DNA Identifications After the 9/11 World Trade Center Attack

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Before the September 11, 2001, World Trade Center (WTC) attack, the use of DNA profiling for victim identification in mass casualties (e.g., plane crashes) was typically limited to situations with fewer than 500 persons (*1–5*). Often, the condition of the remains allows rapid recovery of intact bodies. However, the number and condition of the remains in the WTC attack were unprecedented.

After many deaths, especially in the aftermath of a mass fatality, there is an element of disbelief. Accepting the loss is a component of a complex grief process (*6*). Our motivation was to provide a tangible artifact of remains to survivors to facilitate coping and grief processes. Enabling family participation in some of the identification decisions was a critical component of the effort.

At the time of the attack, no infrastructure existed for rapid, effective victim identification in large-scale disasters (>1000 victims). Processes had to be scaled up to collect and analyze massive amounts of data in order to return identified remains to the families of almost 3000 victims. We summarize some challenges of the DNA-based component of the victim identification process, how these were met, and considerations for the future.

Some mass fatality identification projects begin with a list of victims (e.g., airline flight manifests listing passengers and crew). In contrast, the WTC mass fatality was initially "open" because the number of victims was unknown. Concerns about unreported or fraudulently reported victims made estimates difficult. The condition of the remains ranged from a few nearly complete bodies to multitudes of tiny fragments of charred bone, often difficult to distinguish from inorganic material. The fires affected the remains with temperatures exceeding 1000°C (*7*) that burned for more than 3 months. The towers' collapse fragmented and commingled victim remains and admixed building material. Many tissue fragments were retrieved months after the crashes, and bacterial and other processes further compromised the DNA. These factors made it difficult to isolate and genotype the DNA from the specimens.

Identification of human remains by DNA typing requires reference samples for comparison. These and other sources of information formed a deluge of material and data to be cataloged, archived, and analyzed. Preexisting sample collection and identification methods were insufficient for these needs.

Identification of WTC victim remains was the responsibility of the New York City Office of Chief Medical Examiner (OCME), which is one of the largest and most sophisticated in the country. Yet, its resources and scope of experience had to be expanded. The New York State Police Department (NYSP) was responsible for DNA analysis of reference samples. Several private DNA laboratories also tested samples, and software vendors helped to develop data analysis and compatibility tools. The NYSP and the OCME asked the National Institute of Justice (NIJ) to convene a group of scientific and medical experts [the Kinship and Data Analysis Panel (KADAP)] to advise them in the DNA identification effort. KADAP included experts in forensics; bioinformatics; and molecular, medical, statistical, and population genetics. The KADAP's charge was to assist the OCME in the development of procedures, standards of evidence, and processes related to the DNA identification effort. The final determination of a specific identification rested with the OCME (*8*).

DNA identification technologies. A bat-tery of genetic identification markers, the Combined DNA Index System 13-locus short tandem repeat (STR) panel (CODIS) (*9*), was initially selected because it was established in forensic and legal systems and was compatible with forensic software packages. The first round of STR genotyping had a relatively low yield, because of DNA damage and other factors. Therefore, KADAP recommended several other approaches.

Because there are many more copies of mitochondrial DNA (mtDNA) than nuclear DNA, mtDNA analysis can be successful when DNA is limiting (*10, 11*). Although mtDNA typing is generally labor-intensive, mtDNA typing with semiautomated analysis was provided for this project. Alone, mtDNA typing is insufficient to meet the threshold of identification and could only be used in conjunction with STR profiles. KADAP also recommended use of "mini" STR markers (*12–16*) that encompass the same CODIS STRs, but use shorter amplicons, which makes them more likely to be successful on fragmented DNA. Finally, technology for typing single-nucleotide polymorphisms (SNPs) was considered. Similar in concept to the use of mini-STRs, SNP typing can work with fragmented DNA because the amplicons are small.

Statistical criteria. About 5000 persons were initially assumed missing, so we used 1/5000 as the prior probability that a tissue fragment was from a particular missing person. This prior was lowered to 1/3000 as the victim list was refined. Direct DNA profile comparisons were used to test for identity

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PLANNING FOR FUTURE DISASTERS

Further research and development of forensic DNA typing systems is needed.

Software must be able to integrate analytical, database, and workflow functions.

Information technology infrastructure must be adequate to interconnect data-gathering, analysis, archiving, and reporting functions (*22, 23*).

A kit should be developed for mass-fatality reference sample and kinship data collection, as well as for family education and counseling.

External prioritization requests should be minimized.

Processes should be designed to test and to validate novel identification processes concurrent with their development.

Criteria for determining end points should be designated early in the identification process (*24*).

of tissue samples to reference samples from victims when these could be obtained. The CODIS marker set typically produces a random match probability (the probability of finding the same DNA profile in a randomly selected, unrelated individual) that is much less than 10^{-10} in most populations. This random match probability, when combined with the prior probability, yields the final match probability (17).

KADAP chose an initial minimum random match probability threshold (10^{-10}). This was stringent, but there was consensus that the approach would permit the OCME to make the easier identifications promptly and with high confidence. As the project progressed, fewer novel DNA profiles were generated, and the victim count decreased. Therefore, the required random match probability was reduced to 2.5×10^{-10} when DNA-based gender testing gave no results, to 5×10^{-9} for females, or 5×10^{-10} for males when gender markers were available (18). Details of these methods are described elsewhere (19). Although they represented a wide range of continental origins, many victims' backgrounds were unknown, so data on allele frequencies were not available for all groups. KADAP recommended that random-match probability be calculated using frequency data from each of four major population groups. The result that gave the most conservative random match probability was used (20).

Kinship analyses (comparison of a WTC remains DNA profile to those of biological relatives of the victims) were used when victim reference samples were not available or to confirm or increase confidence in direct comparisons. A kinship likelihood ratio was calculated for a victim sample by using family reference data, calculated jointly when multiple kin samples were available (17). This likelihood ratio compares the probabilities of observing a given DNA profile if a victim sample belonged to a particular missing individual (based on the stated genetic relationship of kin providing reference samples) to the chance of observing the profile if it was from an unrelated person. This likelihood ratio, when multiplied by the prior odds, yields the posterior odds (of kinship). To reach KADAP's goal of high confidence of correct identification, sufficient loci and family members were needed to reach a likelihood ratio of at least $3 \times 10^6{:}1$ (in favor of the stated genetic relationship).

Software issues. Matching thousands of victim sample genotypes (and partial genotypes) to next-of-kin and/or reference samples meant new software tools were needed. Data format incompatibilities and difficulties interconnecting the data set were major technical challenges. Contractors wrote custom "middleware" interfaces to unify several database and analytic functions.

This provided a virtual tool linking many previously independent functions and freed the analysts from manually moving data among different platforms. Prioritizing samples was critical to maintaining high throughput. Work flow and tracking software modules were also developed. Protocols and software for evaluating and performing quality control of the software and analytic processes were designed and implemented (21).

The OCME recognized that its computers and data communication facilities were inadequate for this project. Data transfer between the NYSP and the OCME required new information technology infrastructure and support. A primary data repository allowing shared access to analysts outside OCME was set up on a secure server at the National Center for Biotechnology Information, in Bethesda, Maryland.

Because collection of personal reference and kinship samples was implemented rapidly, ~1/6 of the initial data had to be corrected or resampled. The KADAP developed new kinship and personal reference collection forms and components for standardized sample collection kits for future collections.

Summary of the effort and thoughts for the future. The OCME cataloged 19,913 putative victim tissue fragments from 2749 individuals reported missing. The fragment count increased to 20,120 because anthropological review identified commingled fragments (confirmed by DNA profiles). The DNA identification project generated more than 52,000 STR, 44,000 mtDNA, and 17,000 SNP profiles. As of September 11, 2005, about 850 of the 1594 victim identifications established for the 2749 WTC victims were based solely on DNA results. Most DNA identifications used standard CODIS genotypes. Although many CODIS genotypes originally failed, technical improvements leading to better DNA yields from damaged samples gave useful DNA profiles in 40% of the samples for which standard procedures failed. Modifications included a two-stage drilling process to isolate uncontaminated bone powder from the compromised specimens and modifications to the wash incubations, buffer concentrations, and elution times for the DNA isolation kit (16). Beyond standard CODIS STR typing technologies, more than 20% of the DNA identifications were made solely from mini-STRs. SNP analysis alone identified about 10 individuals with 10 more identifications made when SNP genotypes were used to supplement partial STR profiles. Additional identifications were made when mtDNA typing results were used to screen for potential matches, followed by DNA re-extraction and mini-STR retyping. No DNA-based identifications were accomplished by mtDNA

analysis alone, as expected. The rate of new identifications has become negligible. The OCME and the KADAP believe that additional large-scale efforts are scientifically unwarranted at this time.

In looking toward the future, the KADAP panel recognized several major needs for improvements in technology and infrastructure (see table, p. 1122) (22). There is no doubt that improved preparedness and enhanced mass fatality forensic infrastructure would lead to more rapid and efficient identifications in the event of future mass disasters or terrorist attacks.

References and Notes

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18. About four times more males than females worked at the WTC, so the statistical threshold of a female profile (by the amelogenin marker) was higher than for a male. The reduction in stringency also meant if one fragment sample was matched to a reference sample by the criteria, a second fragment with a partial profile could also be matched if the random match probability of the two victim fragment samples was at least 1×10^{-8}.
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20. The population group reference DNA panel comprised 525 samples previously collected by the OCME; African American ($n = 126$), Caucasian ($n = 123$), Hispanic ($n = 151$), and Asian ($n = 125$).
21. National Center for BioTechnology (www.ncbi.nlm.nih.gov/IEB/Research/GVWG/OSIRIS/index.htm).
22. A "Lessons learned" document will be available through the National Institute of Justice: (www.ojp.usdoj.gov/nij/).
23. B. Budowle, F. R. Bieber, A. J. Eisenberg, *Leg. Med. (Tokyo)* **7**, 230 (2005).
24. KADAP attempted to determine an end point early in the process, but this could not be done because of unknown variables and the overwhelming nature of the attacks. The development of principles to facilitate such determinations would be useful.
25. We dedicate this to the memory of the victims of the WTC attack, to the families, and to everyone whose efforts made the identifications possible. We gratefully acknowledge Mark Dale for his advocacy and support.