Science & Justice 59 (2019) 214–216

Contents lists available at ScienceDirect

Science & Justice

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

Case review

Identification of an exhumed corpse by DNA extraction from bulb swab. A disputed parentage case report

Anna Rocchi[a,*], Silvano Presciuttini[b], Enrica Chiti[a], Simone Pierotti[a], Isabella Spinetti[a]

[a] University of Pisa Department of Medicine- section of Legal Medicine, Pisa, Italy
[b] University of Pisa Department of Medicine, Pisa, Italy

1. Introduction

Genotyping procedures from post-mortem remains, including those that had undergone putrefaction, are generally performed for human identification, after a mass disaster or in parentage disputes involving deceased people. The environmental conditions accelerate the decomposition of human remains and, in these situations, human hard tissues such as bones and teeth represent the most common DNA sources [1–4]. Some authors suggest to use femur shafts and teeth, since cortical bone and dental tissue with the calcium matrix, unlike spongy bone, provide protection to DNA from post mortem degradation, guaranteeing a good yield of adequate quality DNA and, consequently Short Tandem Repeat (STR) DNA profiles befitting for identification purposes [5]. Even if these tissues are considered the most suitable material for genetic identification, however, their processing and DNA extraction have some special requirements that make them time-consuming. In particular extraction from a bone sample involves several critical steps before DNA analysis [6]. First of all bone surgical removal invasiveness from the deceased and risks for staff when using saws during samples retrieve. Secondly, the procedure requires refrigerated storage for samples. Another step is sample decalcification that takes a few days to be performed [7]. Furthermore a combination of two extraction method (organic extraction and purification with silica beads) is often performed to increase quality and quantity of recovered DNA, involving the use of hazardous chemicals and a prolonged time of the procedure [8]. Finally multiple required steps increase the risk of errors or contamination.

For identification purposes, several studies use toenails as an alternative source of DNA from decomposed remains. As the bone, these tissues are quite resistant to environmental damage and to effects of decomposition but their collection is easier and non invasive and their processing requires less time [9,10]. Sometimes, however, DNA extraction from nails fails and it is necessary to use other tissues. In one of these cases, for getting over the impasse, authors have tried to obtain another easy-collectable sample from putrefied ocular bulb, comparing subsequently this tissue with other standard DNA source as concerns extraction and profiling.

2. Material and methods

We performed a paternity test between a man and an alleged father who was deceased 20 years ago. The forensic team reported a corificated corpse enclosed in a metallic coffin. Cadaveric material was collected from ocular bulb using 4N6FLOQSwabs™ COPAN and from other tissues: nails, muscle tissue (quadriceps femoris), patella and incisors. Prior to processing, all the samples were stored at −20 °C. DNA extraction was performed with a silica-based extraction protocol using QIAamp DNA Investigator Kit-QIAGEN, according exactly to manufacturer's recommended instructions. Ocular bulb swab collected from the orbital cavity was processed following QIAamp DNA Investigator handbook protocol “Isolation of total DNA from surface and buccal swabs”. All the samples were eluted in a final volume of 100 Ml of buffer ATE. Determination of total human DNA and human male DNA was carried out using Plexor® HY System kit (Promega Corporation). Reactions were performed in an IQ5 Multicolor Real-Time PCR Detection System (Bio-Rad) and analyzed using Plexor® HY software version 1.5.6.7 (Promega Corporation). 0.5 ng of DNA template from muscle tissue and ocular bulb swab were amplified by PCR with PowerPlex® Fusion and PowerPlex® ESX17 kit System (Promega Corporation) following manufacturer's instruction. Amplified products were separated by capillary electrophoresis with POP4 polymer and detected in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) according to the parameters indicated in PowerPlex® Fusion and PowerPlex® ESX17 manual protocol.

Data were analyzed with Genescan v. 3.7 and Genotyper v. 3.7 software. Allele designations were determined by comparison of the amplified DNA fragments with the allelic ladders supplied in the respective kits. Data Signal intensities exceeding 50 RFU were regarded as valid.

3. Results

Among the five collected samples, only the ocular bulb swab (OB) and the muscular tissue (MT) provided sufficient DNA for analysis (500 pg). We applied the same experimental protocols to both samples,

* Corresponding author.
E-mail address: anna.rocchi@neuroscienze.unipi.it (A. Rocchi).

https://doi.org/10.1016/j.scijus.2018.10.001
Received 6 April 2018; Received in revised form 4 October 2018; Accepted 8 October 2018

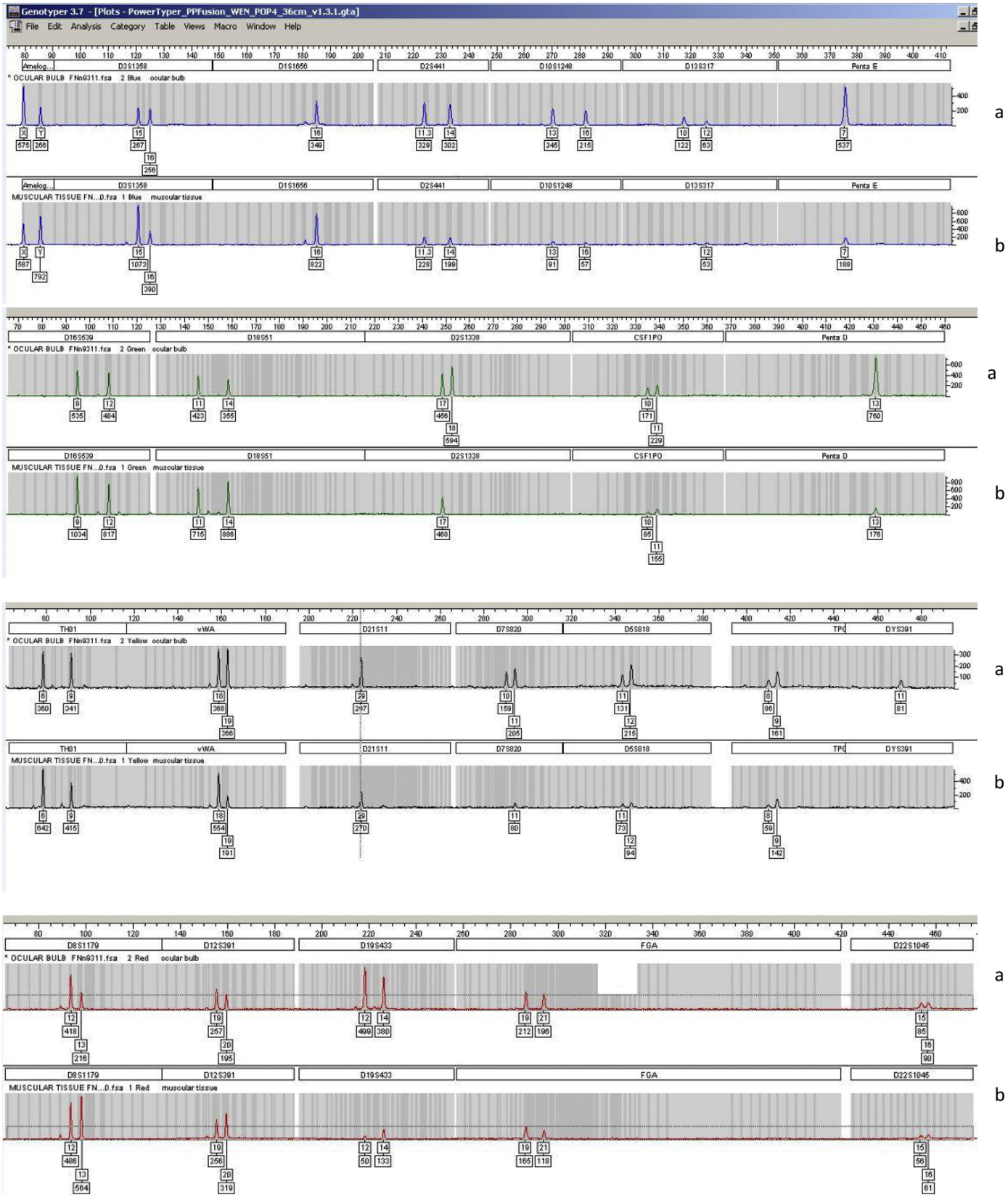

Fig. 1. Genotyper data from PowerPlex® ESX17 kit System. a) ocular bulb b) muscle tissue.

and obtained the electropherograms of 24 STR loci, 15 of which were duplicated (as they are common to the ESX and FUSION kits). Perhaps unexpectedly, the OB showed better results compared to MT. This was observed 1) at the level of mean peak height, 2) for the number of drop out alleles, and 3) for heterozygote peak unbalance (Fig. 1).

1) Among the 64 total allele peaks that were observed in both samples, 47 (73%) were considerably higher in the OB sample, and this

aspect was even more striking for the alleles with amplicons longer than 150 bp, for which the rate was 40/47 (85%). We considered the peak ratio of each allele in the two samples (OB/MT); the overall mean value, calculated as antilog of the log difference, was 1.4, which was significantly higher than 1.0 (single sample *t*-test: $P < 0.001$).

2) Even more importantly, the MT sample showed several allelic dropouts compared to OB, whereas the opposite did not occur. Among the 16 ESX loci, four showed dropouts (three of a single allele, one of both alleles), whereas among the 23 FUSION loci, four showed a single allele dropout.
3) For the unbalance of peak height in heterozygous loci, we considered the mean ratio of the higher/lower peak in the OB vs. the MT sample; these resulted to be 1.24 ± 0.32 and 1.78 ± 0.83, respectively ($P < 0.01$, two sample *t*-test assuming unequal variance).

4. Conclusions

The present work shows that the DNA retrieved from a corpse inhumated 20 years ago and preserved by spontaneous corification, was much better preserved in an ocular bulb sample than in a muscular tissue sample. While this single result cannot be considered of general validity, it suggests that the globe of the eye could represent a more protective environment with respect to other tissues. Other independent studies are necessary to confirm or disprove this appealing hypothesis.

References

[1] A. Alonso, et al., DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples, Croat. Med. J. 42 (2001) 260–266.
[2] S.M. Edson, et al., Naming the dead: confronting the realities of rapid identification of degraded skeletal remains, Forensic Sci. Rev. 16 (2004) 63–90.
[3] A. Alonso, et al., Challenges of DNA profiling in mass disaster investigations, Croat. Med. J. 46 (2005) 540–548.
[4] M. Prinz, et al., DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI), Forensic Sci. Int. Genet. 1 (2007) 3–12.
[5] A. Milos, et al., Success rates of nuclear short tandem repeat typing from different skeletal elements, Croat. Med. J. 48 (2007) 486–493.
[6] A.Z. Mundorff, et al., DNA preservation in skeletal elements from the World Trade Center disaster: recommendations for mass fatality management, J. Forensic Sci. 54 (2009) 739–745.
[7] J.E. Stray, J.G. Shewale, Extraction of DNA from human remains, Forensic Sci. Rev. 22 (2010) 177–185.
[8] S. Desmyter, et al., Organic extraction of bone lysates improves DNA purification with silica beads, Forensic Sci. Int. 273 (2017) 96–101.
[9] M. Allouche, et al., Genetic identification of decomposed cadavers using nails as DNA source, Forensic Sci. Int. Genet. 3 (2008) 46–49.
[10] A. Schlenker, et al., Toenails as an alternative source material for the extraction of DNA from decomposed human remains, Forensic Sci. Int. 258 (2016) 1–10.