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USE OF DNA ANALYSIS TO IDENTIFY UP HUMAN REMAINS AND CADAVERS

Summary
Identification of UP (Unidentified Person) corpses and human remains is a process aimed at establishing the identity of a person whose body (body parts) or remains have been found in circumstances that do not allow his or her personals to be established. It is a multi-faceted process, using research methods from many areas of forensic science. Among them, genetic research is important, allowing the analysis of a variety of biological material, including archival material.

Keywords: UP corpse, human remains, identification methods

Introduction

The identification of UP remains and human remains is a process aimed at establishing the identity of a deceased person. In some cases, due to the circumstances, time, and place of disclosure, the corpse undergoes post-mortem transformations (decomposition, fragmentation, charring, or vitrification). As a result, visual identification of the body by demonstration is not possible. In this situation, the only way to establish identity becomes genetic analysis, which involves determining the DNA profile of an unknown person and comparing it with the genetic profiles of his alleged relatives[1]. No matter what identification methods are used, their effect should be to establish the identity of the corpse, which in turn makes it possible to take legal action to declare a person dead.

[1] E. Kapinska, Z. Szczerkowska, *Ustalenie tożsamości nieznanej osoby w oparciu o określenie profilu DNA z ekshumowanych szczątków ludzkich*, „Archiwum Medycyny Sądowej i Kryminologii" 2008, vol. LVIII, pp. 32-36.

Procedure for dealing with the discovery of an UP corpse

According to the Order of the Chief of Police No. 48 of June 28, 2018, in the event of the discovery of a UP corpse, the duty officer of the police unit shall order the securing of the place where the UP corpse was found, ensure the arrival of a doctor to determine death, direct an investigation team to the scene, and inform the locally competent prosecutor of the fact that the UP corpse was discovered. At the scene, the investigation team performs an inspection of the John Doe corpse, secures the traces and objects found, and takes photos of the corpse including four shots of the head, silhouette, and left auricle, as well as photos with special attention to the characteristics, including scars, tattoos, birthmarks, etc., following the rules of scale photography. Subsequent steps include fingerprinting of the hands and fingers and taking a biological sample for genetic testing. The key issue of the further direction of the investigation is to obtain a preliminary assessment by a medical expert as to the cause of death, so the participation of a forensic doctor in the inspection is justified. The police unit with the local jurisdiction to conduct identification activities is the unit in whose territory John Doe's corpse was discovered. If the death occurred in an inpatient facility, identification activities will be carried out by the police unit where John Doe resided before hospitalization. The order under review in Chapter 8 lists 10 methods for identifying an UP corpse:

1) fingerprint comparison;
2) DNA profile comparison;
3) comparison of drawing features and distinguishing marks, including scars and tattoos;
4) comparison of medical data on past medical and surgical procedures;
5) recognition of personal belongings;
6) recognition based on documents revealed with the John Doe corpse or remains of the John Doe corpse;
7) recognition by witnesses, family members, or friends;
8) examination of dentition and other odontological data;
9) radiological examination;
10) anthroposcopic examination.

When conducting the identification of a UP corpse, it is first necessary to make preliminary findings aimed at identifying the UP corpse, which include an analysis of the circumstances under which the corpse was found and the results of interviews conducted. This is followed by the typing of the John Doe, which involves comparing the drawing, including the specific marks of the John Doe, with those registered in the National Police Information

System (KSIP). During the forensic autopsy, a forensic technician performs photographic documentation and, if possible, takes finger and palm prints, as well as samples of biological material for genetic testing. The order directs that a fingerprint interview be conducted immediately. In the case of a negative dactyloscopic interview or when fingerprints could not be taken due to advanced post-mortem lesions, the police officer orders the determination of the DNA profile and registration in the DNA database. In justified cases, the skull is secured for anthropometric and anthroposcopic studies.

A John Doe corpse is considered identified when it has been recognized by a member of the immediate family, a legal guardian, or at least two persons who directly knew the deceased. In addition to the above identification methods, it is possible to establish the identity of a John Doe by obtaining a positive fingerprint interview or a positive fingerprint report and collecting other evidence establishing the identity of a John Doe, including a positive DNA profile comparison or an expert anthroposcopic examination.

An important issue is the proper documentation of the identification activities carried out. A sample, "Doe identification file" should contain two types of documents. The first, related to the identification of the John Doe corpse, consists of copies of the inspection reports of the John Doe corpse, clothing and the place where it was found, a copy of the autopsy protocol of the John Doe corpse, photographic documentation, including signature photos, photos of clothing, objects found with the John Doe corpse, tattoos and distinctive marks, and auricles, fingerprint card with fingerprints of the John Doe corpse along with the result of the search in the AFIS automatic fingerprint identification system, documentation regarding the collection of biological material for DNA testing, ordering its determination and registration of the DNA profile in the DNA database, expert reports, opinions and other findings or copies thereof that may contribute to the identification of the John Doe corpse. The second group of documents includes a note on the date and location of the burial of the John Doe and notes on other activities performed in the case, confirmations of the registrations and checks made and typing concerning the John Doe in the KSIP, as well as the control and supervision sheet of the service[2].

[2] Order of the Chief Commander of the Police of June 28, 2018 on the conduct of the police search for a missing person and proceedings in the event of the discovery of a person of undetermined identity or the discovery of unknown corpses and human remains, Official Journal of the KGP.2018.77

DNA analysis in non-coding regions

Due to the possibilities of determining the DNA profile in a variety of biological materials, such as blood, saliva, hair, or bones, and performing tests on material secured many years ago, genetic testing according to the International Society for Forensic Genetics (ISFG), guidelines has been placed at the forefront of identification methods used. This has to do with the fact that it is not always possible to take fingerprints from a corpse[3].

In the case of John Doe cadavers and human remains, genetic testing involves determining the DNA profile and then comparing it with profiles obtained from secured material *ante mortem* or DNA profiles of potential relatives (parents, siblings, children). Genetic testing is not an individual test, but it allows one to rule out the identity of a corpse with 100% certainty. In contrast, in the absence of exclusion, the result of the comparative analysis is based on statistical analysis.

The decision as to what type of biological material needs to be taken from a John Doe should be made on a case-by-case basis depending on the specific situation. For example, dried blood samples, hair, fragments of the shaft of the femur or fragments of the humerus (about 5 cm), teeth, or tissue fragments can be used for tests to determine the genetic profile[4].

For the purposes of personal identification, analysis of polymorphic microsatellite sequences of nuclear DNA, i.e. *short tandem repeats*(STR) markers, has been used for more than 20 years. Using the multiplex polymerase chain reaction (multiplex PCR) method, it is possible to analyze multiple STR markers simultaneously, even in very small and highly degraded biological traces. This method can be used to test all types of biological material. DNA analysis using multiplex STR sets has high discrimination power. However, in cases where we are dealing with DNA samples with a significant degree of degradation, mini-STR and SNP*(Single Nucleotide Pol*ymorphism) markers are used[5].

To establish kinship between a UP corpse and family members, Y chromosome polymorphism testing is also used, the characteristic feature of which

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[3] M. Prinz, A. Carracedo, W.R. Mayr, N. Morling, T.J. Parsons, A. Sajantila, R. Scheithauer, H. Schmitter, P.M. Schneider, *DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI)*, "Forensic Science International: Genetics," 2007, no. 1, pp. 3-12.

[4] I. Soltyszewski, B. Mlodziejowski, R. Ploski, W. Pepinski, J. Janica, *Kryminalistyczne i sądowo-lekarskie metody identyfikacji zwłok i szczątków ludzkich*, „Problemy Kryminalistyki" 2003, no. 239, pp. 7-12.

[5] J. Drabik, *Markery miniSTR jako nowa technologia badania śladów biologicznych w kryminalistyce*, „Problemy Kryminalistyki" 2007, no. 258, pp. 56-62.

is inheritance only in the male line, which makes it possible to establish kinship between father and son, grandfather and grandson, etc. Another test that can be performed for these purposes is the analysis of the polymorphism of the X chromosome, making it possible to determine the kinship of, for example, a mother with a presumed son. For highly degraded biological material that cannot be used for nuclear DNA analysis, mitochondrial DNA (mtDNA) sequence analysis is used. According to the accepted model of inheritance, all descendants of a single mother have identical mitochondrial DNA haplotypes. Mitochondrial DNA studies in the three hypervariable regions of the D-loop are of great importance in identification analyses of bone (including teeth) and rootless hair. Due to the presence of multiple copies, mitochondrial DNA testing offers a better chance of success for highly degraded biological material that cannot be used for nuclear DNA analysis. However, it should be borne in mind that the statistical analysis of the results should be based on a sufficiently large and representative database of mtDNA haplotypes of the population from which the UP may come (reference base). This is particularly important when estimating the frequency of rare and unique haplotypes[6].

The experience of many countries indicates that DNA databases containing STR-type markers make it possible to use this tool to establish the identity of a John Doe. Thanks to the DNA database information system, it is possible to compare and match the DNA profile determined for a John Doe corpse or human remains with the DNA profiles of the families (parents, siblings, children) of persons reported missing. Oral swabs or blood are taken from these individuals as comparison material.

Statistical analysis of genetic test results

The Familias program[7] is a tool used to estimate the credibility quotient and kinship probability values in cases of disputed paternity, as well as in search and identification cases of missing persons or persons and corpses of undetermined identity, assuming varying degrees of kinship with their alleged relatives. The software allows us to perform calculations of greater complexity, taking into account possible mutations, null alleles, and "drop out"/"drop-in". It allows us to carry out a case analysis to consider such problems as inbreeding, incest, and kinship among a small, closed population.

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6 P. Daca, M. Mielnik-Sikorska, J. Bednarek, T. Grzybowski, *Ocena stopnia wysycenia bazy danych mitochondrialnego DNA dla populacji Polski*, „Archiwum Medycyny Sądowej i Kryminologii" 2010, vol. LX, pp. 263-269.

7 https://familias.no/ (accessed 15.09.2024).

The program provides an opportunity to investigate the degree of kinship between victims of mass disasters and alleged relatives[8].

When analyzing the degree of relatedness, a statistical calculation based on calculating the *likelihood ratio* is used, and then transforming the resulting *likelihood ratio* into a form of probability of relatedness. The LR value equation implies a comparison of the probabilities of establishing evidence from genetic testing under the assumption of two alternative hypotheses (quotient of two conditional probabilities)[9].

$$\mathbf{LR} = \frac{\mathbf{Pr\ (proof/H1)}}{\mathbf{Pr\ (proof/H2)}}$$

Where:
LR - *likelihood ratio* (reliability quotient)
Pr - probability
evidence - allele sets (DNA profiles of analyzed individuals)
H1: hypothesis 1
H2: hypothesis 2

The resulting value is then transformed into a probability of relatedness using the formula:

$$\mathbf{P} = \frac{\mathbf{LR}}{\mathbf{LR+1}}$$

Where:
LR - *likelihood ratio* (reliability quotient)
P - probability of relatedness[10]

The Polish Society of Forensic Medicine and Criminology has issued a recommendation for interpreting the results of the statistical analysis, according to which confirmation of paternity/relation follows an LR score ≥ 1,000,000, which corresponds to a probability of at least 99.9999%[11].

DNA analysis of coding regions

Forensic genetics is a rapidly developing scientific discipline, so new research tools are successively being developed that can also find applica-

8 T. Egeland, D. Kling, P. Mostad, *Relationship Inference with Familias and R Statistical Method in Forensic Genetics*, Elsevier, Amsterdam et al. 2016.

9 J. Drabek, *Validation of software for calculating the likelihood ratio for parentage and kinship*, "Forensic Science International: Genetics" 2009, no. 3, pp. 112-118.

10 https://familias.name/Files/manualFamilias.pdf (accessed September 15, 2024).

11 http://www.ptmsik.pl/komisja-genetyki-sadowej/zasady-atestacji-na-rok-2016 (accessed September 15, 2024).

tion in the process of identifying John Doe cadavers and human remains. Phenotyping is relatively new to forensic genetics and is not used in routine forensic or identification cases[12]. With phenotyping, a person's external appearance characteristics can be determined through analysis of their DNA. Currently, commercial reagent kits are available to determine such appearance characteristics as hair color, eye color, skin color, and biological age. It is also possible to determine the biogeographical origin[13]. This is crucial in the absence of comparative material.

There are a number of aspects associated with limiting the use of phenotyping in forensic cases. The main difficulties include the interpretation of complex genetic data, ethical issues related to privacy, and legal regulations regarding the feasibility of using this technology. Current DNA analysis methods include STR markers located in non-coding regions of DNA. In addition to non-coding fragments, markers located in the protein-coding region of the human genome are used for phenotyping. This fact is a major cause of concern for violating the privacy of the person being tested, as it can reveal individual characteristics of the sample donor. As for the legal aspect of the use of this method, it is not regulated by Polish law, and therefore there are no standards for its use, so it is possible to perform phenotyping for forensic genetics. Since this method is an investigative tool and its purpose is only to assist ongoing investigations, phenotyping should not be the only evidence in a case used to identify individuals. In identification cases of human remains that, due to the degree of preservation of the remains, do not qualify for identification by other methods, phenotyping provides an opportunity to effectively narrow down the group of wanted persons.

The future of DNA phenotyping seems promising, especially with the development of sequencing technology and further progress toward more precise and comprehensive analysis. Already, new predictive markers are being discovered for more appearance traits, such as eyebrow color, freckles, hair texture, hair loss in men, tall stature, and graying. Work is also underway to determine such appearance traits as ear morphology, facial beard type, and facial shape, but phenotyping of the aforementioned traits is not yet effective enough for commercial kits to be developed to determine them[14].

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12 P. Dabas, S. Jain, H. Khajuria, B.P. Nayak, " *Forensic DNA phenotyping: Inferring phenotypic traits from crime scene DNA*," Journal of Forensic and Legal Medicine 2022, no. 88, pp. 102351.

13 P.M. Schneider, B. Prainsack, M. Kayser, " *The use of forensic DNA phenotyping in predicting appearance and biogeographic ancestry*," Deutsches Ärzteblatt International 2019, no. 51-52, pp. 873-880.

14 M. Kayser, W. Branicki, W. Parson, C. Phillips, *Recent advances in forensic DNA phenotyping of appearance, ancestry and age*, "Forensic Science International: Genetics" 2023, no. 65, p. 102870;

Case descriptions

Case 1

In November 2018, in an unoccupied building in the Mazowieckie province, police officers revealed a corpse undergoing late metamorphic changes. Forensic medical examination of the corpse determined that it belonged to a man of about 65-70 years old. During the investigation, it was found that no man of similar age and physique was reported missing during the period under review. The actions taken by the police officers of the criminal division allowed them to identify that the John Doe man may be Rafal M., who has no permanent residence.

Due to the condition of the corpse, identification by demonstration could not be carried out, as well as a fingerprint interview - so genetic testing proved necessary. The research first required DNA profiling from a fragment of a male John Doe's femur. The next step was to locate members of his surviving family to collect biological material in the form of oral swabs, determine his DNA profile or profiles, and perform kinship analysis. Unfortunately, the search for a living family was unsuccessful. The man had no children or siblings, and his parents were cremated after his death. In this situation, the only relative from whom a sample of comparative material could be taken was his alleged grandmother on his mother's side, who died in 1984. The exhumation carried out in April 2021, made it possible to retrieve a tooth and three long bone fragments. Genetic testing using a set of STRs showed that the UP man with a probability of $P = 0.98$ is the grandson of the exhumated woman. However, the result of the statistical analysis was not sufficient to unequivocally confirm the relatedness of the subjects. It was necessary to perform mitochondrial DNA testing to establish relatedness in the maternal line. As a result of the research, taking into account the results obtained during the preparation of the earlier opinion, it was determined that with a probability of $P > 0.999$, the male John Doe is related to the exhumed woman from whom the biological material was taken.

M. Kukla-Bartoszek, E. Pośpiech, A. Woźniak, M. Boroń, J. Karłowska-Pik, P. Teisseyre, M. Zubańska, A. Bronikowska, T. Grzybowski, R. Płoski, M. Spólnicka, W. Branicki, *DNA-based predictive models for the presence of freckles*, "Forensic Science International: Genetics" 2019, no. 42, pp. 252-259; A. Alshehhi, A. Almarzooqi, K. Alhammadi, N. Werghi, G.K. Tay, H. Alsafar, *Advancement in human face prediction using DNA*, "Genes" 2023, no. 14(1), p. 136.

Case 2

In September 2020, the corpse of a male John Doe was discovered in the forest complex. The corpse was covered by advanced post-mortem lesions. The steps taken in the case led to the hypothesis that the deceased was a nursing home resident who had been wanted for two months and had been reported missing by the center. Due to the state of preservation of the corpse, it was not possible to perform a presentation to family members, so it became necessary to perform genetic testing. A femur fragment was taken from the corpse, and oral swabs were taken from the alleged brother and half-sister of the deceased as comparison material. The study was conducted using a reagent kit that contains 23 autosomal STRs and 3 STRs located on the Y chromosome. As a result of the research, it was determined with a probability bordering on certainty ($P > 0.999999$) that the John Doe male is the brother and half-brother of the persons from whom the comparison material was taken.

Summary

Nowadays, it is difficult to imagine the activities of identifying a UP corpse without the participation of DNA analysis, which is eloquently confirmed by the statistics of the expertise performed in this area. Current reagent kits using STR panels of autosomal and Y and X chromosomes allow, in many cases, the identification of UP cadavers and human remains with probability bordering on certainty. A positive result of genetic identification largely depends on the quality of the biological material taken from a human cadaver or remains. The result of the statistical analysis of relatedness is significantly influenced by the degree of kinship between the analyzed individuals. The range of tests that can be performed in the forensic laboratory adequate to the needs of the client is also not insignificant.

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