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Picking up the Pieces: DNA Identification of Mass Disaster Sites

Josef Stalin once said that "the death of one man is a tragedy and the death of millions is a statistic" – but the anonymous figures behind that statistic are becoming lucid with the advent of mass disaster DNA identification. This new era may be in its infancy, but for humanity, it is the culmination of a scientific process that has lasted for centuries. Burial sites have a long history, going back thousands of years to the Neanderthals. The practices remain in mutated form today, a catharsis after the dramatic loss of a loved one or friend. That need for release, for understanding, becomes even more important with the death of many. As societies merged and evolved throughout the Middle Ages, mass disasters seemed to become more prevalent. Some historians refer to the 14th Century AD as the "Disaster Century" for its famines, wars and plagues. The Mendicants became popular, and many asked why such trauma was hitting society. However, the scale of horrors that have targeted humanity in the last century, from the machine guns in World War I, the death chambers of the Nazis and the massive earthquakes and hurricanes of powerful weather patterns have changed the meaning of mass casualty. The anonymity of the past that these images evoke demanded better tools for identification, and DNA answered that call.

DNA's ubiquity has revolutionized many fields, and this is no less the case in mass casualty identification. The development of more sophisticated and efficient identifying equipment has allowed organizations for the first time to identify bodies from these sites in a systematic way, rebuilding communities and providing closure after horrific moments of despair. In this paper, I will provide a

summary overview of the use of DNA identification techniques at mass disaster sites. First, I will present an overview of the major goals of DNA identification and provide context for these methods. Second, I will discuss some initial considerations of DNA identification, such as what fragments should be identified and how a team should approach degraded DNA. Third, I will present a technical summary of the current techniques used, followed by a wide variety of case studies showing these methods in action. Finally, I will look at some of the innovations and developments in technology that will shape this field in the years to come.

The Objectives of DNA Identification

There are several objectives in identifying remains at a mass casualty site. The first and most important one is closure, both for the victims' families and for the national and ethnic groups linked with the site. The classic Kubler-Ross grief cycle posits that only when someone reaches the final stage of acceptance will the stress of a traumatic incident subside.[1] Reaching that stage, however, can be difficult when a victim's remains have not been identified. Part of the coping process often includes creating alternative possibilities, such as the victim forgetting to board a plane before it crashed. In the worst cases, this avoidance of reality can lock the victim's family or loved ones in the very first stage of the grief process – denial. DNA identification does not lessen grief, but it can slowly initiate the healing process. Beyond individuals, there is the wider social context that must be taken into account. Identifying the bodies at a mass grave, for instance, can provide a different kind of closure – one that ends the chapter of a long nightmarish history. Newspaper accounts often mention the importance of providing closure to families and ethnic groups who were targeted, a goal that has become more realistic with DNA identification methods.[2] In cases where a dictator denies the existence of a mass grave,

victims can now use DNA identification to provide counterevidence, assuring them a proper acknowledgment of history.

The identification of mass graves goes beyond the need for closure though, as war crime tribunals can now use the evidence to convict their perpetrators. DNA evidence has become more common in American court cases, and international court cases are increasingly considering DNA evidence. A recent example comes from the tribunal of Saddam Hussein, the former leader of Iraq. Proving the charges against him, while perhaps obvious to most observers, is significantly more difficult in a court setting where standards of evidence are required. Witnesses can be intimidated and perpetrators often hide or destroy physical evidence. These evidential matters are of less consequence due to new evidence from DNA. As written in the USA Today, "… prosecutors should have ample evidence when Saddam goes to trial [… as] DNA will help to clearly establish the identity of many of Saddam's victims who ended up in the country's mass graves."[3] With DNA evidence, these issues are no longer barriers to a final sentence.

For individuals staggering from the loss of someone at a mass casualty site, financial reasons may also play a small but significant part of the need for DNA identification. A death certificate will most likely be required to access a life insurance policy. In order for a Medical Examiner to file a death certificate, a positive identification is often required unless corroborating evidence can be collected.[4] DNA can significantly increase the speed of this process, especially at a mass casualty site. For this reason, the National Institute of Justice has encouraged MEs to use this information in making their determinations.[5] Another financial benefit can come from a compensation fund. After September 11, the US Government created a Victim's Compensation Fund tasked with dispensing about $6 billion to families.[6] As part of the process, the fund's investigators had to place victims at the site to avoid

fraudulent claims. They used DNA evidence when available, which increased the speed of the process for those families.[7]

One final objective is clearing up the historical record of ancient sites. Scientists and historians are often forced to make conclusions about ancient plagues and massacres without significant evidence. Today, these two fields are attempting to use DNA techniques to better evaluate history. One example is the recent identification of the Romanov family. For years, there were questions about the final fates of the children of Tsar Nicholas II. DNA evidence has now confirmed their final burial place at a small gravesite, indicating the final resting site of the bodies.[8] Further clarification of the past will only increase with better methods. Scientists have just published the first draft of the Neanderthal genome,[9] and when the techniques developed for retrieving this ancient DNA are used elsewhere, further identifications of mass casualty sites will be found in the near future.

Initial Considerations

Before a mass casualty strikes and DNA identification is underway, there are significant considerations that a chief medical examiner must evaluate if the process is to be successful. First, there are significant ethical and policy related questions that should be addressed before a disaster occurs. The National Institute of Justice (NIJ) has created a list of such questions to guide crime labs across the United States.[10] One of its major questions is whether scientists should identify each person or every fragment recovered at a site. This question is generally the hardest to answer with foresight, but is incredibly important nonetheless. In the aftermath of a large disaster, families of the victims will demand the lab identify every fragment. However, this is far more than is necessary from a legal standpoint. As briefly mentioned previously, identification at a site requires the recovery of a single DNA fragment for an ME to sign a death certificate. Any additional identification is beneficial for

emotional closure. The balance between the identification of as many fragments as possible and the financial impact of such a decision should be carefully considered before the decision is needed in practice.

Another set of questions revolves around the importance of setting expectations. Crime labs need to make decisions such as defining a minimum sample size and a timeline for the recovery and identification period. Following a disaster, emotions will often reach extraordinary levels. Labs will be under incredible public and political pressure to speed up the process, to identify more fragments and to announce greater numbers of positive identifications. The public's goals must be mediated by scientific reasoning. Generating a false positive identification is one of the worst failures possible and must be avoided at all costs. Therefore, promulgating proper procedures for identification, and if disaster strikes, providing the public with accurate information is crucial for satisfying the demands of stakeholders. No laboratory should compromise these principles, regardless of the pressure involved. Making these decisions early and codifying them into standardized procedures will improve accuracy and reduce pressure from politicians.

Another consideration is defining the actual identification procedures, and whether DNA should even play a role in such an effort. According to the most recent standards, the first step in an identification process is having a forensic archaeologist/anthropologist evaluate the remains. The expert should identify any visible traits, such as age, weight, height, gender or other physical features. The investigator should also attempt to separate samples as best as possible. From there, the forensic archaeologist can refer samples to various specialists, such as odontology, radiology and fingerprint experts. As should be clear, DNA identification is not the perfect tool in all circumstances, especially when easier and cheaper methods are available. Despite the increasingly cost-effective technologies developed, DNA identification can still be quite expensive. In addition, the results of DNA are not

easily identifiable by a victim's family. They cannot see the DNA as they can teeth or bones, and it is that physical reassurance that can be crucial for sustaining trust in the identification. If possible, labs should use multiple methods to provide additional sources of data, especially traits that can be used as confidence factors by the victims. Overall, this standard procedure places DNA at the end of the process for identifying bodies, although the NIJ knows that this will also be the most common method at disaster sites.

These decisions have occurred before a disaster, but there are also considerations that must be evaluated after a mass casualty identification effort is underway. The most important one is determining the type and extent of damage to the DNA samples. Depending on the disaster, samples may be hard to collect, hard to identify or both. The Armed Services DNA Identification Laboratory has done extensive work on these types of samples, and has identified some of the largest difficulties with identifying remains.[11] Among bones, the femur and ribs were the best for collecting a sample of usable DNA. The cranium was the worst, since the porous structure of the bone provides more surface area for environmental degradation. Even if a sample is collected, it may have been destroyed by a number of contaminants such as acids in the soil, jet fuel, weather elements, or a number of other possibilities. Beyond the specifics offered by Edson's paper, there is simply the larger issue of separating out the different samples. While the NIJ has charged such a task to the forensic archaeologist, it may simply be impossible to separate samples individually. This makes it even more difficult to sequence the samples through PCR and STR-analysis. Degraded DNA can throw off PCR primers, and with multiple samples, it can be impossible to tell which base pairs come from which sample. This discussion leads back to the earlier question of setting expectations – DNA evidence may be difficult to obtain in certain situations, and lab personnel should take the unique properties of a disaster into account.

Procedures in Identifying DNA

DNA identification at mass casualty sites is very similar to the process used in typical crime investigations. The basic premise of any DNA identification test is to make a match between the test sample and a reference sample. Since no sample is entirely sequenced (which at best estimate would cost $50,000), forensic investigators have developed shortcuts for comparing a sample to a reference. The most common method is quantifying the variants at Short Tandem Repeat (STR) locations. These sites have a variable number of repeated base pairs that are detectable through lab techniques.[12] While there are hundreds of these sites in the human genome, the FBI has selected 13 of them to make comparisons easier between samples.[13] Today, automation has made sequencing these STRs easier. Polymerase Chain Reaction can amplify the DNA at these sites, and STR typing technologies like gel electrophoresis, mass spectrometry or microchip CE can count the number of repeats at each locus.[14] Identifying the number of repeats is the first phase of identification. The true utility of STRs comes from the wide variation of these repeats in the human population. Surveys were taken of different races and ethnicities to determine the probabilities for each of the CODIS STR loci. By multiplying out these percentages, scientists can determine a probabilistic estimate of whether the reference sample matches the test sample. In practice, 13 loci are sufficient to make this determination.

While this technology is sound for typical samples found at crime scenes, mass disaster samples are not usually as complete. Whole parts of the DNA may have disappeared, eliminating one or more of the loci detected by typical lab equipment. This can lower the discriminating power of the statistics involved, preventing a clear positive match with a reference sample. Scientists have addressed this problem in multiple ways. First, more STR loci can be sequenced to provide additional reference locations for comparison. Carolyn Hill of the National Institute of Standards and Technology has looked into the issue of degraded DNA and has determined an additional 26 loci that are possible sites of

identification.[15] She and her colleagues chose STR loci that were significantly distant from the 13 CODIS ones and spread throughout the genome, increasing the likelihood that enough loci could be determined. More importantly, the team developed primers that are incredibly close to the loci. Since PCR uses these primers to amplify a strand of DNA, they are much more likely to be successful in damaged DNA if they are closer to the desired sequence than further away. Lastly, the team performed population studies to determine the variable expression of the different alleles at each of the 26 STR loci. This table makes these sites meaningful, since the statistical power comes from the comparisons between the sample, the reference, and the general population. With 39 possible sites to choose from, scientists have a higher chance of determining a link between two samples, even with degradation.

If adding additional STR loci is ineffective, another method is to sequence mitochondrial DNA (mtDNA). MtDNA is located in the mitochondria, and each cell has thousands of copies of it, increasing the chance that a full copy survived degradation. Forensic scientists have developed multiple sites of interest in mtDNA. Instead of STRs though, they use Single Nucleotide Polymorphisms (SNPs), or a single change to one base pair that can be determined and compared statistically to a population in order to generate a match statistic. The development of these sites has been a long development over the last decade. Michael D. Coble, a member of the Armed Services DNA Identification Lab, has sequenced the mtDNA from several hundred Caucasians and has determined a variety of SNPs of interest for identification.[16] In the paper, he has generated eight panels with several SNPs a piece that can be used to identify an mtDNA sequence. Once the sequences of the test sample and reference are determined, the math is very similar to that in STR analysis – the individual SNPs are compared to probabilities within the population and multiplied. With the number of SNPs in this analysis, the discriminating power is usually high enough to make a definite match.

Case Studies and History in Mass Disaster Identification

September 11, 2001 was the beginning of one of the largest DNA identification efforts in history, and one of the first major uses of these techniques in American history. The magnitude of the site was enormous. Almost 3,000 victims were believed to be in the towers when they collapsed.[17] The wreckage itself did little to make the victims easier to identify. As the scientists who advised the DNA identification process wrote in Science:

> "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 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."[18]

Furthermore, there was no city, state or even national infrastructure for identifying so many samples, which at final count surpassed 40,000.[19] Dr. Charles S. Hirsch, as Chief Medical Officer of New York, had the unenviable task of identifying all of those samples while keeping the media and public heat away from the city's morgues.

In this case, the team followed the standard procedures for identifying DNA: collecting samples from the scene and from family members and beginning to make comparisons. The largest issue for the team, and the one this case study focuses on, is the management of those thousands of samples of DNA. At the time of the disasters, no software package was designed to handle the vast amount of data that this site required. The Chief Medical Examiner hired the Gene Codes Corporation to write the software program necessary to store and present the results.[20] The time requirements for such a software program were quite unusual. Software is generally planned well in advance of its deployment, with enough time to create a software design document and to control for quality. This was not the case here. Cash and his software team needed to write a product immediately, as the DNA identification was already

underway and data needed to be quickly stored in a very dependable system. He selected a programming methodology known as Extreme Programming, which at the time was in its infancy. The goal of Extreme Programming is to write high-quality code in an incredibly short period, focusing on better communication between the customer and developers and higher satisfaction with better code.[21] This programming methodology was perfect for the task according to Cash. Every week, his team released an update to the software with the latest changes and additions asked for by the identification team in New York. This meant that if a problem arose or a feature was missing, the development team had the ability to quickly address the problem and release a new version. By the end of the process, the software was greatly assisting the OCME in keeping track of the data, from the actual base pairs to metadata such as where a sample was found and what characteristics it had.

9/11 was the vanguard for mass DNA identification in the United States. Following the conclusion of the process, the US Department of Justice created a task force charged with designing common standards and best practices in the aftermath of a major disaster. Software packages and data management now underlie many of those standards.[22] The process may have rocky in the initial months, but the OCME was not without significant successes. By the end of the identification process, it had identified 1,585 victims (58%) at the site through DNA evidence.[23] Nonetheless, this number poignantly demonstrates how difficult DNA identification can be at mass disaster sites. The process, though, is not ending. The OCME has saved the DNA samples in protective storage, and it will attempt to sequence them when better technology is available.

Mass disasters, unfortunately, are not exclusive to the United States. In the Balkans, numerous wars, conflicts and genocidal campaigns occurred during the 1990s. These conflicts have left many dead, most famously the 8,000 killed at Srebrenica. International outrage at the human rights abuses, now codified in agreements, led to the goal of identifying the victims and returning remains to victims'

families. The Dayton Peace Accords of 1995 created and charged the International Committee on Missing Persons with identifying those who had died in the fighting.[24] At the end of 2006, they employed 200 people and held more than 80,000 reference samples and 18,000 bone samples in their collection from the conflicts.[25] Many of these samples are highly degraded, and in response, the ICMP has developed its own set of primers for PCR to generate better results. Its efforts have led the way for mass casualty DNA identification outside of the United States. Through analysis at its two laboratories, the organization has identified 14,404 individuals at sites throughout the Balkans region – more than nine times the identifications made at the World Trade Center site.[26] Srebrenica, the most infamous mass grave, is also one of the most challenging for the organization. According to the ICMP, bodies at the site were moved in an attempt to scatter the remains and hide the atrocity. The ICMP has used forensic anthropology (the same anthropology that begins the process in American procedures) to put bones back together and attempt to sort through the many secondary burial sites. The efforts have paid off – in 2005, they identified their 2000th victim.[27] ICMP's techniques and expertise have been used after other major mass casualty events, including the 2004 Asian Tsunami and Hurricane Katrina.

Both of these case studies have looked at present sites, but this is not the only time when DNA identification is useful. Historical sites are a major focus too, especially those from World War II. In recent years, there has been increasing focus on the people of World War II, sometimes colloquially referred to as the "greatest generation."[28] This interest has led to new resources and renewed efforts to identify missing war veterans and attempt to clear up ambiguities in the historical record. A group of scientists led by Damir Marjanović attempted to identify 27 bodies from World War II in Slovenia.[29] The team considered other identification techniques, but the degradation of the bodies practically required DNA techniques. The team collected teeth and bone samples from the femurs and brought them back to the lab for processing. As is typical in these instances, the bone fragments are sanded to

remove exterior contaminants. This increases the chance for a successful identification (one of the reasons why bones from the cranium are often difficult for identification). Optimized agents were used to extract a sample, and once it was obtained, the DNA sample was placed through PCR and electrophoresis to create a test sequence. The team then compared it to suspected familial DNA. This comparison is difficult, since a direct match is impossible. Nonetheless, the team was able to identify several of the fragments with a high degree of certainty. This study was completed in mid-2007, and represented one of the first times that nuclear DNA had been recovered from an historical mass grave and used to make a positive identification.

The Onward March of Technology

Technology's rapid advance has greatly increased the efficiency and quality of DNA identification at mass casualty sites. One of the most important and ongoing developments has been the creation of more specific primers for STR loci and mtDNA SNP scans. As mentioned before, degraded DNA can block primers from properly binding with the DNA, preventing PCR from amplifying the sample. Primers that are more accurate allow scientists to get samples from otherwise useless DNA fragments. The Slovenian DNA analysis used better primers and managed to secure nuclear DNA identifications from World War II. As our understanding of the genome and degraded DNA expands, more accurate primers will be possible. Another continued development will be the creation of primers for non-standard STR loci. As mentioned with the Hill article earlier, scientists have now identified another 26 loci as significant for identification purposes, and they designed primers to bind as close as possible to the desired sequence. These developments of primers are rarely revolutionary, but each small increase in quality can lead scientists to better and greater number of more identifications.

Scientists are not just creating better tools, but new ones as well. SNP analysis is an increasingly sophisticated and accurate way to scan the genome. The Affymetrix GeneChip has the ability to scan 500,000 SNP sites throughout the genome.[30] In addition, scientists have a population-wide probability table for each of these sites, making it useful to compare two samples. The benefits are enormous. With hundreds of thousands of SNPs, a forensic investigator does not need to be able to sequence long lengths of a sample in order to generate a match. Instead, he or she can generally genotype the entire sequence and see what areas of the genome emerge as viable. This should increase the number of samples that come back with useful data. Another advantage is that SNPs can have phenotypic consequences. For instance, a base pair change at a certain SNP may be highly correlated with a single ethnicity. This evidence could be used to identify the bodies at a mass grave for instance, providing proof that there was a specific group targeted.

For all of the positives of SNP analysis, there are significant barriers and ethical issues that need to be addressed in order for the technology to be more commonly deployed. There still remains a significant cost barrier that is far higher than typical STR analysis. Considering that cash-strapped crime labs and private non-profit organizations handle most of the wide-scale identifying projects, such costs remain a large concern for these organizations. There is also a different financial issue. Companies have rapidly developed SNP analysis with the intention of determining different health outcomes for a sample. This can have broad implications for a test sample. Should life insurance companies have this data in order to block a claim? If a victim's compensation fund administers money based on future earnings potential (as the 9/11 one did), should they that take into account likely adverse health factors? These issues need significant pre-planning through regulation in order to be effective.

Related to SNP analysis, more development is expected in high-throughput database designs. There are 13 STR loci and there are 500,000 SNPs. While much of that data is compressed, comparing

thousands of samples with each other is a monumental task, and one that can still use additional IT support. Like other facets of mass DNA identification, further research in this area will continue to expand the capabilities of labs and ensure a steady improvement in DNA technology for the next disaster.

Conclusion

It was not so long ago that a major disaster would end with the massive burial of every fragment left at a site. That anonymity of the past is being eliminated with the advent of increasingly sophisticated DNA identification techniques available to scientists and organizations. What were once lost individuals of a terrorist attack are now properly buried remains – providing closure during a national nightmare. People once killed for their ethnicity or politics and left buried and forgotten beneath the land are now being exhumed – and the evidence left behind is now being used to bring justice to their killers. In an article entitled, "Genomic sequencing in the service of human rights," Kelly N Owens discusses the impact of these technologies: "Human rights violators have used increasing levels of technology to perpetrate their crimes. Genomic tools provide an opportunity to use advances in biotechnology to combat these crimes worldwide."[31] Justice is finally coming to the Balkans, in Latin America, and in almost all areas of the world. This identification is a morbid science, but one with a happy ending. There is another famous Josef Stalin quote, "Death solves all problems - no man, no problem." Problems are no longer forgotten with death – they have a new life in the light.

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