Missing persons genetic identification

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Abstract:
The paper presents identification of missing persons from poorly preserved post-mortem remains using molecular genetic methods. Highly polymorphic and individually specifical genetic markers that allow for identification of missing persons are microsatellites on autosomal chromosomes and on Y- chromosome, and mitochondrial DNA control region. For genetic profiling comparison biological material from post-mortem remains and reference samples have to be collected. When post-mortem remains are found shortly after presumed death of a missing person, his or her personal items are used for comparison. If these are not available, reference saliva samples can be collected from the missing person's relatives. Tissues stored in health institutions can be used if the person had had a diagnostic biopsy earlier in life. When reference samples are not available, genetic identification is not possible. The type of the biological material sampled from the deceased depends on the condition of human remains. Blood, soft tissues, nails, teeth or bones are most commonly used for genetic identification; the time required for DNA extraction depends on the type of the biological material available. Extracting DNA from teeth and bones is the most demanding and time-consuming method; it is used in cases when only skeletal remains are available, or when we cannot get sufficient amount of DNA from other tissues for genetic identification. When the genetic profiles of post-mortem remains and reference samples of the missing person match, the strength of genetic evidence has to be statistically evaluated and the probability of identification has to be reported.

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1. Introduction

The results of DNA identification have been recognised worldwide as evidence material in civil and criminal legal proceedings for nearly thirty years. In Slovenia DNA identification tests have been utilised for two decades. Molecular genetic methods are used not only to identify biological traces in criminal cases and to confirm family relationships, but also to identify missing persons. These may be victims of mass disasters (such as earthquakes, floods, fires, tsunamis), aircraft crashes, train accidents and terrorist attacks. Also, genetic analysis is used to identify charred, drowned or hanged persons, found long after death and unidentifiable by traditional forensic identification methods. A missing person's personal items, samples collected from his or her relatives or his or her tissue samples archived in health institutions can be used for identification purposes. If none of these are available, genetic identification is not possible (1). Before the discovery of highly polymorphic DNA markers, identifying missing persons was based on traditional forensic and anthropologic approaches. To-

day, these methods are complemented by DNA analysis, which represents the final phase of the identification procedure (2). Genetic identification is frequently the only method available for identifying missing persons, particularly in cases of poorly preserved post-mortem remains (3). DNA analysis can be used to identify missing persons thanks to the DNA molecule, which is unique for each individual and remains unchanged throughout one's life. In bones and teeth, DNA remains relatively well preserved long after death (4) therefore genetic material can be obtained from very old bone remains. In Slovenia, the oldest skeletal remains used for genetic identification were 300-year-old skeletons from Auersperg tomb (5).

The success of genetic identification depends on the quantity and quality of the isolated DNA; i.e. on how well the biological materials obtained from human remains are preserved. Whenever possible, length polymorphisms in nuclear DNA, which is highly polymorphic (autosomes and Y-chromosome) are used for analysis. Nuclear DNA in ancient and problematic biological samples may be severely degraded and present in insufficient amounts. In these cases, we resort to mitochondrial DNA (mtDNA) analysis. The circular conformation of mtDNA makes it less vulnerable to environmental degradation. It is present in multiple copies per cell, which facilitates its use in old and poorly preserved samples (6). Genetic methods are used to identify skeletal remains and poorly preserved or badly damaged remains of victims of aircraft, train or road traffic accidents, charred human remains recovered from fire and explosion sites, victims of natural disasters, terrorist attacks, wars, victims of post-war mass killings found in mass graves and others. MtDNA typing along with nuclear DNA analysis used in routine investigations allow for a comprehensive approach to molecular genetic identification of biological materials (7).

Identification of a missing persons using DNA analysis comprises four phases: a) collection of biological materials from human remains, b) collection of reference samples for comparison with human remains (personal items, archived tissues and samples from biological relatives), c) DNA analysis of post-mortem remains and reference samples (DNA extraction, determination, quantification, amplification and genetic profiling), and, d) comparison of genetic profiles from human remains against reference samples. The latter phase involves interpretation and statistical evaluation of genetic evidence and kinship probabililty calculation (8). The obtained genetic profiles of the human remains investigated have to be compared with the elimination database profiles from individuals involved in any phase of the identification process, and the purity of the extraction and amplification negative controls has to be checked.

2. Biological materials collected from human remains

The degree of DNA preservation decreases with the age of samples, and greatly depends on the local environment in which the body was lying from death to discovery. The complexity of the identification process depends on the condition of human remains. Identifying victims of aircraft accidents or explosions is a very difficult task. Human remains may be badly degraded, dispersed or intermixed (e.g. in explosions, mass graves may hold the remains of different individuals) (3). Sampling plays a key role in fragmented body remains (each body part must be stored and labeled individ-

ually). Genetic analysis may be used to determine whether different body parts belong to the same individual. Parts of the body with the same genetic profile come from the same person (9).

A forensic genetic laboratory should use different techniques for the identification of DNA from various biological materials. The choice of biological materials for genetic analysis depends on the condition of human remains. Isolation of DNA from blood samples is the least time-consuming; followed by DNA recovery from soft tissues and nails. DNA identification of fully skeletonised human remains, in which only bones and teeth are available for DNA analysis, is most time consuming. Only blood and soft tissue samples obtained shortly after death are suitable for genetic analysis. Blood of fairly good quality can be collected from the aorta of individuals ran over by a train or burnt to death in traffic accidents (2). In severely burned bodies, deep red muscle samples, hip cartilage and swabs from inside the urinary bladder can be used for genetic examination (10). Nail samples are collected from badly degraded bodies, in which skeletonisation has not yet taken place. In heavily skeletonised bodies, discovered seveal years after death, bone and teeth samples are used for DNA extraction. Extracting DNA from skeletal remains is a most time-consuming procedure and requires the greatest amount of preparatory work (3).

3. Comparison or reference materials

Reference samples may take the form of a missing person´s personal items, direct reference material (tissue samples archived in health institutions) or biological materials from his or her relatives (3). Direct reference materials are biological samples from a documented source which shows an unequivocal association with the missing person. In most cases, these materials (biopsy samples) were obtained while the missing person was still alive. The advantage of direct reference samples is that they come directly from the missing persons and that their quality allows obtaining of full genetic profiles (3). In the absence of appropriate source documents, due consideration should be given as to whether or not to use these direct biological materials. Items thought to have been used by the missing person, such as a toothbrush, razor, comb or worn clothing, are employed for DNA analysis (3). Unlike direct reference materials, personal items have no documented source which would confirm their authenticity. Items belonging to the missing person may have been used by other family members, which accounts for mixed genetic profiles obtained from reference toothbrushes. It is therefore very important to get accurate information on who else used the items belonging to the missing person. If the missing person´s personal items are found to have been used by several other persons, these individuals´ genetic profiles should be determined for elimination purposes when comparing post-mortem remains and personal items of the missing person. Personal items do not necessarily provide enough biological traces to allow obtaining of full genetic profiles, yet in most cases full profiles can be determined. In addition to direct reference materials and personal items, biological samples obtained from relatives of the missing person can be used for DNA identification (3). A missing person's relatives can be a source of high quality biological samples (e.g. blood or saliva, usually collected in a health institution) that can provide full genetic profile. However, there is always

a possibility that the missing person and her or his relatives are not genetically related (e.g. a non-biological father or adoption) (3). Genetic identification using reference materials collected from genetically unrelated relatives may yield false-negative results (11). In the study by Hartman el.al. (12), a great majority of missing persons (82 %) were successfully identified using reference samples from their relatives. Samples obtained from close relatives allow a more precise genetic identification than those collected from distant relatives (13). If there are no close relatives available for identification, sampling of a larger number of distant relatives is required, which markedly increases the costs of genetic examination (9). Samples from maternal cousins, nephews and nieces may be used to verify a mtDNA match, which is passed down unchanged through the direct maternal line over the generations. Y-chromosome testing is used in known distant relatives on the father´s side because this chromosome is passed unchanged from father to son and as a result all male descendants carry an identical Y-chromosome. It is with this fact in mind that we approach the living relatives of missing persons or victims of post-war mass killings, and explain to them that biological samples from distant relatives can also be used for DNA analysis. Including samples of several living relatives increases the rate of statistical probability required for a positive identification (7). In all mass disasters in which identification of missing persons is started immediately after the accident (aircraft crashes, train accidents, terrorist attacks), personal items and achived tissue samples represent the most suitable reference samples for DNA testing. These samples allow direct comparison of genetic profiles, which is the most economically rational approach considering a small number of reference samples and high probability of positive identification. In victims of mass catastrophes (e.g.World War II mass graves in Slovenia, mass graves in Bosnia and Herzegovina) which occurred in remote past, personal items and archived biological samples are frequently unavailable, therefore biological samples from living relatives are used for DNA analysis (9).

4. Chronological documentation of the identification process

Chronological documentation of DNA analysis is essential to ensuring the authenticity of a sample. It starts with the collection of samples which should be followed throughout the identification process. The collected samples have to be stored and labeled appropriately. Participation of experts from different fields should be documented as well. Appropriate storage of evidence materials and chronological documentation of the collection, transport and analysis of samples play a key role in correct genetic identification and form the basis of identity reports, which are often presented in court (3).

5. DNA analysis for missing persons identification

DNA is the carrier of genetic information in living beings and is found in the nucleus of eukaryotic cells (14). DNA molecules are also present in mitochondria, organelles which produce cell energy (15). Each cell contains approximately 500 mitochondria and each mitochondria 5 to 10 DNA molecules. MtDNA in humans accounts for approximately 0.3 % of genomic DNA. It is a double-stranded, covalently closed

circular molecule, composed of external heavy chain (H chain) and internal light chain (L chain). Mitochondrial genome is relatively small in size, and contains about 16,500 base pairs. Heart, muscle and liver tissues comprise a high number of mitochondria. Each mitochondrion comprises multiple copies of its chromosome. People share similar structural and functional features and therefore have similar DNA molecules. It is possible, however, to distinguish one person from another on the basis of DNA regions that are unique to an individual. This finding serves as the basis for missing persons identification. In nuclear DNA these regions occur as microsatellites or short tandem repeats (STRs) which represent length polymorphisms. In mtDNA, sequence polymorphisms in control regions are studied (3).

Microsatellites are short repeating nucleotides, which occur in human genome in multiple identical or closely related copies. They are present in noncoding regions of DNA as introns and nucleotide repeats between genes. STRs repeat units are 2 to 7 base pairs in length, their number being less than 100 (16). Repeated sequences represent 3 % of human genomic DNA (approximately 90 million base pairs). Microsatellite markers on autosomes and Y-chromosome are used for the identification of missing persons. When only distant relatives on the mother´s side are available for DNA typing or when dealing with heavily degraded DNA that precludes nuclear DNA analysis, mtDNA polymorphisms are used for the identification.

5.1. Studies of autosomal microsatellites

Autosomal microsatellites, which are inherited codominantly are present on any of the 22 pairs of autosomes, ie. chromosomes that are not involved in sex determination. The level of microsatellite variability within the population is so high that the use of a larger number of loci makes it possible to differentiate between any two individuals except between identical twins. Polymerase chain reaction (PCR) is used for their amplification and it effectively amplifies both alleles of a heterozygous pair because of their similar size and length of up to 400 base pairs (14). In addition, PCR allows amplification of a larger number of STRs, which increases the speed of analysis and reduces the consumption of DNA extracted from post-mortem samples. According to human identification recommendations, at least 12 autosomal STRs are needed for the identification of a missing person when biological samples from the relatives are used as reference samples (12). In practice, different kits are used for simultaneous amplification of 15 or more microsatellites (18). In samples with heavily degraded DNA, it might be impossible to amplify longer STRs. In these cases we use the commercially available sets for the amplification of STRs that are less than 250 base pairs in length (3).

5.2. Studies of Y-chromosome microsatellites

In addition to STRs autosomal microsatellites, of sex chromosomes can be analysed in the DNA identification process. Y–chromosome, which is smaller in size than autosomes and X-chromosome, also comprises a smaller number of microsatellites. Y-chromosome is the second smallest human chromosome; it is 60 million base pairs long (16). Y-chromosome does not undergo recombination, therefore Y-chromosome microsatellite markers are useful for tracing parental lineages: all descendants of the same fa-

ther have identical Y-chromosome haplotypes. Haplotype is a fragment of DNA molecule inherited from one parent only. Thanks to Y-chromosome analysis, reference samples from distant relatives on the father´s side can be used for the identification of missing persons. This analysis, however, is of no use in the identification of female victims (19).

5.3. Mitochondrial DNA analysis

For identification purposes, sequence polymorphisms in the mtDNA control region are used. The advantage of using mtDNA in the identification of highly degraded human remains is that it is less susceptible to environmental degradation than autosomal DNA thanks to its circular conformation and mitochondrial membrane, and because it is present in multiple copies per cell. The mitochondrial genome has a much higher mutation rate than the nuclear genome because of mtDNA exposure to reactive oxygen compounds generated during oxidative phosphorylation (20). As mtDNA passes from mother to child, testing mtDNA allows only tracing the maternal line (3). If only mtDNA profile is obtainable from highly degraded post-mortem remains, identification can be based on the typing of reference samples from maternaly related relatives. Also, MtDNA analysis is used for the identification of ancient human remains, when the only living relatives available for genetic typing are those on the mother´s side several generations apart. Because mtDNA does not undergo recombination, the mtDNA analysis gives a lower identification probability than the probability of identification using nuclear DNA typing. The probability of identification using nuclear DNA is almost 100 % compared to 98–99 % for mtDNA analysis. The same is true of Y-chromosome which is passed on from father to male descendants and is not subject to recombination (21).

For genetic typing DNA has to be extracted from post-mortem remains. There follows determination of the amount of DNA in samples (DNA quantification) and DNA amplification using PCR. Genetic profiles of autosomal nuclear DNA are obtained by amplifying sutosomal STRs and Y-chromosome haplotypes by amplifying Y-STRs. MtDNA haplotypes are obtained by sequencing mtDNA control region.

6. DNA extraction from post-mortem remains

6.1. Recovery of DNA from blood, soft tissues and nails

Isolation of DNA from blood is one of the less demanding extraction methods employed in forensic genetics and allows for fast recovery of DNA. Haemoglobin is a PCR inhibitor and has to be eliminated from the extract (22).

Efficient DNA purification involves binding DNA to magnetic beads and irrigation performed in an automated fashion (Biorobot EZ1;Qiagen). The advantage of this method over organic extraction, a method frequently used in forensic analysis, is that it obviates the use of toxic organic solvents, such as phenol and chloroform This automated procedure lasts only 20 minutes. Nucleic acids bind to silica-coated magnetic beads in the presence of chaotropic salts that induce lysis of cells and protein denaturation, inactivate nucleases and enhance DNA binding to magnetic beads (24). In comparison to DNA extraction from bones, which takes several days to complete, DNA recovery from blood samples is considerably faster and can be completed in about an hour (25).

Soft tissue samples are used for analysis whenever DNA extraction from blood is no longer possible because of poorly preserved post-mortem remains. Tissue samples taken from the heart, muscles, liver and kidney are most suitable for genetic identification (17).

For the identification of highly degraded human remains, genetic materials are obtained from teeth or bones. Nails can be used as alternative source. They contain keratin and only rare microorganisms feed on this nail constituent. In comparison to DNA in soft tissues, DNA in nails is less susceptible to degradation and remains preserved long after death. Toenails are thick and therefore most suitable for analysis. Recovering DNA from toenails is less time-consuming and less demanding than extracting DNA from bone and tooth samples. In fresh nails, the possibility of obtaining full profiles is relatively high, whereas in old nail samples the quality of profiles greatly depends on local environmental factors. Keratinised tissues contain disulphide bonds and have to be incubated in extraction buffer with added DTT. Using the method of alkaline extraction in TNCa buffer, lysis of keratinised tissues is completed in only 2 to 5 hours (21).

6.2. Recovery of DNA from skeletal remains

When the remains of missing persons are poorly preserved or when many years have elapsed since theis discovery, DNA extraction from blood and soft tissues is no longer possible because of degradation. After death DNA gets degraded by lysosomal nucleases, bacteria and fungi. DNA present in bones and teeth is bound to hydroxyapatite and degrade slower; therefore it may remain available to analysis for centuries. The amount of DNA preserved in human remains depends on the given local environment. Environmental factors which play a major role in preservation of DNA molecules are temperature, humidity, UV radiation, pH, the presence of microorganisms and chemical characteristics of soil in which the body was burried (26). Low temperatures, low humidity and rapid drying are beneficial to DNA preservation. Skeletal remains with best preserved DNA are found in soil with neutral or slightly alkaline pH, low humidity, increased concentrations of salt and low humic acids levels. Also important is the way in which skeletal remains are stored after exhumation. Identification is best done immediately upon the digging up of skeletal remains. Keeping skeletal remains at room temperature has negative effects. If the identification procedure cannot be started promptly, the best solution is to freeze bone and teeth remains (1).

Long bones that contain a large proportion of compact bone tissue and hydroxyapatite to which DNA molecules are binding are best suited for DNA extraction. The binding process most probably occurs as follows: hydroxyl groups in hydroxyapatite molecule bind to phosphate groups on DNA. The femur and the tibia are the most suitable, and flat bones, such as the skull bones, are the least suitable bones for genetic investigation (1).

If the whole femur is sent to the laboratory for investigation, a 8–9 cm long and 2–3 cm wide piece of bone is cut for identification. After cleaning the earth off the bones these are washed with distilled water with added detergent. There follow multiple rinses in water. Proper labeling of bone samples is crucial for genetic identification process. The next step is grinding off the surface layer with a grinder. Liquid nitrogen is employed

to prevent bone overheating. After mechanical cleaning, the bones are cleaned chemically with water, detergent and ethanol to eliminate possible traces of contamination from the surface (27). As a result of oxidative action of detergents exogenous DNA gets split into short fragments or bases, whereas endogenous DNA remains intact thanks to its binding to hydroxyapatite. Then the bones are ground to a fine powder to allow for efficient decalcification. Liquid nitrogen is used to prevent overheating during grinding. For extraction 0.5 g of bone powder decalcified in 0.5 M EDTA is used. EDTA helps hydroxyapatite to dissolve and forms complexes with calcium ions (1) The aim of mechanical and chemical cleaning is to obtain endogenous DNA and eliminate exogenous DNA from the bone surface. It is therefore imperative to prevent contamination of bone samples. Working in the laboratory requires the use of protective coat, cap, mask and double gloves. All grinding and cutting equipment is wiped with sodium hypochlorite, distilled water and ethanol, sterilised and UV irradiated overnight. After the procedure all work surfaces are cleaned with bleech, water and ethanol. In all procedures negative controls are used to check the cleanliness of isolation and amplification reagents and laboratory plastics. Another important step is the creation of elimination database (24).

7. Determining the amount of DNA in samples

Before DNA amplification, the amount of DNA in a sample is determined with real-time PCR assay. On the basis of the assay results it is possible to decide whether the sample is suitable for nuclear DNA typing or just for mtDNA analysis. When short and long DNA fragments are amplified simultaneously, from their ratio the level of degradation of DNA can be calculated. The results of quantitative PCR reaction allow for the choice of genetic markers for typing. In addition to the amount of DNA and the level of its degradation, the presence of PCR inhibitors in the extract can be determined using new DNA quantification kits (29). Very low initial DNA concentrations in a sample may increase the error rate in PCR-based DNA amplification due to stochastic effects. The possibility of allelic drop-out due to failure of amplification must therefore be taken into consideration in the interpretation of results.

8. PCR-based DNA amplification

PCR-based DNA amplification should be the first step in DNA analysis, regardless of whether nuclear DNA or mtDNA is to be investigated. PCR assay is very useful in forensic genetic investigations as it allows for the use of very small quantities of DNA, which is often heavily degraded. A PCR assay amplifies DNA molecules in the same manner as they are amplified in the cell. The assay is based on annealing and elongating two oligonucleotide primers delimiting the target DNA region that will be amplified. The first step is denaturation that breaks the DNA double helix, and DNA separates into single strands. There follows hybridisation of oligonucleotids primers with complementary DNA strands. Meanwhile the strands are extended by thermostable DNA polymerase. Under opimal conditions from one molecule 2^n molecules of PCR product is obtained; where »n« is the number of cycles; usually 28–30 (17). Fluorescent labeling of amplified DNA fragments during the assay allows for detection of products

in capillary electrophoresis, and for differentiation between fragments that differ in length only by one nucleotide (3). Fluorescent labeled DNA fragments are separated according to molecular weight. PCR products are injected into a capillary which contains a denaturating polymer. Electrical current that passes through the polymer makes negatively charged products migrate towards the positive end of the capillary and DNA fragments are detected by laser beam. DNA fragments with lower molecular weight move faster than larger ones. The simultaneously amplified microsatellites can thus be differentiated by their length and fluorochrome labeling (3).

9. Statistical evaluation of the strength of genetic evidence and interpretation of results

Laboratory testing as part of missing persons identification is followed by interpretation of the results. A genetic profile obtained from post-mortem remains is compared with a reference sample to confirm or refute a potential match (3). When a match is found between the DNA profile from post-mortem remains and that obtained from a reference sample (a personal item of the missing person, e.g. a toothbrush) the next step is to determine how probable it is that both DNA profiles have the same source, i.e. how likely it is that the genetic profile from a person selected at random from the Slovene population match to that obtained from the biological material found on the toothbrush investigated, or to determine the probability of a random match between the DNA profile from post-mortem remains and biological traces recovered from the toothbrush (9). A match between two DNA profiles is statistically estimated on the basis of the likelihood ratio (LR) calculation. The likelihood ratio is inversely proportional to the genetic profile frequency (LR = 1 / frequency DNA profile) (9). A comparison is made between the likelihood that the biological material on the toothbrush comes from the missing person (numerator), and the likelihood that the biological traces from the toothbrush come from a person selected at random from the Slovene population, provided that the missing person is Slovene (denominator). The numerator likelihood equals 1, and the denominator likelihood equals the DNA profile frequency. The likelihood ratio indicates how much more likely it is that the biological traces from the toothbrush come from the missing person than from the person selected at random from the Slovene population (9). The calculation is done using different forensic statistical programmes (30).

10. Cases of missing persons identification

Aircraft accident in Spitsbergen is an example of how genetic methods were used for the identification of missing persons. Olaisen et.al. (31) identified 139 of the total of 141 Russian nad Ukrainian victims by comparing samples from their remains with those from their relatives. The most suitable were samples obtained from the victims´mothers, fathers and children. When only siblings were available for genetic analysis, reference materials were recovered from several brothers and/or sisters. Thanks to its high success rate (98.6 %) fast analysis and relatively low costs (3–5 % of the entire operation), genetic identification has proved a very suitable method for identifying mass disaster victims.

DNA analysis was used also in the identification of most victims of mass

killings ion the territory of ex-Yugoslavia (32,33). Forensic DNA analysis was successfully used in the identification of victims of post-war mass killings in Slovenia (34,35), where more than 600 mass graves have been documented. Victims from ten mass graves were identified using molecular genetic methods. For these graves lists of victims were available, which allowed for collection of reference oral swabs from their living relatives. The Konfin I grave is the largest mass grave from which skeletal remains of 88 victims were obtained in 2006. For the identification of victims buccal swabs from living close and distant relatives were collected for 44 victims from the list. DNA samples were recovered from the femurs of the victims, and the obtained genetic profiles were compared with samples from living reference individuals (siblings, daughters, sons, cousins and nephews) and in 32 victims match with living relatives was observed. Statistical estimate showed that the probability of kinship in all 32 victims exceeded 99.9 %, the criterion required for positive identification. Prior to the start of molecular genetic analysis, elimination database was created in order to ensure traceability in the case of contamination. The database contained genetic profiles of all individuals who came into contact with skeletal remains during exhumation, sample storage and anthropologic and genetic studies. Typing of autosomal nuclear DNA, Y-chromosome and mtDNA was performed in skeletal remains samples and typing of autosomal nuclear DNA in samples from living relatives. In addition, in maternal relatives typing of mtDNA and in relatives from the paternal lineage typing of Y-chromosome STRs was performed. In persons included in the elimination data base, mtDNA was analysed in addition to autosomal nuclear DNA. In males typing of microsatellites of Y-chromosome was performed. It was proved that DNA analysis of a larger number of genetic markers (autosomal microsatellites, Y-chromosome microsatellites and control region of mtDNA) and DNA typing of close and distant relatives of vicitims allows positive identification of World War II victims, whose close living relatives are usually not available for analysis because of the rather long time span since the second World War. We succeeded in predicting eye and hair colour from victims of post-war killings found in Slovene graves as well (37).

Genetic identification of missing persons killed in a hot air baloon crash that occurred at Ljubljana marshes on 23 August 2012 was performed in the Laboratory of Molecular Genetics, Institute of Forensic Medicine Ljubljana. Sudden wind shear thwarted safe landing and the baloon hit the ground and bounced off several times. Several people fell from the baloon and those who remained on board were severely, some even fatally, injured in fire. There were 32 people on board; 12 suffered severe and 14 light injuries. Six people died, four at the crash site, and two later at the hospital (38). The four dead at the scene and one person who died later were charred beyond recognition, and could only be identified using genetic methods. For genetic profiling reference samples were obtained from the victims' close relatives (brothers, sisters, sons, mothers, fathers), and aortic blood samples were taken from the charred bodies. Identification of the missing victims was completed in 36 hours.

11. Conclusion

Genetic identification is a modern identification method that began developing after the discovery of highly

polymorphic DNA segments and today complements other forensic and anthropologic methods. It yields the most accurate results and allows for identification of unrecognisable heavily degraded, fragmented and skeletonised human remains.

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