Duarte Nuno Vieira • Anthony Busuttil
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M. J. Pereira [1], **M. L. Pontes**[1], **M. F. Pinheiro**[1,2,3]

[1] *North Branch, National Medico Legal Institute, Oporto, Portugal*
[2] *Fernando Pessoa University, Oporto, Portugal*
[3] *Biomedical Sciences Institute "Abel Salazar", University of Oporto, Portugal*

THE INTEREST OF NAILS IN GENETIC IDENTIFICATION OF HUMAN DECOMPOSED CADAVERS

Abstract: The ability to recover DNA and STR data from bones and teeth exposed over time to a variety of environmental conditions, has become a valuable tool for individual identifications and/or kinship analysis. However, nails can be an advantageous alternative of these remain samples, since it is easier to perform the analysis process, without the need of powdering those mineralized elements. On the other hand, the high success rates for nuclear STR typing reported here, further confirmed that STRs could be considered a method of choice in casework involving skeletal remains. Nevertheless, when the DNA from the samples were degraded another approach to trying to recover information from them, is to reduce the size of the PCR products by moving primers as close as possible to the STR repeat region.

Introduction

A frequently encountered challenge in forensic casework is the analysis of highly degraded DNA that requires extraction from difficult material such as bones and teeth from long dead individuals (1). Forensic scientists are usually confronted with many problems, working with bones and teeth, such as insufficient quantity of DNA, high level of DNA degradation, and the presence of polymerase chain reaction (PCR) inhibitors. Therefore, careful optimization of all the stages of the procedures employed in the analysis of this type of samples is obligatory. The selection of appropriate procedure to promote the identification of human remains depends on the circumstances and the state of the examined material (2). Bone and teeth samples clearly protect DNA through their physical and/or chemical robustness to environmental degradation and/or biological attack. An elementary manifestation of this is that bone and teeth are often the only surviving material that can be tested (3). Nevertheless, shortly after death, blood and muscle can be easily collected from cadavers, as a DNA source for genetic identification. However, as previously stated, when the time elapsed between the death and the discovery of the body (*post mortem* interval) increases, the availability of these samples and the quality of the DNA decrease, which can hamper the establishment of a DNA profile. In such situations, DNA can be extracted from bones or teeth. Sampling is, in this case, invasive and DNA extraction from hard tissues will require

supplementary time consuming, for example, steps like powdering and decalcification of bones or fragmentation of teeth. Therefore, nails are very easy to collect and contain large amounts of good quality DNA that can be extracted within a few hours (4).

Short tandem repeat (STR) markers are the primary means used today for human identity and forensic DNA testing. However, with highly degraded DNA specimens a loss of signal is typically observed with larger sized STR products, either due to PCR inhibitors present in the forensic evidence or fragmented DNA molecules. Size reduction of STR markers, and thus improved success rates with degraded or inhibited DNA samples, may be accomplished by moving PCR primers in as close as possible to the STR repeat region (5).

The Forensic Genetic and Biology Service from the North Branch of the National Institute of Legal Medicine deals with different kinds of issues, such as kinship analysis, criminal cases and individual genetic identification, mainly human remains (Figure 1). A survey on genetic identification of human remains in the last four years (2005-2008), is referred to paternity testing (Table 1) or individual identification (Table 2) since the genetic analysis is required when the traditional methods failed. In the former situations, the human remains exhumation of the putative father (the more frequently absent in a trio) is required when close family members aren't available. Blood or muscle can be used as a DNA source for the genetic identification of recently deceased persons. However, if the *post-mortem* interval increases, bones and teeth are used, but in these cases, collection and DNA isolation is more difficult and time consuming. So, nails are an alternative genetic material source for the identification of decomposed cadavers, as referred before.

Material and Methods

In 26 human remains cases studied, 10 of them are related to paternity testing and 16 to genetic individual identification. The corpses, mainly those for individual identification, were found in different environments. In all the studied cases, bones (n= 25) are the most common sample sent to the laboratory. However, teeth and nails are also samples frequently collected. When nails (n= 17) were available, since it is easier to provide its DNA extraction without the need of previous treatment, they were cut with appropriate scissors. Before beginning the extraction procedure, all kinds of foreign substances were removed, and the nails were then washed with abundant quantity of sterile water at room temperature. Finally, to take away any exogenous or endogenous DNA, the nails were placed under a UV light during 30 minutes. DNA was extracted using a modified organic method followed by a Microcon® purification procedure. The DNA extraction from the reference samples was performed using the Chelex method. Autosomal STR profiles were obtained after amplification with the AmpFlSTR® Identifiler kit and the AmpFlSTR® MiniFiler™ kit. In the majority of cases we also used the AmpFlSTR® Y-filer™ kit to complement the information obtained from those STR loci, since the involved remains were from males (except three). The amplified products were detected and separated by capillary electrophoresis on an ABI PRISM® 3100 (Applied Biosystems). Fragment sizes were determined automatically using the Genescan® Analysis Software v 3.7 and allele designations using Genotyper® Software v. 3.7 (Applied Biosystems) typed by comparison with an allelic ladder.

Results

In twenty five of the total studied cases (n= 26) a full autosomal STR profile was obtained, in twenty four of them using the AmpFlSTR Identifiler® (Applied Biosystems). In one case, in which the sample was a femur from a deceased man buried 24 years ago (putative father), the DNA only performed results using the MiniFiler® (Applied Biosystems); in this situation the mitochondrial DNA analysis wasn't informative, because it was a paternity test in which a female descendant was involved. Only in a case where the analysis was made in two little bones found in a cemetery, sent to the laboratory without complementary information, including the *post-mortem* interval, no results were observed either with nuclear or mitochondrial analysis. The total of the results (n= 25) was obtained using bones, teeth and/or nails. Nails were the preferred sample used to make the study, although other type of sample was used, when available, to confirm the results (Table 2).

Discussion

In our routine casework we observed an increasing demand for paternity testing, when the putative father is deceased. Subsequently, the only option is the exhumation of the cadaver. We have also had deceased human bodies to establish the genetic identity, by comparing their genetic profile with personal items (direct comparison) or with relatives (indirect comparison).

When soft tissues were decomposed, bones and teeth were usually collected. However, DNA extraction from hard tissues requires supplementary time consuming, including steps like powdering. Previous studies showed that the most successful samples for STR testing were intact teeth and mid-shaft sections of femur. Bones that performed less well tend to be less dense and/or have a greater proportion of soft portions. For this reason, there was a laborious work to do before starting the DNA extraction process. Despite ease of collection and good resistance to decompose, nails are an advantageous sample used as a DNA source for cadaver's genetic identifications. In our case, when nails were available we used them, and the results obtained were similar to the ones found with other body sample, in accordance to this study.

STRs are highly polymorphic and capable of generating typing results from very little material through multiplex amplification, using the polymerase chain reaction (PCR). All the studied cases but one, were successfully concluded with the autosomal and Y STRs. However, with highly degraded DNA biological material, no results or allele dropout is typically observed with larger sized STR products, either due to PCR inhibitors present in the forensic evidence or fragmented DNA molecules. The AmpFlSTR® MiniFiler™ PCR Amplification Kit increases the ability to obtain DNA results from compromised samples that previously would have yielded limited or no genetic data. This situation occurred with a femur from a deceased man buried 24 years ago (putative father), that provided results with this kit when the analysis of conventional STRs failed.

Conclusion

Nails are very easy to collect and contain large amounts of good quality DNA that can be extracted within a few hours. So, they are an attractive genetic material source for identification of decomposed corpses, mainly because the starting process before the DNA extraction is easy and fast. Despite their utility in the identification of human remains, it is sometimes important to confirm the results with the study of other type of sample.

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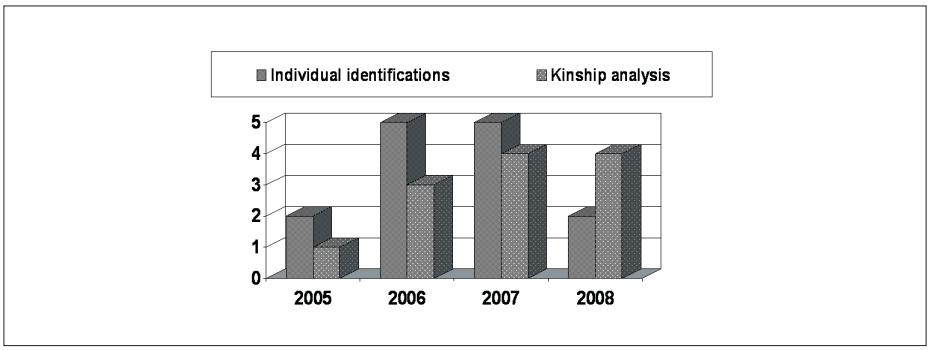

Figure 1 – Identification of human remains distribution

	Human Remains	Time of Burial	Collected Samples	Studied Samples
1	Putative Father	?	Teeth, bone, nails, muscle	Nails
2	Putative Father	+/- 4 years	Teeth, bone, nails, muscle	Nails
3	Daughter	+/- 6 months	Bone, nails	Nails
4	Putative Father	+/- 2 years	Teeth, bone, nails	Nails
5	Putative Father	+/- 25 years	Bone	Bone
6	Putative Father	+/- 5 years	Teeth, bone, nails	Nails
7	Putative Father	+/- 1 year	Teeth, bone, nails	Nails
8	Putative Father	+/- 3 years	Teeth, bone, nails	Teeth, bone, nails
9	Putative Father	+/- 1 year	Teeth, bone, nails	Nails
10	Putative Father	+/- 3 years	Teeth, bone, nails	Teeth, bone, nails

Table 1 – Paternity testing

	Place/ Reason of identification	Collected Samples	Studied Samples	Genetic Markers Results
1	Suicide (train)	bone, nails	Nails	Autosomal and Y STRs
2	Hill	Teeth, bone, nails	Nails	Autosomal and Y STRs
3	River	Bone, nails	Nails	Autosomal and Y STRs
4	Carbonized	Bone, teeth	Bone, teeth	Autosomal and Y STRs
5	River	Bone, teeth	Bone, teeth	Autosomal and Y STRs
6	Carbonized	Bone, teeth	Bone, teeth	Autosomal and Y STRs
7	?	Teeth, nails	Nails	Autosomal and Y STRs
8	House	Bone, teeth, nails	Nails	Autosomal STRs
9	House	Bone	Bone	Autosomal and Y STRs
10	Sea	Bone	Bone	Autosomal STRs
11	Possible exchange of identity	Bone, nails	Nails	Autosomal and Y STRs
12	House	Bone, teeth, nails	Teeth, bone, nails	Autosomal and Y STRs
13	Carbonized	Bone	Bone	Autosomal and Y STRs
14	Woods	Bone	Bone	Autosomal STRs
15	Cemetery	Bone	Bone	No Results
16	Hill	Nails	Nails	Autosomal and Y STRs

Table 2 – Individual genetic identification