestion buffer (200 mM sodium acetate, 0.5% SDS, 2 mg/ml Proteinase K, 100 mM DTT—final volumes) at 56 °C up to 2 days with repeated additions of fresh Proteinase K. DNA was always purified using the phenol–chloroform method, resuspended in 30–50 μl and amplified using commercial kits (AmpflSTR Profiler, AmpflSTR SGM Plus, Applied Biosystems; Powerplex16, Promega) in a final volume of 15 μl, 30 cycles. Fragments were resolved on an ABI 310 Genetic Analyzer with either automatic (Genotyper) or manual allele call. Statistical analyses with Italian-specific frequency data [9] were carried out in forensic cases by calculating paternity index (PI) and probability of paternity (W). Extraction and amplification negative controls were always carried out.

3. Results

For one bone only (17 years old) the phenol–chloroform extraction method yielded DNA for successful amplification, whilst this procedure gave good results for all the 15 nails examined. Commercial kits proved effective for bones aged 48 and 6 years old after failure of the organic procedure. No results were obtained for the bone sample aged 7 years old with either methods, whereas the correspondent nail sample gave good results. Allelic drop-out for HMW loci and/or peak imbalance was occasionally observed. Negative controls always gave negative results.

4. Discussion

In our routine we observed an increasing demand for paternity testing when the putative father is deceased, when the only option is the exhumation of the body. When soft tissues are badly decomposed, bones and teeth are usually considered the tissues of choice for extracting DNA. It is well known, however, that environmental condition may influence the degree of DNA decay within bones and teeth even fairly shortly after burial, thus leading to unsuccessful amplifications [2,3].

In this study we report the results of 8 forensic paternity cases on exhumed bodies, for 4 of which bone samples were analyzed. The phenol–chloroform method yielded too little amount of DNA in 3 out of these 4 cases. Commercial extraction kits were therefore used and proved to be effective in all but one bone sample (7-year-old sample). In this case DNA was extracted from nail samples which had been collected after exhumation. The derived STR profile matched the one of the living putative son at each of the 15 loci investigated (PI > 100,000; W > 99.999%). Even high molecular weight loci (e.g. D2S1338) gave good results.

In each of the following 4 cases investigated, DNA was extracted from nails only and the STR profiles matched those of the putative living sons/daughters, thus confirming the reliability of the method used. Penta D and PentaE alleles were also positively typed.

The extracted DNA from nail clippings stored at room temperature for 10–12 years gave good amplification results both using forensic kits and in the clinical setting which the DNA was extracted for (molecular diagnosis of genetic diseases).

This study thus confirms that nails are a suitable source of DNA, even after long time of inhumation. The DNA extraction procedure is less tedious and less time consuming than bone DNA extraction.

It is likely that the preservation of DNA in nails may be due to a positive microenvironment surrounding the hands after burial: in our tradition the hands of a deceased are cross-positioned onto the abdomen, above the corpse, with no or little contact with other putrefying tissues. We therefore suggest to collect fingernails in all cases when these tissues are available.

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