Original Article

FORENSIC ANTHROPOLOGICAL EXAMINATION AND DNA ANALYSIS IN THE IDENTIFICATION OF HUMAN REMAINS IN RWANDA

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ABSTRACT

The identification of human remains plays a big role in solving legal and social challenges. To date, significant strides have been made to help positively identify human body remains following both natural and man-made disasters as well as reported cases of missing individuals. Thorough anthropological examination and DNA analysis of the remains can be used to conclusively link the profiles of the remains to persons if a potential living match is available even after a long period of time.

We present cases of excavated human remains and samples from Rwanda that were part of both legal and social disputes. Following anthropological examination and DNA analysis, the disputes were conclusively settled. This case report also highlights the possibilities as well as challenges of identifying victim remains of larger calamities such as the 1994 Genocide perpetrated against the Tutsis in Rwanda in which an estimated one million Tutsis lost their lives.

Keywords: Identification of human remains – forensic anthropological examination -DNA analysis and profiling

INTRODUCTION

The identification of human remains plays a big role in solving legal and social challenges. However, the actual identification is not a straightforward exercise since it is not only resource but also time consuming. To begin with, determination of whether the remains are actually human is of vital importance. Various environmental factors such as heat, moisture, nature of soils, etc. greatly influence the state of the remains and in turn, the whole identification process.

Significant strides have been made to help positively identify human body remains following both natural and man-made disasters as well as reported cases of missing individuals (1, 2, 3, 4). Personal identification of the actual remains by family members, friends or witnesses is also widely used to date even though it has sometimes led to errors and cannot be relied upon in cases where the remains are highly decomposed, burned or mutilated. With the current advances in the field of forensic anthropology, a number of features such as age, ancestry, sex and individual stature can be determined from a thorough examination of the available skeletal remains. DNA analysis, first applied in forensic casework investigation in 1985 (5) remains the most accurate and most reliable method for identification. Nonetheless, it is highly dependent on the availability of a match as well as quality of the available samples. Samples for analysis can be derived anywhere from blood or other body fluids, teeth, bones, and hair. Information gathered from these methods when put together, can provide a considerable level of accuracy needed in establishing the identity of the remains being analyzed.

We present cases of excavated human remains and samples from Rwanda that were part of both legal and social disputes. Anthropological examination and DNA analysis were done at the Institute for Legal Medicine, University Medical Center Hamburg-Eppendorf, Germany, between 2012 and 2016. This has been enabled by cooperation between the Institute for Legal Medicine, University Medical Center Hamburg-Eppendorf and various Rwandan institutions most notably the University of Rwanda, College of Medicine and Health Sciences, Rwanda National Police and the Rwanda National Public Prosecution Authority (NPPA) with support from the German Academic Exchange Services (DAAD) through its "Partnerships for the Health Sector in Developing Countries" program.

METHODS

Case presentation,

Case 1

Bone remains found beneath a house under renovation were at the center of a dispute between two families with one party claiming that the remains were of an old man who happened to be their grandfather while the other party claimed the remains belonged to a young woman who had gone missing without trace for over 5 years. A forensic anthropological examination and DNA analysis were requested to estimate the age and to determine if the remains were male or female. This was done between August 2015 and January 2016.

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Anthropological examination

The identifiable bone parts were: skull with right upper jaw (fig. 1a-b, fig 2h), parts of the cranium (fig. 1c), the lower jaw (fig. 1d), teeth (fig. 1e), one cervical vertebra (Atlas) (fig. 2g), shaft of one clavicle, right humerus (fig. 2i), parts of both pelvic bones, both femora (both missing the distal parts, different length) (fig. 2j), shaft region of both tibiae (broken off at different lengths), two shafts of fibulae (broken off at different lengths), several parts of long bones, tarsal bones.

Illustrations

Anthropological examination

Fig 1: (a-b) Skull with right upper jaw ***(c)*** Cranium ***(d)*** Mandible ***(e)*** Teeth

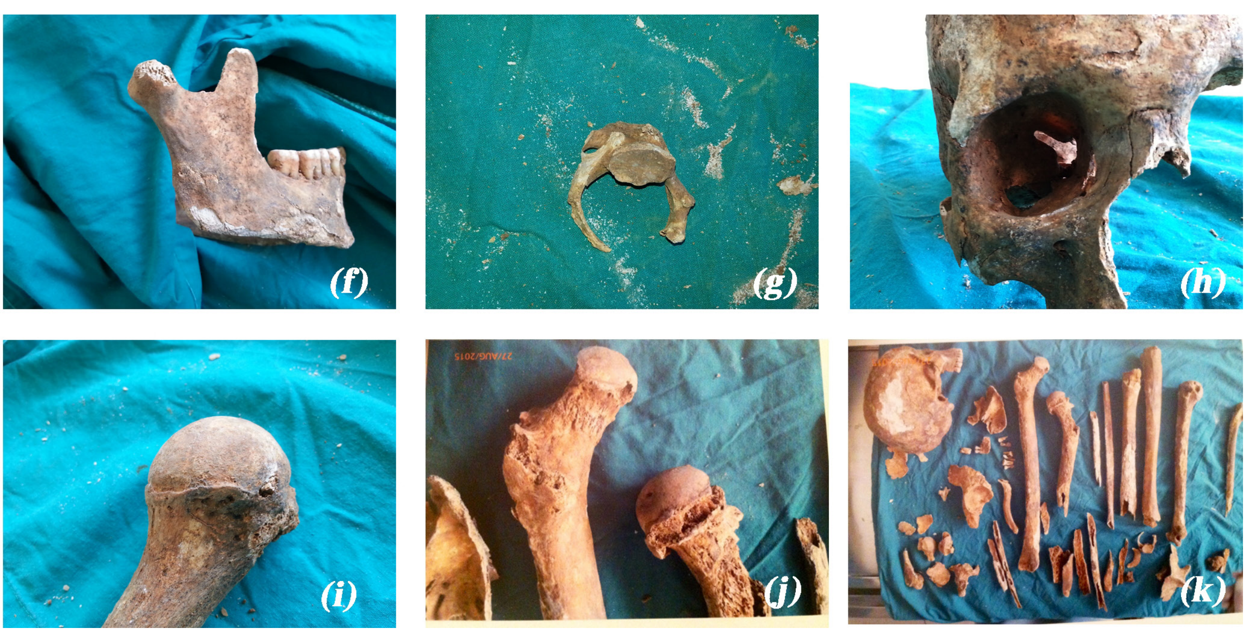

Fig 2: (f) Mandible ***(g)*** Cervical vertebra ***(Atlas) (i)*** Right humerus ***(h)*** Orbit
(j) Both femora showing extensive arthrosis.

Case 2

A young woman requested for DNA testing to determine whether bone remains that were found and initially presumed to be animal bones belonged to her mother or not. The analysis was done between November and December 2015.

Case 3

A bone sample from the right tibia of a dead newborn was analyzed together with a saliva sample collected on cotton swabs from the putative father between June and July 2015. The DNA-analysis was requested to find out if indeed he was the father of the dead baby or not.

Case 4

A brother and sister claiming ownership of a dead body they believed belonged to their father had their saliva samples taken. A part of the femur bone of the dead body was also taken. A DNA-analysis was requested to find out if indeed the dead man was their father. The analysis took place between October 2014 and January 2015.

Case 5

Bone samples from a dead newborn as well as saliva samples from the putative father and mother collected on cotton swabs were analyzed between August and September 2012. The DNA-analysis was requested to ascertain whether the putative father was indeed the father of the dead newborn or not.

PCR and Sequencing analyses

DNA extraction

For Case 1, DNA was extracted from the teeth and bone using standard protocols by means of CrimePrep Adem-kit (Adem Tech).

For Case 2, saliva samples of the young woman were then collected on cotton buccal swabs and DNA extracted by means of the Chelex-based method. DNA from the bone sample suspected to belong to the child's mother (bone sample labeled No 1) was extracted by means of CrimePrep Adem Kit (AdemTech).

For Cases 3 and 4, DNAs were then extracted from the saliva samples by means of the Chelex-based method. The DNA from the bone was extracted using CrimePrep Adem Kit (AdemTech); while for case 5 DNA was then extracted from the samples by means of the QiaAmp Kit. PCR and sequencing analyses

PCR- amplification of 18 Short Tandem Repeats (STRs) was performed using Powerplex 16 (Promega) and Decaplex-SE multiplex (Qiagen) kits. Visualization of the alleles was performed using capillary electrophoresis on genetic analyzer ABI 3130 using Genemapper software.

RESULTS

For case 1, anthropological examination was inconclusive due to the missing parts of the skeleton but from what was left (less bossed frontal region, large mastoid process, relatively square orbits and a large acetabulum) pointed towards male characteristics.

Age determination was based on the presence and conditions of the teeth as well the state of the joints. Both wisdom teeth in the lower jaw were noted but since most of the upper jaw was missing, the state of the dentition could not be determined. All the available teeth showed signs of heavy wear on the chewing surface and tartar on the buccal surface. No dental fillings or prosthetics were visible. The missing teeth were lost post-mortem.

On the available bones, all epiphyseal grooves appeared closed. Bone ridges and decalcification noted on the femoral and humeral joints as well as sockets, were signs of advanced arthrosis. DNA analysis showed an XY genotype. Genetic sex determination showed a male genotype supporting the anthropological (albeit inconclusive) findings.

Skeletal and dental examination was suggestive of long years of hard labor with ensuing wear. This assumption was supported by the objectivated build-up of bone material as ridges at the joints and degradation of the surface. A conclusion that the remains were of an advanced-age male was made.

For Case 2, the DNA profile was established as shown in Table 1.

DNA-STR-Systems	**Child**	**Bone Sample No1**
D3S1358	16/17	15/17
D19S433	14/14	12/14
D2S1338	17/20	17
D22S1045	10/16	10/15
D16S539	11/11	11/12
D18S51	19/19	19
D1S1656	16/16	11/16
D10S1248	13/14	13
D2S441	11/14	11
TH01	7/8	8/9
VWA	17/20	16/20
D21S11	29/29	29
D12S391	18/19	18/19
D8S1179	14/15	14/15
FGA	22/26	22
SE33	17/17	17

Table 1: DNA analysis results

In every STR-system, the bone sample No1 has one allele that the named child must have inherited from her mother. Thus the person to whom the bone belonged cannot be excluded from maternity. The calculated probability of maternity was 99.99999% (Maternity Index: 59.018.605); this was calculated on the hypothesis that no closely related relative of the person to whom the bone belonged can either be the mother of the child in question. From a forensic point of view, there was no doubt that the bones were indeed the remains of the young woman's mother.

Table 2 shows DNA analysis results for Case 3

DNA-STR-Systems	Bone sample from unborn Child	Saliva sample from putative father
D3S1358	15/16	14/17
D19S433	11/15.2	13/13.2
D2S1338	16/19	23/26
D22S1045	14/17	15/16
D16S539	11/14	9/11
D18S51	17.2/20	18/19
D1S1656	15.3/16.3	15.3/16.3
D10S1248	14/16	12/15
D2S441	11/12	14/14
TH01	8/9	6/7
VWA	16/19	17/18
D21S11	29/32.2	28/31
D12S391	15/18	18/19
D8S1179	15/15	14/17
FGA	20/25	22/23
SE33	17/18	27.2/29.2

Table 2: DNA analysis results

There were inconsistencies with paternity in 13 out of 16 DNA-Short Tandem Repeat systems (D3S1358, D19S433, D2S1338, D22S104, D18S51, D10S1248, D2S441, TH01, VWA, D21S11, D8S1179, FGA, SE33). The putative father does not share an allele with the baby in these 13 systems and hence he cannot be the father.

For Case 4, DNA-alleles from the bone sample could not be detected. Therefore, it was not possible to make a kinship-analysis regarding the question whether the dead man was the father of the brother and sister in question.
For Case 5, Calculation of the probability of paternity was done by the method of Essen-Moeller using Genoproof software (Qualitype/Dresden). Table 3 shows DNA analysis results.

DNA -STR-Systems	Bone samples of dead newborn	Mother	Putative father
D3S1358	16/17	16/17	16/17
TH01	9/9	9/9	7/9
D21S11	30/32	27/30	32/34
D18S51	16/17	17/18	15/16
Penta E	No result	8/15	15/15
D5S818	11/12	10/12	11/13
D13S317	12/13	10/12	13/13
D7S820	9/10	10/10	9/10
D16S539	11/11	11/11	11/12
CSF1PO	No result	7/8	9/10
Penta D	No result	2.2/14	8/9
VWA	18/19	18/19	19/19
D8S1179	12/14	12/16	14/16
TPOX	9/11	8/11	8/9
FGA	22/23	22/25	23/24
D2S1338	18/19	18/19	19/19
D19S433	14/14.2	13/14	14/14.2
SE33	17/19	18/19	11.2/17

Table 3: DNA analysis results

The putative father has in every STR-system (15 systems gave a result with the bones) the allele that the baby must have inherited from its father. Thus the man in question cannot be excluded from paternity. The calculated probability of paternity was 99.9999% (Paternity index 3.165.005). The probability was calculated on the hypothesis that no closely related relative of the man said to be the putative father could either be the father of the baby. From a forensic point of view, there was no doubt that the putative father was indeed the father of the baby.

DISCUSSION

Detailed analysis of the different elements of a single skeleton can be used to estimate the biological aspects such as age, sex, stature, ancestry and identity of an individual. Bone is a dynamic tissue that allows for growth during development of the individual. It is shaped and re-shaped by cells that reside within it. It is due to this reason that the overall morphology of bones and teeth may vary between individuals.

The four major factors that lead to variation in the human skeletal anatomy are: 1) growth, 2) sex, 3) population based (geographic), and 4) individual (idiosyncratic) variation defined as normal variation between different individuals of the same age, sex and population.

The determination of an individual's age in skeletal remains involves estimating the age at the time of death rather than the amount of time that has elapsed since death. Seven age classes commonly used to segregate human osteological remains are: fetal (before birth), infant (0-3 years), child (3-12 years), adolescent (12-20 years), young adult (20-35 years), middle adult (35-50 years) and old adult (50+ years) (6). Dental development (eruption and wear) is the most widely used technique when trying to estimate individual age due to the fact that teeth are the most commonly found remains in forensic work. Qualititatively, sufficient DNA can also be extracted from the teeth (7). Tooth formation begins in the embryo between 14-16 weeks after conception then dentition emerges in four distinct periods. First, most deciduous teeth emerge during the second year of life. The two permanent incisors and the first permanent molar usually emerge between 6 and 8 years. Most permanent canines, premolars, and second molars emerge between 10 and 12 years. Finally, the third molar emerges around 18 years and not earlier than 16 years of age. With knowledge of these stages of emergence, the age can be estimated by comparing the unknown individual with a pre-established chart or atlas showing the mean stage of development of the entire dentition. It should also be noted that, once a permanent tooth erupts, it begins to wear. Rate and patterns of wear are governed by tooth developmental sequences, tooth morphology, tooth size, internal crown structure, tooth angulation, non-dietary tooth use as well as diet (8). Hence, the rate and extent of wear is a function of age and can be used in assigning age, for example, as we noted in our case report (case 1). Other methods for estimating age include cranial suture closure (9), epiphyseal closure (10), nature of the pubic symphysis surface (11), sternal rib end (12) as well as radiographic analysis of the cancellous (spongy) and cortical bone structure (11).

Sexual identification of human skeletal material on the other hand is generally most accurate after the individual reaches maturity. Sex differences in humans tend to be concentrated and most extreme on the elements of the pelvis and skull.

Analysis of the pubic features can orient towards a particular sex. The sacrum and os coxae of females are smaller and less robust than those of males while female pelvic inlets tend to be relatively wider than male ones. The greater sciatic notch on female os coxae is relatively wider than the notch on male bones. Females also tend to have relatively longer pubic portions of the os coxae, including the superior pubic ramus than males. The subpubic angle, formed between the lower edges of the two inferior pubic rami, is larger in females than in males.

The acetabulum tends to be relatively larger in males.

Sex determination based on parts of the skull on the other hand, follows the observation that males tend to be more robust than females. Relative to female crania, male crania present more robusticity. Supraorbital ridges and glabellar regions are more prominent as well as heavier temporal and nuchal lines. Male frontals and parietals tend to be less bossed than female ones. Males also tend to have relatively large, broad palates, squarer orbits, larger mastoid processes, larger sinuses and larger occipital condyles than females. Male mandibles tend to have squarer chins, deep mandibular rami and more rugose muscle attachment points when compared to female mandibles. Studying the posterior border of the mandibular ramus could be used to sex unknown individuals with an average of about 92% accuracy (13)

Much information such as evolution of mankind, ways of life and stature of ancient humans too, can be got from anthropological analysis of human fossils that are even thousands of years old. In a study about the behavioral inferences from the Skhul/Qafzeh early modern human hand remains, Niewoehner's observations supported earlier assumptions that Neanderthals were more heavily muscled, had stronger upper-limb bones and possessed unusual shapes and orientations of some upper limb-joint complexes relative to the Skhul/Qafzeh hominids; two groups of humans found in the Near East about 100,000 years ago. On the other hand, the most significant difference that the Skhul/Qafzeh hominids had, was the functionally significant bases of the first and third metacarpals which resembled those of Upper Paleolithic humans not Neanderthals leading to a conclusion that the Skhul/Qafzeh hominid hands were adapted to the Upper Paleolithic-like manipulative activities and appeared to have used less somatic effort to accomplish upper-limb related subsistence tasks than did the Neanderthals (15). Another recent discovery of fossils in South Africa of an extinct species from the genus Homo named Homo naledi described Homo naledi as being similar in size and weight to a small modern human, with human-like hands and feet. And, even though its skull had several unique features, it had a small braincase that was most similar in size to other early hominin species that lived between two million and four million years ago (16).

In a recent study on 27 human remains found in mass graves in Slovenia in which DNA was isolated from the teeth and bones, 4 strong matches of victims of World War II with potential living relatives were made (14).
Through reconstruction and DNA analysis aided by matching with living relatives, scientists and forensic pathologists at the University of Hamburg were also able to establish the identity of an American pilot as well as an Italian soldier, victims of the second World War, about 70 years ago, whose remains had been buried in Germany (18, 19). In another study by Qiaomei, et al, DNA analysis from a 37,000-42,000 year- old modern human from Romania, six to nine percent of the genome of the individual was found to have been derived from Neanderthals more than any other modern humans sequenced to date (17)

CONCLUSION

The cases described above clearly indicate the importance of both anthropological and DNA-analysis in the identification of dead or missing persons even after long periods of time, as well as helping to understand the evolution of mankind. With the recent advances in both forensic anthropology and DNA analysis techniques, identification of human remains can be conclusively made if the remains are well preserved and samples well handled, in turn, playing a key role in solving both legal and social disputes. This also indicates that even with bigger catastrophes such as the 1994 Genocide perpetrated against the Tutsis in Rwanda, in which about one million people were killed, identification is possible even though, the availability of infrastructure and skilled personnel is equally crucial.

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