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Identification of scattered skeletal remains

Combined dental and DNA-based identification

Introduction

In the course of history, dental identification has been commonly used since Roman times [1]. Compared to other identification means, dental identification is relatively less time and money consuming [2] and characterized by its simplicity [3], high efficiency and procedural rapidity [4]; however, several challenges and obstacles can be encountered during dental identification, such as the possibility of charting mistakes in antemortem records [5, 6], the low quality of antemortem records, for instance, the lack of dental X-rays, unintelligible abbreviations on dental charts and confined documentation on the areas of treatment disregarding the material used, treated surfaces and other existing conditions [2], identification of children, adolescents or edentulous individuals [4, 7], difficulty to conduct quantitative interpretations presented to the court [8], adverse post-mortem conditions, such as fragmented or commingled corpses in situations after explosions or airplane crashes [9], considerable number of missing teeth post-mortem, severe antemortem bimaxillary trauma [4] or post-mortem animal scavenging negatively influencing dental evidence and scattered skeletal fragments resulting in laborious matching between different components. Therefore, a combination of DNA analysis and conventional dental identification is required to confirm the identity under special conditions, such as identifying disarticulated scattered human skeletal elements and to exclude false conclusions.

Due to the fact that DNA fingerprinting can be generated from any nucleated biological sample [10, 11], DNA methods can effectively be used to re-associate scattered or fragmented elements while other techniques cannot; however, DNA degradation still represents the main limitation. DNA degradation causing a downgrading in quality and quantity of DNA under adverse post-mortem conditions makes bones and teeth samples almost the best biological material available for DNA profiling [12] owing to the protection afforded by their mineral matrix [13, 14].

Case report

In December 2016 a corpse of an unidentified man was found in a small forest

Fig. 1 ▲ Retrieved mandible from different views. **a** The lower jaw at the location of discovery. **b** Frontal view. **c** Lateral right view. **d** Lateral left view

Fig. 2 ◂ Scattered human remains at the scene of discovery

Fig. 3 ▴ The reconstruction of the skeletal remains for anthropological analysis

in the north of Germany. A mandible (▫ **Fig. 1**) from human remains was incidentally discovered by a dog accompanying a passer-by. Near to location of discovery incomplete scattered human bones (without a skull), remains of shoes and clothing but no ID card or other personal belongings were found by the local German Criminal Police in Cuxhaven during the crime scene search (▫ **Fig. 2**). The preliminary police investigations were oriented to the assumption that the missing person was a male who had been missing since August 2016 from his dormitory. The presumptive missing man was 59 years old. All human remains were sent to the department of Legal Medicine for purpose of identification and routine autopsy procedures (▫ **Fig. 3**).

Material and methods

The anthropological parameters of the pelvis, the long bones and the lower jaw were compatible with a male person. To establish the identity, dental identification and DNA profiling were undertaken.

Dental identification. Forensic odontological examination by visually detecting and describing each tooth of the mandible, taking photographs, and applying radiological methods of panoramic radiography has been utilized to match the mandible to the data of the presumed missing person. Antemortem dental records comprising 10 intraoral x-rays (5 for lower teeth and 5 for upper teeth) were retrieved during the criminal police investigations to be compared with the post-mortem panoramic radiograph (▫ **Fig. 4**).

DNA testing. Prior to DNA extraction, to eliminate environmental contaminants and exogenous DNA [15], the external surface of bone samples (from femur) were mechanically removed. The teeth (36, 45 and 46) were extracted and air-dried. Being preserved in their alveolar sockets, dental samples did not require treatment with bleach or root surface removal to avoid any negative impact of cementum on DNA [16]. Thereafter, the teeth were horizontally sectioned and the crowns were removed. Only the roots were used as a source of DNA, since the roots of teeth yield better DNA than the crowns [9, 17, 18].

Bone and teeth samples were ground into powder and 100 mg of each sample was used for extraction according to the manufacturer's instructions of the Crime Prep Adem-Kit (Ademtech SA, Pessac, France) and 50 µl of the final eluate obtained was used for analysis. For DNA quantification for both teeth and bone samples, real-time PCR (PowerQuant™ System, Promega, Madison WI, USA) using a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) was applied. After multiplex PCR amplification, capillary electrophoresis was performed using an ABI 3130 Genetic Analyser (Applied Biosystems). For short-tandem repeat (STR) DNA analysis multiplex commercial kits (Powerplex® ESI 17, Powerplex® ESX 17, Promega) were used. The ABI GeneMapper™ ID v3.2. software was utilized for data analysis and visualization.

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Identification of scattered skeletal remains. Combined dental and DNA-based identification

Abstract

The resistant nature of bones and teeth to environmental insults highlights their importance in identification investigations. The DNA preserved in bones and teeth can play a crucial complementary role to comparative dental identification in some forensic scenarios. We report on a case where an isolated mandible and scattered skeletal remains without a skull, were found in a small forest after a post-mortem interval of approximately 4 months. This case illustrates a situation in which two reliable identification modalities, dental identification and DNA profiling, were necessary to reach the confidence level of personal identification and to exclude any false conclusions. Dental identification was established by sufficient concordant dental features in the lower jaw. A comparison of DNA profiles generated from teeth and bone samples of the human remains with DNA profiles generated from a toothbrush and beard hairs as reference samples showed matching profiles. This emphasizes the effectiveness of combining DNA and dental identification for assigning scattered skeletal fragments and identifying human remains. To economize efforts dental comparison, if available, should be performed as a first step prior to DNA genotyping.

Keywords

Short tandem repeats · DNA profiling · Odontological identification · Forensic dentistry · Forensic anthropology

Identifizierung eines verstreuten Skeletts. Kombinierte dentale und DNA-basierte Identifikation

Zusammenfassung

Die Widerstandsfähigkeit von Knochen und Zähnen gegenüber zersetzenden Umwelteinflüssen unterstreicht ihre Bedeutung in Identifizierungsuntersuchungen. Die in Knochen und Zähnen erhaltene DNA kann in manchen forensischen Situationen eine wesentliche Ergänzung zur vergleichenden dentalen Identifizierung darstellen. Im hier vorgestellten Fall wurden ungefähr 4 Monate post mortem ein einzelner Kiefer und verstreute Skelettreste ohne Schädel in einem kleinen Waldstück gefunden. Mit der dentalen Identifizierung und dem DNA-Profiling waren zwei verlässliche Identifizierungsverfahren erforderlich, um die Person mit hinreichender Sicherheit zu identifizieren und falsche Rückschlüsse auszuschließen. Die dentale Identifizierung wurde durch die ausreichende Übereinstimmung dentaler Merkmale des Unterkiefers erzielt. DNA-Profile aus Zahn- und Knochenproben der menschlichen Überreste stimmten mit den DNA-Profilen einer Zahnbürste und von Barthaaren überein. Der Fall verdeutlicht, wie effektiv die Kombination der DNA-basierten und dentalen Identifizierung bei der Zuordnung verstreuter Skelettfragmente und bei der Identifizierung menschlicher Überreste ist. Um den Aufwand zu reduzieren, sollte der dentale Vergleich, soweit durchführbar, in einem ersten Schritt vor der DNA-Genotypisierung erfolgen.

Schlüsselwörter

Mikrosatelliten · DNA-Profiling · Odontologische Identifizierung · Forensische Zahnmedizin · Forensische Anthropologie

A set of 17 markers (16 autosomal markers as well as the amelogenin marker) were used for matching DNA profiles generated from a toothbrush and beard hairs of the potential missing person. The latter profiles were generated by the police laboratory and were sent to be compared with teeth and bones DNA profiles. Statistefix software version 2.3 was applied for statistical calculations.

Results

Comparative dental identification

As shown in **Table 1**, positive identification could be confirmed as a result of sufficient matching similarities and no unexplainable discrepancies discerned between antemortem and postmortem dental records [19]. Further features could also be included and the high individual discrimination potential of dental details is represented in the morphological characteristics of the dental treatment of tooth 37. This revealed uniqueness in position, design, shape, extension and dimensions.

According to Keiser-Nielsen [20] regardless of the frequency of occurrence in several age groups, race, and sex, the combinations representing the simultaneous occurrence of 7 intact teeth, 3 crowns, 2 missing teeth, 1 filling, 1 root filling and 1 post within one and the same person in the mandible during life can be obtained by multiplying the possible combinations of each dental feature C (n, k), where n represents the maximum and k represents the variable:

$$C\ (16{,}7) \times C\ (9{,}3) \times C\ (6{,}2) \times C\ (4{,}1) \times C\ (3{,}1) \times C\ (2{,}1)$$
$$= 11{,}440 \times 84 \times 15 \times 4 \times 3 \times 2$$
$$= 345{,}945{,}600$$

This value of the random match probability in an unidentified population, without consideration of dental status related to certain populations, generations and age can be enormously increased if tooth surfaces, materials of the filling, materials of the crown and a root of a tooth presenting with a post are included.

This leads to the conclusion that the lower jaw matched to the missing person; however, the assignment of both the jaw and the bones to the missing person could only be verified by DNA analyses which is considered the best tool to match different body parts.

DNA findings

Reference samples of the missing person including a toothbrush and beard hairs were obtained from his bathroom. The genetic profiles of the aforementioned samples generated in the police laboratory could be directly compared with the DNA profiles of the bones and teeth. The genotype comparison revealed a complete match of 17 markers (including the amelogenin sex-determining marker) out of 17 between the beard hairs and the bone samples as shown in **Table 2**. The toothbrush had a mixed sample of at least two persons, one of which was matched

Table 1 Comparative dental examination of the antemortem and post-mortem dental charts

Antemortem dental chart in July 2012		Code DVI system	Post-mortem dental chart in December 2016		Code DVI system	Comparison	Concordant dental features
31	Intact	nad	31	Intact	nad	Similarity	1
32	Intact	nad	32	Intact	nad	Similarity	1
33	Intact	nad	33	Intact	nad	Similarity	1
34	Amalgam filling	mcf	34	Amalgam filling	mcf	Similarity	1
35	Intact	nad	35	Missing post-mortem	mpm	Explainable discrepancy	–
36	Crown	mtc	36	Crown	mtc	Similarity	1
37	Root filling, metallic post, crown	rfx, pox, mtc	37	Root filling, metallic post, crown	rfx, pox, mtc	Similarity	3
38	Extracted[a]	mam	38	Missing ante-mortem	mam	Similarity	1
41	Intact	nad	41	Intact	nad	Similarity	1
42	Intact	nad	42	Intact	nad	Similarity	1
43	Intact	nad	43	Intact	nad	Similarity	1
44	Intact	nad	44	Intact	nad	Similarity	1
45	No information	non	45	Amalgam filling	mcf	–	–
46	Crown	mtc	46	Crown	mtc	Similarity	1
47	No information (according to x-ray no root filling)	non	47	Root filling, retained root	rfx, rov	Explainable discrepancy	–
48	Extracted[a]	mam	48	Missing ante-mortem	mam	Similarity	1
Concordant dental features in the lower jaw							15

DVI Disaster victim identification, *nad* no abnormality detected, *mcf* metal coloured filling, *mtc* metal crown, *rfx* root filling, *pox* post; *mam* missing antemortem, *non* no information, *mpm* missing post-mortem, *rov* retained root
[a]According to the dentist's handwritten notes in antemortem records

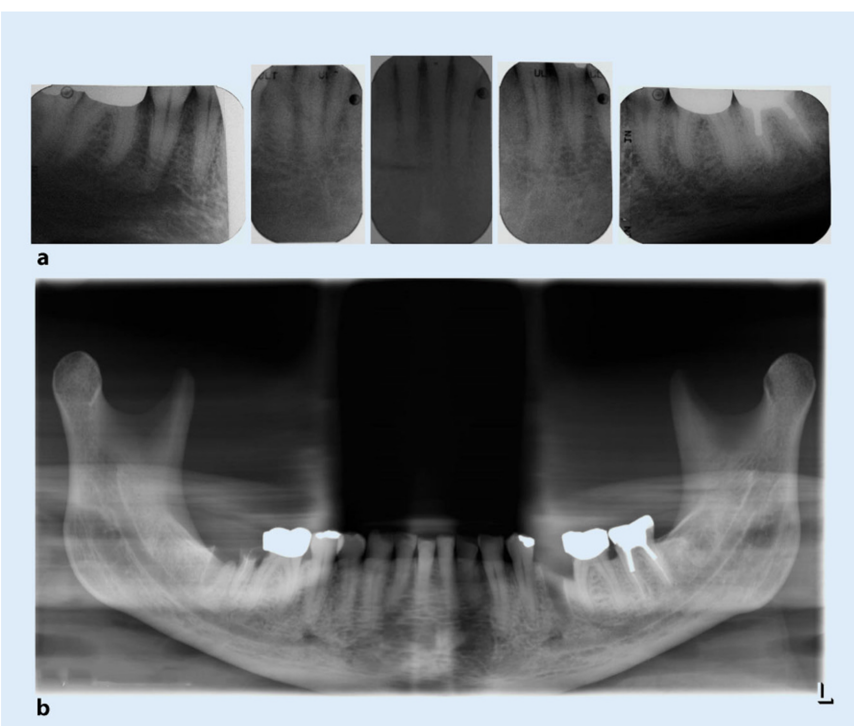

Fig. 4 ▲ **a** Antemortem intraoral x-rays from July 2012. **b** Post-mortem panoramic radiograph performed in December 2016

to the bone sample as well. Compared with the DNA profile obtained from dental samples 14 markers out of 17 were matched, while one allele in locus SE33 could not be detected and for FGA and D21S11 one additional allele could be detected.

According to the guidelines of German Committee for Genetic Diagnostics [21] the DNA results conclusively confirmed that the recovered mandible and the scattered human skeleton bones originated from the same person (**Table 2**).

Discussion

To ensure these bones were from a single person, it was verified if every bone of the human skeleton was represented only once. Furthermore, several correspondences in the biological profiles could be observed in the recovered skeletal remains. The harmony of left and right sets of remains (**Fig. 3**), the consistency of the observed degenerative age-related morphological changes, the consensus of morphological sex parameters

Table 2 Short tandem repeat genotyping and statistical analysis using Statistefix software

Loci	Missing person's toothbrush	Missing person's beard hairs	Unknown corpse bone samples	Unknown corpse teeth samples
Amelogenin	XY	XY	XY	XY
D19S433	13/14/*15*	13/14	13/14	13/14
D2S1338	18/*23*/*24*/25	18/25	18/25	18/25
D16S539	*9*/11/12	11/12	11/12	11/12
D22S1045	15/16	15/16	15/16	15/16
D12S391	*18*/19/21	19/21	19/21	19/21
D10S1248	13/14	13/14	13/14	13/14
D2S441	14	10/14	10/14	10/14
D1S1656	12/*13*/14/*16*	12/14	12/14	12/14
D18S51	11/*13*/15/*16*	11/15	11/15	11/15
D8S1179	*10*/13/14	13/14	13/14	13/14
D3S1358	15/16	15/16	15/16	15/16
FGA	*20*/*21*/21.2/22.2	21.2/22.2	21.2/22.2	(21/21.2)/22.2
TH01	6/*7*/*9*/9.3	6/9.3	6/9.3	6/9.3
VWA	15/16/*17*/*19*	15/16	15/16	15/16
D21S11	*27*/28/*29*/30	28/30	28/30	(28/29)/30
SE33	*19*/29.2	17/29.2	17/29.2	17/–
Random match probability			6.893×10^{-24}	
Likelihood ratios (1)	The probability that beard hairs (reference sample) and bone samples are from the same person (hypothesis H1)/the probability that someone other than the provider of the beard hairs (reference sample) is the unknown dead person (H2)			1.43204×10^{23}
Likelihood ratios (2)	The probability that beard hairs and teeth samples (H1) are from the same person/the probability that someone other than the provider of the beard hairs is the unknown dead person (H2)			3.84377×10^{16}
Likelihood ratios (3)	The probability that one of the persons contributing to the mixed sample of the toothbrush (reference sample) and the one providing the bone sample is the same person (H1)/the probability that someone other than the persons contributing to the mixed sample of the toothbrush (reference sample) are the unknown dead person (H2)			7.28983×10^{11}

in the bones and the consistent length of long bones indicated that the bones likely belonged to the same individual [22, 23].

Despite the missing maxilla, it was certainly prudent to begin with dental identification since antemortem records were available. Determining a certain number of concordant features for dental identification is a controversial issue. Pretty and Sweet [24] stated that positive dental identification can be established without fulfilling a minimum number of concordant dental features. Whereas Keiser-Nielsen [20] mentioned that 12 concordant features, even being uncharacteristic, are the minimum requirement to conclude a proof of identity. Moreover, Adams [25] emphasized the high individuality of the combinations of dental features. The distinct prevalence of dental caries or injuries in different teeth should be considered. Modesti et al. [26] utilized the statistical information about the dental status of the Brazilian population in calculating the frequency of forensic dental features. In our case 15 consistent dental features were identified and no unexplainable discrepancies could be discerned. This confirmed the identity of the lower jaw.

Despite the positive comparative dental identification, other skeletal elements, which might belong to another person, led to keeping the case still open to question. Therefore, DNA genotyping was required. Personal effects such as a toothbrush [27–29] or hair comb [29] are commonly considered a potential source for recovering reference samples. Birngruber et al. [30] found in their retrospective study that nearly 66% of antemortem DNA samples included personal belongings (e. g. hairbrush, toothbrush and razor) for comparative DNA identification; however, using personal belongings as sources of antemortem DNA samples is currently under discussion [31]. Schwark et al. [32] concluded that personal hygiene items should be restricted in use for DNA identification to cases of no known blood relatives as they can complicate DNA investigations [32] and are a potential risk for a cross-contamination or false profiles [33–35]. In the presented case no known biological relatives were available. Beard hairs found in the bath of the dormitory where the missing person lived, yielded an uncontaminated full DNA profile. A complete match between the missing person's beard hairs and the bone samples from the corpse could be confirmed.

Regarding the DNA profile generated from dental samples, additional alleles were found in the FGA and D21S11 loci. Those alleles are most likely artifacts due to degradation and do not represent a triallelic patter [36–38] as the markers are not present in the non-degraded samples. On the other hand, the DNA profile generated from the missing person's toothbrush showed a mixture of DNA of most probably two individuals (the potential missing person as well as another individual). This leads to the possibility of a contaminated toothbrush (i. e. it has also been used by an individual other than the missing person). Although the comparison with toothbrush and teeth samples revealed a lower probability because of the contamination and the additional alleles, the identity could be confirmed. Thus, re-association of the scattered skeleton elements and the mandible with the potential missing person could be legally validated.

Conclusion

This case report emphasises the value of teeth not only as a powerful tool in the conventional comparative dental identification but also in matching different scattered human remains. To reach a high confidence of correct identification of scattered human skeletal remains, a combination of DNA and dental identification is required.

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