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World Trade Center Human Identification Project: Experiences with Individual Body Identification Cases

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Aim. To present individual body identification efforts, as part of the World Trade Center (WTC) mass disaster identification project.

Methods. More than 500 samples were tested by using polymerase chain reaction (PCR) amplification and short tandem repeat (STR) typing. The extent to which the remains were fragmented and affected by taphonomic factors complicated the identification project. Anthropologists reviewed 19,000 samples, and detected inconsistencies in 69, which were further split into 239 new cases and re-sampled by DNA specialists.

Results. The severity and nature of the disaster required an interdisciplinary effort. DNA profiling of 500 samples was successful in 75% of the cases. All discrepancies, which occurred between bone and tissue samples taken from the same body part, were resolved by re-sampling and re-testing of preferably bone tissue. Anthropologists detected inconsistencies in 69 cases, which were then split into 239 new cases. Out of 125 "split" cases, 65 were excluded from their original case. Of these 65 cases, 37 did not match any profiles in M-FISys, probably because profiles were incomplete or no exemplar for the victim was available. Out of the 60 remains not excluded from their original case, 30 were partial profiles and did not reach the statistical requirement to match their original case, because the population frequency of the DNA profile had to be ≤1 in 10^9 for men and ≤1 in 10^8 for women.

Conclusion. Due to transfer of soft tissue and other commingling of remains, DNA testing alone would have led to problems if only soft tissue would have been tested. This was one of the reasons that forensic anthropologists were needed to evaluate the consistency between all linked body parts. Especially in disasters with a high potential for commingling, the described anthropological review process should be part of the investigation.

Key words: *bone and bones; disasters; DNA; forensic anthropology; polymerase chain reaction; tandem repeat sequences*

The World Trade Center (WTC) attack on September 11, 2001, followed by the collapse of two of the five highest buildings in the world, resulted in a so far unprecedented challenge in the field of victim identification after a mass catastrophe. The focus of the identification efforts in this project was, and still is, to identify the highest possible number of recovered human remains. At most disasters, the standard of care is to identify each victim, not each remain. In the case of the September 11 mass disaster, there is no definitive victim list. That is why WTC is considered to be an open population. As a result, an attempt had to be made to identify each set of remains recovered, based on the conservative assumption that this may represent the only remain recovered for an individual. In other words, because the bodies recovered from the WTC site were highly fragmented, a single sample theoretically could represent the only identifiable remain of an individual. All circumstances, such as the impact of the aircrafts and abnormally high temperatures due to the fuel explosion, collapse of the towers, prolonged exposure to different weather conditions, fire and water, as well as the use of heavy equipment in the recovery effort, led to an extraordinary high level of destruction of the human remains and a lot of friction and contact of one body part with another. This led to a high level of commingling, which was an obvious impediment to the main goal of the project. The necessity to respond to the commingling problem led to the development of an

innovative interdisciplinary protocol, which should be considered as one of the possible procedures in the event of other mass casualties.

The original protocol employed by the New York City Office of Chief Medical Examiner (NYC OCME) involved the following steps (1,2):

1. analysis of the remains by a forensic pathologist and triage by an anthropologist,

2. separation of any remains that were not connected by soft tissue,

3. the assignment of a tracking number/bar code to each separate specimen,

4. removal of soft tissue from bone surface, and

5. extraction of most appropriate specimen from each of the separate pieces for DNA analysis.

At the early stages of the project, soft tissue was preferentially used for DNA analysis instead of skeletal remains, which consecutively resulted in several cases of false association based on the DNA profiles. For example, two fragments were presumptively linked by DNA profiles, although it was clear that both pelvic parts came from the same (right) side (Fig. 1). The soft tissue typed for one set of pelvic bones must have been transferred from another body part. DNA testing of the bone tissue confirmed the two different identities.

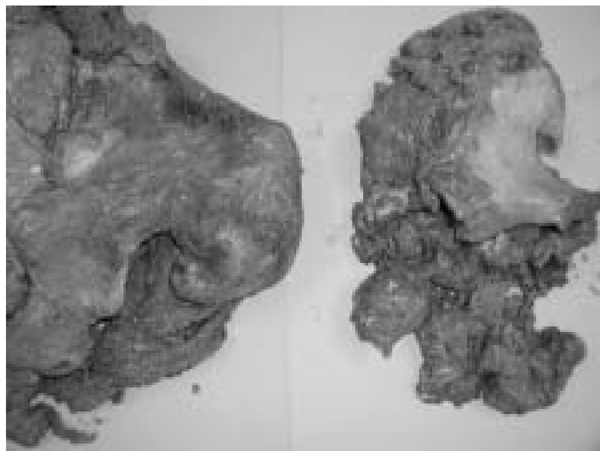

Figure 1. Two pelvic parts came from the same (right) side, but were linked by DNA profiles generated for the adhering soft tissue.

To maximize the accuracy of the DNA identification results, the NYC OCME responded to the commingling problem by initiating an interdisciplinary project involving anthropologists, medical examiners, and DNA analysts. The Anthropology Verification Project was founded as a means to verify the minimum number of individuals in each set of remains. The process involved the review of more than 19,000 separate human remains; detection of duplicate elements by using standard physical anthropological techniques; review of all associated inventory paperwork; adding details to the existing description, such that future re-sampling could be done by DNA experts (e.g., details regarding anatomical orientation, part of body and completeness of skeletal elements help DNA personnel to sample the correct item); creating new cases by splitting original sets of remains where unconnected body parts were found; and re-sampling of bone matrix in any case in which duplicate elements were detected.

The DNA re-sampling project was initiated to support the Anthropology Verification Project in order to maximize the number of possible identifications. DNA testing was performed in all cases in which a duplication or an inconsistency was detected either by anthropologists or by previous DNA analysis (Figs. 2 and 3).

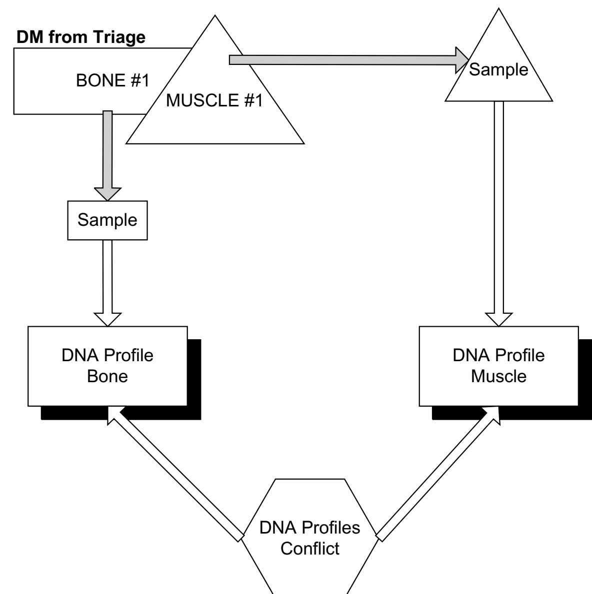

Figure 2. Bone and soft tissue sampled (grey arrows) from the same body part (DM). DNA testing can yield inconsistent results.

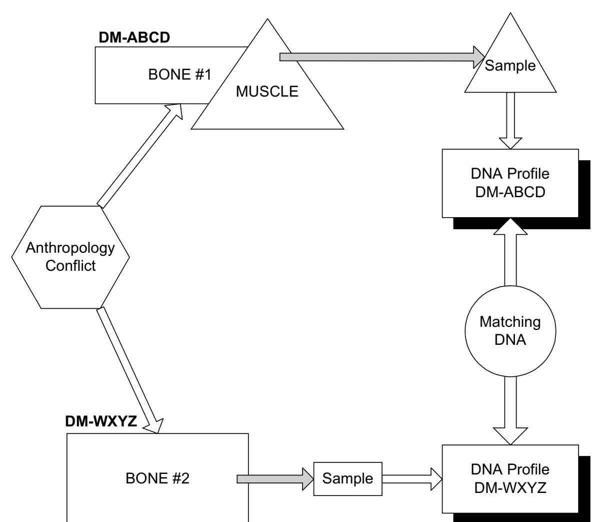

Figure 3. Bone and soft tissue sampled (grey arrows) from different remains (DM). Although muscle #1 and bone #2 showed matching DNA profiles, anthropological review could result in a conflict, e.g., if bone #1 (ABCD) and bone #2 (WXYZ) were both left hip bones.

Methods

Triage of the Biological Samples

Based on previous experience regarding the amount of DNA extracted from bones (3,4), the re-sampling protocol preferred sampling of the compact bone tissue over spongy bones. Morphologically, the samples were in different states of preservation, ranging from very well preserved, with even preserved bone marrow, to semi-burned and/or burned (Fig. 4). Due to that fact, each single specimen was treated as an individual case per se. The aim was to triage specimens and judge the amount of bone tissue to be taken for the extraction procedure. This step was established empirically. The main idea of the presented protocol is to use as much tissue as needed (within the circumstances of each single case) to obtain a sufficient amount of DNA suitable for PCR amplification, and to "un-trap" osteocytes from their original positions in compact and spongy bones.

DNA Extraction Procedure

After sampling the bone specimens in the morgue, care was taken to clean them vigorously and minimize contamination with any external DNA (5). Every single specimen was cut with an electrical autopsy saw, which was repeatedly cleaned using 10% bleach. After labeling, the specimens were put on a clean bench pad under a biological hood and cleaned by using scalpels, brushes, and dH_2O, followed with another dH_2O step where disposable tooth-brushes were used. After sonication in a 5% Terg-a-zyme solution (Alconox Inc., New York, NY, USA) and drying, the outer surface of the bone specimen was sanded by using a Dremel tool (Racine, WI, USA) equipped with an emery disk, so that the outer surface appeared completely free of dirt and debris. The emery disks were used just once and then discarded. This step was followed by fresh changes of 5% Terg-a-zyme, until most of the dirt had been removed, followed by fresh changes of sterile dH_2O and drying in the oven at 36 °C. Using a Dremel rotating tool equipped with a cut-off wheel (one per bone specimen), the bone specimen were cut into pieces of an approximate size of 0.5×0.5×0.5 cm (Fig. 5). The total amount of bone tissue per sample used for the extraction was established by multiplying 2g of bone tissue, depending of the actual state of the bone piece itself. Bone pieces were frozen in liquid nitrogen and ground in the 6750 SPEX CertiPrep Freezezer Mill (SPEX CertiPrep, Methucen, NJ, USA). Plastic vials of the mill, as well as metal end-parts and impactors, were cleaned vigorously following a specific procedure, with 0.1% sodium dodecyl sulfate (SDS), 10% bleach, sterile dH_2O, and 100% ethanol, followed by UV sterilization.

Upon grinding, the bone dust was divided into several 50-mL conical tubes (2 g of bone dust per tube), and incubated at 56 °C in 3 mL of organic incubation buffer per tube (10 mmol/L Tris, pH 8.0; 50 mmol/L EDTA, pH 8.0; 100 mmol/L NaCl; deionized H_2O, with 2% SDS and 20 μg/μL Proteinase K) overnight in a horizontal or vertical shaker. We extracted two times 500 μL of the supernatant from each conical tube, using a Phenol-chlorophorm-isoamylalcohol (24:24:1) organic extraction procedure and Phase Lock Gel tubes (Eppendorf, Hamburg, Germany), after which multiple washing and concentrating on Microcon 100 microconcentrators (Amicon, Inc., Beverly, MA, USA) were performed. The use of multiple Microcon concentrators over a single Centricon vial helped to avoid clogging of the membranes with bone dust. All microconed samples of a single case were combined together and an additional concentrating step was performed. All extraction volumes were kept low to maximize DNA concentration.

Quantitation, PCR Amplification, and Analysis

To conserve the extract, only 10 μL were employed for quantitation. After the QuantiBlot (Roche Molecular Systems, Alameda; Applied Biosystems, Foster City, CA, USA) quantitation, the samples were amplified by using the PowerPlex®16 multiplex STR system (Promega Corp., Madison, WI, USA), and the GeneAmp® PCR System 9700 (Applied Biosystems), following the manufacturers' recommendations. Samples with zero Quantiblot values were still amplified, whereas maximized sensitivity was obtained by using the highest recommended cycle number (10+22). The DNA input was maximized by reducing the mastermix volume to 20 μL DNA volume in 25.8 μL total

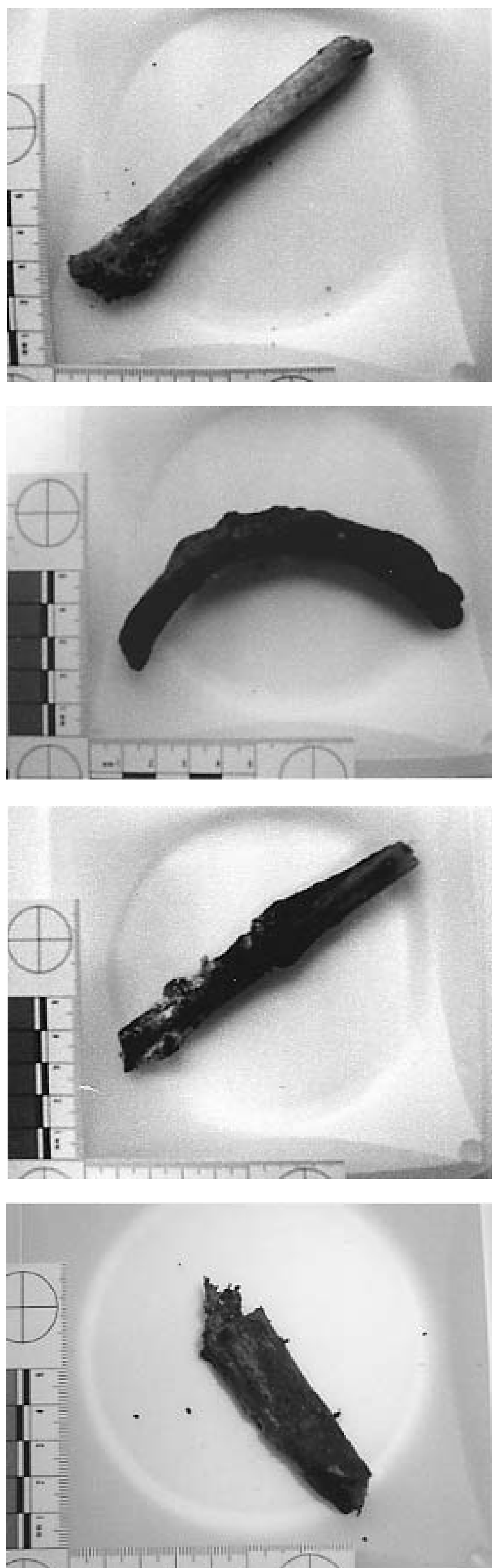

Figure 4. Bone samples in increasingly worse states of preservation (top to bottom).

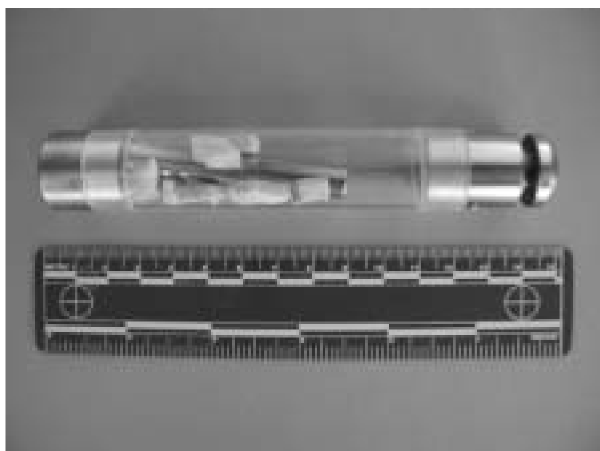

Figure 5. Bone sample cut and prepared for grinding process.

mix. The amplified products were analyzed in the ABI PRISM® 3100 Genetic Analyzer with GeneScan and Genotyper software (Applied Biosystems), according to the manufacturer's recommended protocol. Allele designations were determined by comparison of the sample fragments with those of the allelic ladders supplied with the original kit. All profiles were loaded into the Mass Fatality Identification System software (M-FISys) (Gene Codes Forensics, Ann Arbor, MI, USA) and compared with its matching tools.

Results

After performing more than 500 analyses in the described way, the Special Projects Group of the WTC Human Identification Project obtained usable profiles in about 75% of cases, whereas profiles of more than 13 loci were obtained in about 50% of all analyzed cases. Although the presented procedure was time-consuming and hazardous (due to the use of phenol-chloroform), it has proven to be highly efficient and useful for nuclear DNA-based identification of compromised bone tissue.

The described Anthropology Verification/DNA Re-sampling Project was initiated on May 28, 2002, and continued until the end of August 2002. During the course of these three months, the anthropologists reviewed more than 19,000 separate remains, and detected inconsistencies in 69 cases, which were then split it into 239 new cases and underwent re-sampling by DNA specialists.

Of the 125 "split" cases analyzed by April 15, 2003, 65 were excluded from their original case. Of these 65, three new victims were identified, 17 remains were matched to other previously identified victims, 8 remains were linked to previously identified victims but lacked the statistical requirements for a definitive match, and 37 remains did not match any profiles in M-FISys (Fig. 6).

The failure of 37 samples to match other remains, victim exemplars, or family references might be due to the fact that these profiles were incomplete or that no exemplar for that victim was available (not all of the families of the victims donated samples for DNA typing). Out of 60 remains that were not excluded from their original case, 30 met the statistical requirement to match their original case, whereas 30

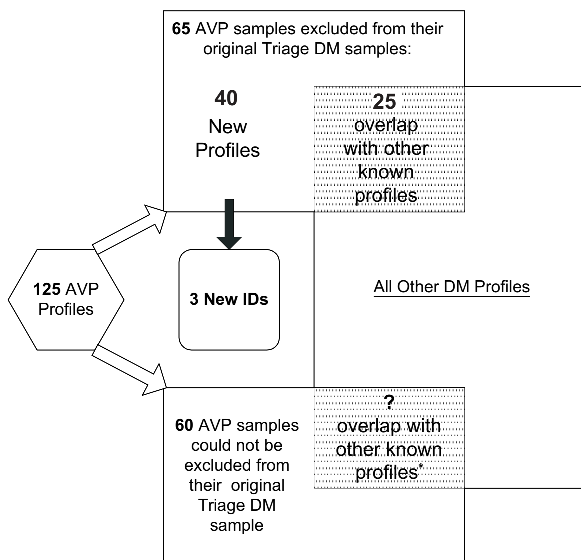

Figure 6. DNA results of Anthropology Verification Project (AVP) as of April 2003. DM – same body part. Asterisk designates samples to be determined with further testing.

were partial profiles and did not reach that value. At this point, the population frequency of a DNA profile has to be equal or rarer than 1 in 10^9 for men and 1 in 10^8 for women for a match to be considered valid.

Discussion

The success rate of nuclear DNA analysis on bone samples was similar to other identification efforts dealing with compromised samples (3,4,6-10). It can be expected that typing results could be improved by mtDNA testing (11). The WTC disaster was unique in both its magnitude and its circumstances, and the NYC OCME was forced to be innovative in a number of ways in its strategy for dealing with this uniquenesses. The severity and nature of the disaster required an interdisciplinary effort. The extent to which remains were fragmented and affected by taphonomic factors complicated the identification project. The emphasis during the entire process was on DNA identifications, thus the role of the anthropologist was modified. Although DNA analysis is a highly discriminatory method and the method of choice in the identification effort, it is not self-sufficient and could not replace an anthropological evaluation. In this project, the anthropologists were not looking for evidence of manner and cause of death. Recorded data regarding the potential sex, ancestry, and/or age of skeletal remains were not used for the identification effort beyond allowing for the separation of incorrectly associated remains. What was ultimately the most important information recorded by anthropologists at triage was accurate inventory information. It could not be assumed that the remains delivered in a single bag were those of a single individual. For each body bag delivered to the triage room, the idea was to determine the minimum number of individuals represented in each set of remains. To avoid the potential complications that could have resulted from commingling problems, as well as in the interest

of maximizing the total number of identifications, the NYC OCME instituted the described process, in which every single set of remains recovered from the WTC site was to be reopened and re-inventoried. In keeping with this, any remains that were not physically attached (either by bone or soft tissue) were to be separated. Each single set of remains, however large or small, was assigned a separate catalog number, and sampled for DNA analysis. The number of new identifications and re-associations of body parts clearly show that the collaboration of the anthropological review team and the DNA laboratory was a successful effort.

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