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SHORT COMMUNICATION

Genetic identification of highly putrefied bodies using DNA from soft tissues

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Abstract The identification of putrefied bodies is a common task in forensic routine work. The deceased are usually identified by dental records, fingerprinting, or—in cases were no such data are available—DNA analysis. However, with progressive putrefaction, DNA integrity is rapidly decreasing. Genetic analysis may then be greatly impaired, if not impossible. The aim of our study was to establish an efficient procedure to successfully extract and amplify DNA from soft tissues of bodies in different stages of putrefaction. Soft tissues—unlike teeth or bones—usually allow the application of fast and easy-to-use extraction protocols. DNA was extracted from different tissues (aorta, kidney, liver, and skeletal muscle) taken at autopsy using a commercially available DNA extraction kit, and DNA quality and quantity were controlled by agarose gel electrophoresis and real-time polymerase chain reaction (PCR). Presence of mitochondrial DNA was tested using a highly sensitive duplex PCR. Short tandem repeat analysis was done using the AmpF*l*STR Identifiler kit. Additionally, mitochondrial DNA sequencing was performed. After DNA extraction from at least two different tissues—preferably the kidney and the aorta—with the extraction kit based on the Nucleobond method, a successful amplification of at least eight loci was possible in 17 out of 18 cases, and 12 or more loci could be amplified in 15 cases.

Keywords STR typing · DNA degradation · Forensic · Soft tissue · Genetic analysis

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Introduction

The identification of human bodies is usually carried out by comparison of ante- and postmortem data, such as specific external features like scars, tattoos, and fingerprints, medical implants or old injuries, and dental examination, and by means of genetic analysis [1–3]. However, there are circumstances under which most of these methods fail or cannot be applied at all, e.g., in mass disasters with extreme conditions regarding temperature development and physical impacts, such as the collapse of the World Trade Center in New York City or the 2004 Tsunami [3–5], or simply a lack of antemortem data from persons without any prior dental or medical treatment [6]. In these cases, genetic analysis may be the only alternative left. Individualization can be achieved by short tandem repeat (STR) typing of the remains and genetic linking of the STR profile to a member of the suspected persons natural family (e.g., "paternity testing"). Under certain circumstances, for example bodies found in their own house or apartment, a comparison with genetic profiles obtained from clinical specimens or articles of daily use that can be clearly linked to the deceased [1] may be sufficient.

Identification of putrefied bodies is a common task in forensic medicine. With advancing putrefaction, however, DNA integrity is rapidly decreasing, and genetic typing might then be greatly impaired. Since DNA stability is generally higher in hard tissues, bones or teeth are frequently used as DNA source in such cases [7, 8]. However, isolation of DNA from hard tissues is much more time-consuming and labor-intensive than DNA extraction from soft tissues. This can be especially important in (forensic) cases where time is short and identification has to be carried out as fast as possible.

The aim of this study was to establish an efficient protocol for fast and easy extraction and amplification of

DNA from soft tissues of bodies in different stages of putrefaction within one working day. The experiments were carried out on routine identification cases, some of which were time critical and required a fast and reliable individualization.

Material and methods

Sample collection and DNA isolation

Five hundred milligrams of each tissue (aorta, skeletal muscle, kidney, and liver) was collected during medicolegal autopsy from 18 bodies in different stages of putrefaction (see Table 1 for further information). Samples were immediately subjected to DNA extraction (50 mg each) using the Invisorb Spin Tissue Mini Kit. If an immediate extraction was not feasible, tissues were stored at −20°C until processing. Samples were incubated at 52°C overnight and vortexed in an Invisorb Gyrator to intensify the lysis process (both Invitek, Germany). The elution volume was 55 µl. DNA for positive controls was isolated from blood drawn from volunteers after informed consent using the Nucleo Spin Blood Quick Pure kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions with a total elution volume of 50 µl. Every extraction was done twice independently.

Testing of DNA quality and quantity

Five microliters of DNA solution per sample was separated on ethidium bromide containing 1% agarose gels. Quantification of high molecular DNA was carried out using a gel imaging system (Geldoc™ EQ, Biorad) and the Quantity One 1D analysis software by comparison with DNA ladders containing fragments of known size and molecular weight (Hyperladder, Bioline, Germany).

To detect smaller DNA fragments, a real-time polymerase chain reaction (PCR) was carried out on an iCycler iQ™ Real-Time PCR Detection System (Bio-Rad) as described in von Wurmb-Schwark et al. [9]. The presence of primer dimers was controlled by a subsequent melting curve analysis.

All samples were first subjected to a duplex PCR which amplifies a 164-bp fragment specific for nuclear DNA and a 260-bp mitochondrial DNA-specific fragment [10] in order to screen for the presence of longer human nuclear and mitochondrial DNA fragments and to test the amplifiability of extracted DNA.

Table 1 Overview of individuals investigated in this study and respective grade of decomposition and number of STRs successfully amplified per tissue

	Sex	Age	Postmortem interval	Grade of decomposition	Successfully typed STR loci using the Identifiler kit			
					Aorta	Skeletal muscle	Kidney	Liver
1	Male	55	10 days	Decomposition with discoloration and marbling	15	8	15	n.i.
2	Female	Newborn	Several days	Decomposition with discoloration	15	15	15	15
3	Male	61	2 weeks	Advanced putrefaction	5	n.i.	15	n.i.
4	Male	57	Several days		15	15	15	15
5	Male	57	10 days		5	1	2	1
6	Female	Newborn	3 weeks		15	9	15	15
7	Female	71	4 weeks	Advanced putrefaction with maggots	15	8	8	n.i.
8	Male	62	2 weeks		15	3	15	n.i.
9	Male	40	2 weeks		10	7	6	8
10	Male	36	1 week		13	14	14	14
11	Male	66	1 week		15	15	15	15
12	Male	50	3–4 weeks		3	1	0	0
13	Male	58	2 weeks	Advanced putrefaction with maggots, partly skeletonized	11	11	11	11
14	Male	70	2 weeks		15	13	15	13
15	Male	44	7 weeks	Mummified	15	7	15	12
16	Male	54	2 weeks		13	13	12	11
17	Female	39	6.5 months	Adipocere	14	11	13	6
18	Male	41	Unknown	Advanced putrefaction, adipocere, partly skeletonized	8	2	15	2

n.i. not investigated

Genetic analysis of isolated DNA and fragment analysis

One microliter of each sample was employed to multiplex PCRs using the AmpF*l*STR Identifiler™ (Applied Biosystems, Weiterstadt, Germany) according to the manufacturer's recommendations. For fragment analysis, 0.2 µl per sample of the required size marker was used (for Identifiler PCR LIZ500, and for duplex PCR ROX500; both Applied Biosystems). Electrophoresis was performed on an ABIPrism 310. Allele assignment was performed by comparison with commercially available ladders and determination of fragment sizes using the 310 GeneScan 3.1.2 software. Peaks below 50 relative fluorescence units (rfu) were not analyzed in this study. Additionally, 439- and 280-bp fragments of the mitochondrial HVI region were amplified according to Pfeiffer et al. [11]. Sequencing analysis was done on an ABIPrism 310 using a short sequencing program.

Results and discussion

DNA yield from different tissues

DNA quality and quantity of extracted DNA were very different from individual to individual and—as expected—got worse with advancing decomposition of the bodies. Agarose gel electrophoresis showed highly degraded DNA and, in some cases, no DNA-specific fluorescence at all. As determined by real-time PCR, DNA concentrations ranged from 0.01 to 75 ng/µl elution volume. Skeletal muscle yielded the lowest amounts of total DNA with an average of 2.2 ng/µl, while the average amounts of DNA extracted from the aorta, the kidney, and the liver did not differ considerably (8.2, 10.0, and 8.3 ng/µl, respectively). The commercially available DNA extraction kit yielded very pure DNA even from highly degraded tissues. Cell lysis was achieved in 3 h, making the protocol the method of choice in cases where a rapid identification is necessary. In addition, the kit is easy to handle, fail-safe, and less toxic than the frequently preferred and very time-consuming phenol–chloroform-based methods [12, 13] that often show considerably worse genetic typing results [14].

Detection of nuclear and mitochondrial DNA to test DNA quality

Duplex PCR results show a strong correlation with the grade of DNA degradation. The use of the duplex PCR as a pretest prior to STR typing or mitochondrial DNA sequencing may help to save expensive chemistries in cases where PCR failure is likely, as we have shown earlier for other specimens [10]. Samples that did not yield mitochondrial specific fragments in the duplex PCR turned out to be also negative for the 280-bp mitochondrial HVI-specific fragment usable for D-loop sequence analysis. Mitochondrial DNA (mtDNA) sequencing was successful, when the peak height of the mitochondrial fragment in the duplex PCR was over 500 rfu. Correspondingly, in samples in which the amplification of the nuclear specific 164-bp fragment failed, only a few STRs—loci with short fragments, such as D8S1179 or D3S1358, or the gender-determining amelogenin locus—or no STRs at all could be detected.

Genetic analysis using the AmpF*l*STR Identifiler kit

In 17 out of the 18 cases investigated, a minimum of eight STR loci could be successfully typed, and identity of the deceased could be established with a sufficient probability by comparison with profiles gained from personal belongings. A minimum of 12 loci necessary for kinship analysis (according to German guidelines) was gained in 15 cases (see Table 1 for more information; case 13: combined profiles from the aorta and the kidney). DNA extracted from the aorta and the kidney yielded the best results, with an average number of 13 and 12 successfully amplified loci in the two tissues. Typing results for the liver and skeletal muscle were considerably worse, with an average of nine successfully typed loci, respectively (Fig. 1). Typing results were called to be correct and successful, when the alleles could be detected at least three times independently and the corresponding peaks were higher than 50 rfu.

Aortic tissue was shown recently to be especially suited for DNA analysis and called to be one of the most useful samples for forensic identification [15, 16]. Our study supports these results and additionally presents the kidney as also a very usable tissue for genetic analysis.

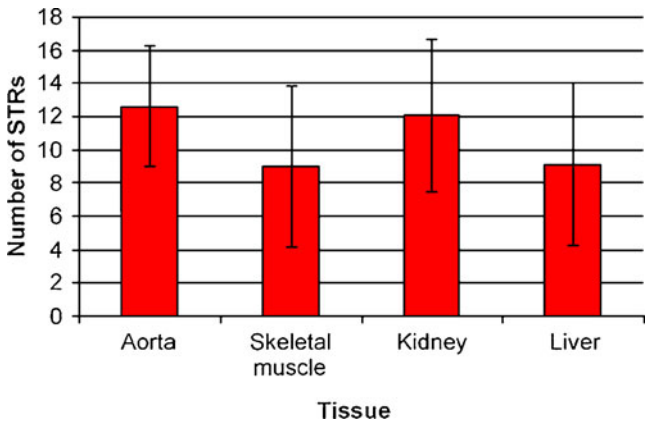

Fig. 1 STR typing from different tissues. Schematic presentation after amplification of 1 µl per DNA solution in the AmpF*l*STR Identifiler™ kit (Applied Biosystems) and capillary electrophoresis on an ABI310 Genetic Analyzer. Shown are the *average numbers* of successfully typed loci and the corresponding standard deviations

As expected, typing results depended strongly on the stage of putrefaction, with corpses showing conserving putrefaction (mummification and adipocere) giving better results than those with sleazy and soggy decomposition.

Amplification of mtDNA for sequencing analysis

For routine D-loop sequencing, a 439-bp fragment of the mitochondrial HVI region was amplified. Sufficient copy numbers for subsequent sequencing analysis of the fragment could be generated from all but two samples. In those cases, a smaller, 280-bp HVI fragment was amplified for sequencing purpose. DNA extracted from the liver tissue gave the best sequencing results, probably due to the high number of mitochondrial genomes in hepatocytes. DNA extracted from the aorta, on the contrary, showed continuously the lowest signal intensities for the HVI fragment, even though STR amplification was successful in most cases.

Conclusions

In summary, putrefied human bodies can, in many cases, be identified by STR analysis using DNA extracted from soft tissues. The protocol presented in this study (DNA extraction out of the kidney and the aorta using a commercially available kit with subsequent STR analysis) will frequently yield sufficient results for identification and should be preferred over DNA extraction from other soft tissues, such as skeletal muscle. Typing results might be improved further by use of kits amplifying shorter fragments (mini-STRs) [17]. In cases that do not allow sufficient STR analysis due to highly advanced putrefaction, mtDNA sequencing or single-nucleotide polymorphism analysis may afford further individualization. DNA extraction from bones or teeth will thus be necessary only as an ultima ratio or in cases where no soft tissues are left.

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