



Relevance of DNA profiling in air crash disasters

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Abstract

In cases of mass disaster, there is often a need for managing, analyzing, and comparing large numbers of biological samples and DNA profiles. This requires the use of laboratory information management systems for large-scale sample logging and tracking, coupled with bioinformatic tools for DNA database searching according to different matching algorithms, and for the evaluation of the significance of each match by likelihood ratio calculations. There are many different interrelated factors and circumstances involved in each specific mass disaster scenario that may challenge the final DNA identification goal, such as: the number of victims, the mechanisms of body destruction, the extent of body fragmentation, the rate of DNA degradation, the body accessibility for sample collection, or the type of DNA reference samples availability.

Keywords: Air crash, forensic, DNA profiling, database

Introduction

The identification of dead bodies in mass casualties has been a Herculean task since time immemorial. Fatal crash of civil or military airliners generally leaves numerous unidentified bodies. As compared to other mass casualties *viz.* earthquakes, floods and collapse of building, the identification of bodies is more difficult post-aircraft accidents as the bodies are mutilated to a large extent and at times decomposed when the aircraft crashes in remote places and recovery is delayed. There have been numerous modalities in identifying the dead in mass casualties like sex, body stature, identification by next of kin, face (if not mutilated), medals (in case worn by the military pilots), identification discs worn by the pilots, personal metal belongings e.g. metal chain, rings and dentition. DNA profiling is the gold standard for human identification. This technique has been widely used successfully not only in forensic and crime investigations but also in identification of victims in mass disasters, including air crash.

A mass disaster is an unexpected event that causes serious injury and death to a number of people. Mass disaster events may be natural disasters (earthquakes, flooding, and tornadoes), accidental disasters (aircraft crashes, train crashes and derailments, and building fires), or intentioned terrorism acts (direct attacks on significant objectives, and bombing of populated areas, including suicide attacks and deployments of chemical and biological weapons). Forensic identification of victims is essential for humanitarian reasons, but also for civil or criminal investigative needs, and it is essentially based on forensic anthropology, fingerprints, forensic odontology, radiology, and DNA typing.

The main task of forensic DNA laboratories faced with mass disaster cases is to help name every anonymous victim, thus bringing closure to surviving family members and friends. This is done by matching DNA profiles of postmortem tissue samples with those of antemortem DNA samples (personal items or biological specimens) or by kinship analysis with living relatives. Generally, mass disaster cases require managing, analyzing, and comparing large numbers of biological samples and DNA profiles (mainly autosomal short tandem repeat [STR] profiles but also occasionally

mtDNA sequence and Y-Chromosome STR [Y-STR] haplotype data) making necessary the use of electronic laboratory information management systems for large-scale sample logging and tracking, coupled with bioinformatic tools for DNA database searching according to different matching algorithms (ie, complete or partial allele sharing on each locus for autosomal STR markers), and software solutions to evaluate the significance of each match by likelihood ratio (LR) calculations.

There are many different interrelated factors and circumstances involved in each specific mass disaster scenario that may challenge the final DNA identification goal, such as: the number of victims, mechanisms of body destruction, the extent of body fragmentation, rate of DNA degradation, the body accessibility for sample collection, or type of DNA reference samples availability.

DNA Sampling and Information Management

Experiences gained from previous mass fatality incidents reinforce the need to make all necessary steps to guarantee sample preservation for DNA analysis and to use suitable protocols for documenting the chain of custody of DNA sampling and body tracking. To help with this purpose, specialized and trained disaster victim identification (DVI) multidisciplinary teams composed of medical examiners, forensic pathologists, anthropologists, forensic odontologists, fingerprint specialists, radiologists, and experts in search and recovery of physical evidence have been developed worldwide. Some examples of federal resources providing aid to local communities in mass disaster response in the USA are the Disaster Mortuary Operational Response Teams (DMORT), the Federal Bureau of Investigation's Evidence Response Team (ERT), and the Office of the Armed Forces Medical Examiner (OAFME). Different Interpol DVI teams have also been developed worldwide and a standing committee on DVI is responsible for recommending measures for improving identification procedures, by encouraging international co-operation and standardization.

Additionally, recent guidelines were published to assist the medical examiner with the whole process of victim identification in mass fatalities, including detailed

procedures for DNA sample collection and data management.

Direct and Family DNA References

Two types of reference samples are usually collected for DNA comparison with mass disaster remains: appropriate family references and direct references, such as personal effects, or antemortem biological specimens, such as biopsies and bloodstain cards.

At present, the simplest and most efficient method of DNA identification is to match each STR multilocus victim's profile to a direct antemortem sample of the victim. Personal items, like toothbrushes, and used shavers and razors have been extensively used as direct references in many cases. The main drawbacks of this strategy are the potential source attribution errors, leading to false exclusions, and the presence of exogenous body fluid or cell debris contaminations leading to mixed DNA profiles. Therefore, these samples cannot be used for exclusionary purposes and, whenever possible, a match obtained with a direct reference should be confirmed through kinship analysis or an analysis of a second direct reference sample.

The possibility of using antemortem biological specimens (like bloodstain cards) as direct references with an accredited and documented chain of custody will overcome the main drawbacks of personal items. Indeed, the establishment of a DNA repository to store reference bloodstain cards has been recommended to facilitate the identification of military personnel in air crash accidents.

Sample preference for family references depends on the type of DNA analysis. The most discriminative power is obtained by using a large number of nuclear STR markers (from 13 to 17 markers) to analyze the following family references: (a) either or both biological parents of the victim, (b) biological mate of the missing person and their child/children, and (c) multiple biological full siblings (sharing the same parent as the victim). The analysis of haploid DNA markers with just maternal (mitochondrial DNA) or just paternal (Y-Chromosomal markers) inheritance allow the use of maternally or paternally-related family members as references. Buccal swabs or blood (collected by venipuncture or by finger stick devices) are the recommended samples for both nuclear and mitochondrial DNA analysis.

The use of standardized collection forms for both direct and family reference samples by trained interviewers, preferably using electronic forms, whenever possible, to avoid handwriting as much as possible, as well as the use of specific sample collection kits will improve the reliability of the sample selection and donor information.

The information data of each reference sample should also be logged, with a unique numbering code using the LIMS system, into the same central database used for victim samples. This allows rapid transference of edited DNA profiling results to each registered sample for subsequent pair-wise comparisons, maintaining and documenting the chain of custody through the whole DNA identification process.

Plane Crash

Modern airplane disasters usually result from a series of improbable, almost random events and pilot-system interactions that are difficult to predict. Mechanical failures are usually a series of events that never happened before.

There are few clearcut, general statements about how planes crash because all the obvious problems have long since been addressed. It is somewhat reassuring to realize how many things must go wrong for the typical crash to occur. It is even more reassuring to know that multiple improvements usually result from the investigation, any one of which would prevent similar future accidents. Fundamentally, large commercial aircraft obtain increased safety from having redundant systems and procedures; there are at least two of everything, including pilots and engines. One event, error, or failure will not crash the plane.

Plane crash is an event related to an aircraft operation arising from the time when one or more persons embark on an aircraft intending to fly until the moment of disembarkation of all persons from the aircraft and the consequences are- death or serious bodily injury to one or more persons on or off the aircraft, destruction of the aircraft, disappearance of the aircraft or unavailability of the aircraft as a result of an accident, damage to the basic structure of the aircraft or greater damage to the property of a third party. The consequences of plane crashes are for persons transported by aircraft, as a rule of extreme difficulty and most often marked by fatal consequences, or by people's suffering. Plane crashes often pose exploratory and accompanying tasks that are inherent to all disasters (search, rescue, finding persons and objects, identification, financial and technical assistance, cause analysis, etc.). Plane crashes, according to the described and other features, belong to the most complex research objects.

Identification of Dead Bodies

Identification of the dead bodies is a compulsory exercise after their recovery and retrieval from the disaster site, before final disposal. Identification of dead bodies is mainly done by comparing and matching the information of the missing person (physical characteristics, personal belongings, place and circumstances of death, etc.) with corresponding information of the deceased mostly with the help of surviving relatives and the community. In case a body is unidentifiable, different techniques for identification are mandatory to be carried out for which adequate infrastructure is needed at all levels, along with availability of trained human resource. The guiding principles for identification and its processes, infrastructural requirements, along with specialized techniques, used singly or in combination, can be classified into primary and secondary methods. The primary methods are laboratory-based specialized techniques used for identification of the dead body, whereas the secondary methods include visual identification, personal description, photography, medical findings and any other evidences found on the dead body.

Human Identification in Major Human Catastrophes

In catastrophes, the main objective is to save the involved living beings. The second objective is to identify the deceased people. The need to identify corpses is related to legal, criminal, civil and moral issues. In the moral and ethical scope, citizens have the right to receive the mortal remains of their relatives, whether they are victims of any type of catastrophe, disaster or emergency. In the civil sphere, for example, the absence of a death certificate due to the lack of a corpse makes all civil procedures difficult, which are essential for families to deal with indemnities or pensions, among others. Concerning the legal and forensic

scope, the absence of an explicit identification leads to questions related to the disappearance of the individual, for example by kidnapping for subsequent human trafficking. For this reason, it is imperative to locate and identify all individuals allegedly involved in the disaster or catastrophe. Human identification is, at its core, a comparative exercise. Based on this methodology, by collecting individualizing and discriminating identification data, it is possible to achieve what is known in forensic sciences as “*positive identification*”, the attribution of a unique and unequivocal identity, usually “*name and surname*”, associated with legal registration in a given country. For this, it is necessary to collect data from the cadaver (postmortem data), allowing forensic scientists to build a biological profile, which can be of different types: dactyloscopy, dental, anthropological and genetic.

Traditionally, anthropology was used as a basis to identify deceased people, due to the simplicity of data collection and the use of a reduced number of materials and equipment. It was based mainly on the observation of postmortem information and the external examination of the remains found, focusing essentially on the analysis of visible phenotypic data (hair color, height, skin color, among others) and identifying physical traits (general characteristics of sex, approximate age, tattoos, scars). Although these techniques were fast, they do not allow for the identification of an individual, instead providing only a guide for the investigation, for example, “*the deceased person is a man 40–50 years old*”. It allows the exclusion or inclusion of the victim from certain phenotypic groups, but it did not permit the identification of the victim.

Currently, the identification of individuals is carried out using three methods, in the following order: first, fingerprints; second, dental records; and third, genetic analysis. Although fingerprints and forensic odontology are indeed considered reliable, fingerprints and/or dental samples are not always available. This is where we find the relevance and usefulness of genetic analysis, a universal technique that can be applied to practically all biological samples, from both living individuals and human remains.

Forensic Anthropology and Plane Crash

A forensic anthropologist may create facial reconstructions to help identify skeletal remains [4]. Facial reconstruction clay is placed and shaped over the victim’s actual cranium, and it takes into account the decedent’s estimated age, ancestry, and sex. With the help of this technique, a composite of the victim can be drawn and advertised in an attempt to identify the victim. Forensic anthropologists are also helpful in identifying victims of a mass disaster such as a plane crash. When such a tragedy occurs, forensic anthropologists can help identify victims using the collection of bone fragments. Usually, the identification of the remains will depend on medical records, especially dental records of the individuals. However, definite identification of remains can be made only by analyzing the decedent’s DNA profile, fingerprints, or medical records. Recovered remains may still contain some soft tissue material, such as the tissue of the hand, which may yield a DNA profile for identification purposes. If the tissue is dried out, it may be possible to rehydrate it to recover fingerprints also.

Except for identical twins, no two people on earth have the same DNA (deoxyribonucleic acid). Advances in DNA

technology have allowed criminal cases to be solved that previously were thought unsolvable. Since the 1980s, DNA evidence has been used to investigate crimes, establish paternity, and identify victims of war and large-scale disasters. Because each human is unique, DNA evidence from a crime scene or from an unidentified body can be traced back to one and only one person. DNA evidence can be used to link a suspect to a crime or to eliminate a suspect. It can also be used to identify a victim, even when nobody can be found. DNA evidence has been used to identify human remains of victims of large-scale disasters, such as plane crashes, tsunamis, and hurricanes. In all life forms—with the exception of a few viruses the basis for variation lies in genetic material called DNA. This DNA is a chemical “blueprint” that determines everything from hair color to susceptibility to diseases. In every cell of the same human that contains DNA, this blueprint is identical, whether the material is blood, tissue, spermatozoa, bone marrow, tooth pulp, saliva, or a hair root cell. Thus, with the exception of identical twins, every person has distinctive DNA.

Small DNA fragments are still useful for analyzing old specimens and crime scene evidence. Short Tandem Repeat (STR) defines a small region (locus) in which different numbers of tandemly repeated core DNA sequences, two to eight base pairs in length, are found. STRs are favored because of the ease with which they amplify by the polymerase (PCR) chain reaction. STR markers have a number of repeats that vary considerably among individuals, thus making them useful for identification. With one nanogram (1ng) of DNA sample, matching probabilities of one in a billion or more is possible. Moreover, the results can be obtained in a few hours, compared to the days or weeks required by RFLP (Restriction Fragment Length Polymorphism). In part, this is the result of automating the analysis of STRs. In addition, with STR methods, owing to the use of PCR chain reaction, both the quantity and quality of the crime scene DNA can be small (0.1 to 1ng) and highly degraded, yet it is possible to obtain distinctive discrimination between unrelated or even closely related individuals, except identical twins. The DNA database now being compiled uses 13 core STR loci in the DNA molecule. The “core” STR loci referred to is the use of the same DNA regions for typing, used by forensic science laboratories in the United States, in cooperation with the FBI in CODIS (Combined DNA Index System). With this uniformity of procedure, they can exchange and compare case work and database typing information. By selecting 13 loci, the power of discrimination rivals that of RFLP analysis, which is expensive and requires considerably more time and DNA to complete. STR markers are now also used for paternity testing. Similarly, STR typing is employed to identify human remains in cases of mass disasters, including high-temperature fires and airplane crashes in which the victims are subjected to obdurate water damage.

Dental DNA Fingerprinting

Molecular biology developed when it was realized that DNA lies behind all the cell’s activities. The development of methods and techniques to study processes at the molecular level has led to new and powerful ways of isolating, manipulating and exploiting nucleic acids. DNA fingerprinting is the result of such an endeavour. This technique is mostly known by its application in forensic medicine, but is also used in transplant medicine, in the

search of hereditary disorders, consanguinity, paternity, and in anthropology. The role of dental restorations, prosthesis and radiological identification as the main stay of forensic odontology has declined lately, whereas molecular biology and laboratory procedures are rapidly increasing in efficiency and availability. The tooth is the most valuable source to extract DNA since it is a sealed box preserving DNA from extreme environmental conditions, except its apical entrance. Teeth has been the subject of DNA studies as the dental hard tissue physically encloses the pulp and offers an anatomical configuration of great durability. Moreover, even a single tooth provides valuable information regarding the individual to whom the tooth belongs.

Teeth are important evidentiary material in forensic cases since they are more resistant to postmortem degradation as well as extremes of environmental conditions. Teeth are also easy to transport and serve as a good source of DNA. Comparisons of antemortem dental records with skeletal remains provide useful means to identify individuals; even in a mass grave. In affluent societies, dental records may be decisive in determining the identity of individual victims. However, in less affluent communities, and these are more likely to be involved in human rights abuses leading to mass murder, dental records are unlikely to be available. In this situation the only option for identification is DNA analysis. It is possible to discriminate one individual from all others with a high level of confidence by starting with only 1ng or less of target DNA, whereas the amount of DNA that can be recovered from molar teeth with pulp volumes of 0,023-0,031cc is nearly 15-20mg.

Identification of bodies or fragments

After the bodies or parts have been rescued, the classification work corresponds. The classification is nothing more than the determination of the identifying tetralogy of each fragment or part that was collected, which is nothing more than the determination of the sex, the ancestral pattern, the height and the age that the subject(s) must have had at death; also known as "Big Four." The quality with which it is made and the accuracy will later determine the speed with which the identifications are achieved.

Sex determination is based on the sexual dimorphism present in the human species. If the corpses or fragments did not include the external genitals, we used other methods. In a general sense, male is more robust and their diameters and circumferences are larger. On the other hand, as a product of evolution, the female pelvis has undergone important changes for adaptation to pregnancy, from the bipedal posture being the most dimorphic region for sexual differentiation.

In terms of ancestral pattern, it is currently divided into three main groups: Whites (European ancestors), Blacks (Sub-Saharan African and Australoid ancestors), Mongoloid (East Asian and Amerindian ancestors) and a mixture of these groups. If the skin, hair or face not exist, the main differences are located in the skull and face, with very specific characteristics of each ancestral pattern, although as cosmopolitan biological beings and with interactions between groups, these patterns are not so strict. What arises is the skull has predominant characteristics of a determined ancestral pattern.

The height from human remains is calculated taking into account regression equations obtained for the different

populations from studies carried out in them, which are mainly based on the lengths of the long bones or their fragments. It is recommended that equations calculated from each population be used in order to have more accuracy when estimating.

To calculate the age, it is first verified whether the remains correspond to a subadult or an adult. If it is a subadult, the method is based on the analysis of the stage of growth and development in which it is found (X-rays are very useful). As life progresses until approximately 28 years of age, growth changes occur in the skeleton; first, ossification points appear, and then the epiphyses begin to fuse with the diaphyses. The order in which each process occurs is reported in the dissimilar investigations carried out on the subject. Likewise, dental evolution has been studied in the same way.

When the subject is already "osteologically" an adult, then the age is calculated by the deterioration that the person suffers over the course of life. There are regions of the body that present gradual changes that are well studied and are closely related to the environmental effects of climate, physical activity and habits. These changes include the processes that occur in the pubic symphysis, the auricular facet, the sternal end of the first and fourth ribs, the sacrum, the acetabulum and, dental wear, less accurately, the closure of the cranial sutures. But in this case of disaster the pubic symphysis, and the auricular facet many times could be broken or difficult to analyze.

In order to make the expert work more effective, our own standards have been developed based on the Cuban population, to estimate parameters of the biological profile (sex, height, age and ancestral pattern). Some of these have been published in Spanish and Mexican anthropology journals. These standards have been included in computer algorithms that speed up the process of estimating the parameters. All of this has been validated in work in other provinces of the country, in air disasters with very good results, and in other countries that have a similar population or as a methodology that is useful for developing their own standards. Likewise, contributions have been made with other entities to improve the software.

In the same way, another group that includes psychologists and psychiatrists is in charge of obtaining antemortem information (data provided by relatives, friends, doctors, dentists, medical records, X-rays, etc.). Computerized axial tomography (CT), magnetic resonance (MRI) and DNA profiles from interviews with relatives and close friends. All that research is later corroborated with what was found in the morgue. There are models made by Interpol for the collection of both antemortem and postmortem data,

Once, the fragments or bodies have been classified, it is now possible to begin to make the absolute identification. First, you start with the extreme individuals: pilots (their topographic location in the place of discovery helps a lot), children, pregnant women, and easily detectable disabled people.

In some countries, positive identification is only considered when it is carried out using fingerprint methods, dental comparison or DNA, and this does not always have to be the case, since in an air disaster, there are often a closed number of victims. Let us give an example: a plane crashes into an uninhabited place, and all 70 people on board die; it is known that among those people, there was only one who had a hip prosthesis. In the search, the fragment or corpse

with the prosthesis is found, and it is not necessary to carry out DNA studies in this case. The same occurs with other elements such as intrauterine devices, certain surgical interventions, and certain tattoos, among other elements.

Genetic Identification

Genetic identification plays a crucial role when the remains to be analyzed are very old or present a very advanced state of degradation, as in the case of air accidents or explosions, not depending on a specific biological sample. One of the main advantages of genetic assessment is that information is found in all the nucleated cells of the body and can therefore be found in very small portions of soft tissue or bone fragments. This lets scientists determine substantially important data such as the victim's autosomal profile, biological sex, biogeographical origin, or external phenotype, or perform kinship analysis.

However, the individual's genetic identification can only be performed if there are samples to be compared with. It is possible to carry out a direct identification when there is a previously confirmed genetic profile belonging to the individual in question, for example, in police databases or biological samples resulting from medical diagnoses, such as biopsies. On the other hand, when it is not possible to access the individual's biological antemortem samples, an attempt is made to carry out an identification either through assigned samples, such as clothing or other types of personal belongings of the person with whom the remains are believed to be associated or through biological relatives. Usually, human identification in catastrophes is performed by resorting to biological kinship analysis. One of the fundamental steps for a correct genetic analysis is the collection of information from the family of the deceased, since it is crucial for the election of the best genetic marker for each case in question to know the relationship of the person with the deceased.

How important is DNA to the identification effort?

The degree to which human remains are fragmented or degraded determines the value of DNA analysis in the identification process. Intact, large body parts lend themselves to identification by less costly methods, such as X-ray, dental examination, and fingerprints. However, DNA analysis is the only viable method for identifying severely fragmented or degraded remains. Even when whole bodies are recovered, DNA analysis still may be the best approach when materials that are necessary for other modalities—for example, dental records or verified body identification by friends or relatives—are unavailable. Remains often are identified by multiple methods, which may or may not include DNA. For example, only approximately 25 percent of the identifications of airline crash victims are generally made by DNA exclusively.

The answer to the question of whether every victim or every fragment of remains will be identified frames the scope of the DNA identification effort. Obviously, intact bodies will require fewer DNA tests than fragmented remains, although decomposing bodies may not easily yield full profiles. For example, in an airplane crash with 50 victims, in which each victim's remains are fragmented into 100 pieces, the identification effort undoubtedly would end sooner if the goal is to identify each victim, rather than each fragment of human remains. Everyone—the public, the policymakers, and the laboratory personnel—needs to understand the

answer to the important question: “When are we finished?” If the policy is to identify all of the victims, DNA analysis would stop as soon as the last victim is identified—which means that some human remains may never be analyzed or returned to the families. However, when the goal of the effort is the attempted identification of all fragments, the work of the laboratory likely will be greater.

It is important to consider that, if a mass fatality incident is so large and devastating that it affects the psyche of a community, a country, or the world, the scope of the identification effort may be broadened to help acknowledge the breadth of the emotional ramifications. After the 9/11 attacks, DNA analysis can be the most for example, the Mayor of directed Office of the Chief Medical to do everything humanly possible to identify every fragment remains. This policy resulted in new DNA analysis techniques and approaches; any biological fragments that could not be identified preserved for potential analysis with future technologies.

The absence of policies guiding the number of DNA tests that will be attempted on severely compromised samples can have enormous consequences. In planning for a future mass fatality, policymakers should consider the impact on the public if technologies at the time are insufficient to obtain DNA profiles on all remains. Lessons learned from the World Trade Center (WTC) identification effort suggest that policymakers need to understand that the broadest testing scale can add years to a DNA identification effort.

Future Perspectives

The new technological revolution in the field of forensic genetics is allowing the implementation in laboratories (both public and private) of the methodology of massive parallel sequencing (MPS), for example, to determine some phenotypic characteristics (skin, eye and hair color). The use of this new technology constitutes a tool of undoubted utility in criminal investigation, as well as in the identification of victims from disasters and catastrophes. Several studies have already evaluated the existence of polymorphisms associated with skin color, hair color, eye color, male pattern baldness, type/shape of head hair, age, facial morphology, height, earlobe folding and hair graying, among others. Predicting other externally visible features, such as facial features for a robot portrait, will be even more difficult and, at present, still impossible because while all features of a person have a genetic basis, facial features are determined by genetics but also by the environment, such as tattoos and scars. Also, with MPS other informative information could be reached, such as autosomal and Y chromosome informative identity SNPs.

Despite the different difficulties encountered in identifying victims resulting from a catastrophe, genetics is the only modern tool with universal character, which can be used in essentially all biological samples, giving an identification of more or less accurate statistical character, depending on whether nuclear or lineage markers are used.

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