HISTORY, PRESENT AND FUTURE OF FORENSIC BIOLOGY

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Annotation

The history of forensic biology is strictly connected with two names – Karol Landsteiner who in 1901 discovered blood types in an ABO system. The other one is Alec Jeffreys who in 1985 came upon polymorphic minisatellite sequences and invented the "DNA fingerprinting" technique[1]. These two separated by time events allow us to understand the road forensic biology followed to become the discipline without which it is impossible to find the offenders. For years serological examinations and enzymes polymorphism analysis has been used to study biological evidence. Nevertheless in many cases such as the situations where changes were caused by natural deterioration processes, physical or chemical factors, the "age" of biological traces left on a crime scene it happens to be impossible to obtain satisfying results. These limitations, however, do not apply to genetic studies. Blood, sperm, saliva are very good sources of DNA. A smaller amount of DNA content in hair, teeth or bones is compensated by greater resistance against unfavourable external factors. The introduction of DNA analysis allowed for the use of the upper skin layer (epidermis) cells in forensic examinations. Prior applied serology methods had not offered such possibilities. It is possible to use also the samples obtained as a result of an autopsy. However, it is worth remembering that the quantity of degraded genetic material grows proportionate to the longevity of *post mortem* time[2]. On some occasions, DNA could be found in the tissues preserved and covered in paraffine. In the end, it is worth mentioning that at the current state of advanced technology and the possibility of applying different examination techniques it is possible to analyse every type of biological trace.

[1] The term „polymorphism" is referring to the existence of two or more variants of the same feature.

[2] *Post mortem – after the death (Słownik Wyrazów Obcych (1980)).*

Keywords: forensic biology, biological material, bloodstains, serology, DNA analysis, forensic phenotyping

Introduction

Forensic biology is using the knowledge from natural sciences to identify and analyse biological traces found and preserved during a crime scene examination to establish the fact of the criminal event, its mechanism and the roles of its participants. It includes different specialized disciplines such as forensic serology, DNA analysis, forensic entomology, forensic botany and microbiology. Its main goal is the identification of the person who left his or her biological material on the crime scene. If the scientific methods or the quality of genetic material do not allow to establish the person's identity, experts strive to identify the group of people, to which the offender might belong. Even though the results of biological examination make it possible to attribute genetic characteristics of a biological trace found on a crime scene to the polymorphic characteristics of the suspect with high probability, this parameter would never reach a value of one, i. e. empirical certainty. This fact follows from the methodology of science – it is impossible to reach certainty examining only a part of reality by extrapolating the results of these examinations on a whole population. Until recently, forensic biological examination allowed only to make the so-called group identification. Based on the biological evidence found on a crime scene – typically blood – it was possible to determine a set of persons (usually small in size) which could be its source. In the beginning, the positive value of forensic biology regarding crime-solving was associated with the possibility of the elimination of the person from a set of suspects, not the identification of the offender. The obtained results were only partially useful for the prosecution. Only the development of cell biology, new examination techniques and their practical application, as well as the creation of the statistical procedures of the interpretation of the results, allowed to make a quality change regarding the genetic analysis. The examination of the fragments of deoxyribonucleic acid with a high variation coefficient among the population has been accepted as a reliable basis for a person's identification.

Forensic serological examination

The scientific grounds of forensic biology were laid down in 1853 when Karol Teichman-Stawiarski, a Polish anatomist and doctor, developed the test which allowed to identify brown stains discovered, for example, on clothing

as coming from the blood[3]. In the following years, other chemical methods to identify blood were created Kastle–Meyer (KM), Benzidine, and Luminol[4]. In 1901 Paul Uhlenhuth developed the method allowing to differentiate human blood from animal blood in the case of micro evidence[5]. Dynamic advancement in this forensic field started with Karol Landsteiner's discovery of blood types(ABO)[6]. A year later, Max Richter stated the first studies on the blood type differentiation considering bloodstains – the work which in 1916 resulted in the development by Leon Lattes the method of the determination of a blood type in the case of bloodstains. In the following years, this method was supplemented by Józef Holzer. Both methods of establishing a blood type were used in forensic biology up until the nineties[7]. Because of the limited evidential value of those studies, scientists tried to supplement the spectrum of the biological markers used in human identification. Somewhat later, in the twenties ofthe XX century, those examinations were supplemented by a group system MN. Twenty years later the next group factor – Rh – was discovered[8]. In the sixties, there were seventeen known group factors, however, not every one of them found its way to forensic biological examinations[9]. A considerable improvement in forensic examinations was the discovery of the polymorphic enzymes in red blood cells in 1963, which are characterized by a genetically determined diversity. This discovery made it possible in the seventies to study several enzyme systems and proteins in blood serum. The progress in haematology was so profound that in the eighties there were around 150 known proteins and enzymes, which might have been used in the criminal justice system. Despite the earlier hopes, in practice, most of them were impossible to use in forensic biology studies[10]. Their law value considering polymorphism, low resistance to degradation and inefficient examination techniques, which did not provide the possibility of obtaining similar results, made it impossible to use the resulting

3 https://www.encyclopedia.com/science/encyclopedias-almanacs-transcripts-and-maps/teichmann-ludwig-karl

4 Spalding, R. (2003). Identification and characterization of blood and bloodstains. In: *Forensic Science. An introduction to Scientific and Investigative Techniques*, 244–245.

5 Gaenslen, R. E. (1983). *Source book in Forensic Serplogy, Immulogy and Chemistry*, 221.

6 *The Future of Forensic DNA Testing. Raport Komisji DNA Departamentu Sprawiedliwości Stanów Zjednoczonych 2000:* http://www.ojp.usdoj.gov/nij.

7 Inman, K., Rudin N. (2001). *Principles and Practices of Criminalistics*, 32.

8 MacDonell, H. L. (1993). *Bloodstain Patterns. Coroning NY*, 35.

9 Ramsland, K. *Serology: It's in the blood:* http://www.crimelibrary.com/criminal_nied/forensic/serology.

10 *The Future of Forensic DNA Testing. Raport Komisji DNA Departamentu Sprawiedliwości Stanów Zjednoczonych 2000:* http://www.ojp.usdoj.gov/nij.

information in the identification procedure. At the end of the era of forensic serology examinations, the following polymorphic features of blood were used:blood type AB0, Rh factor, GM1and GM2 factors, polymorphic enzymes such as phosphoglycerate mutase (PGM), acid phosphatase (AcP), esterase D (EsD)[11].

Together with the development of forensic serology studies, there was a scientific exploration of the human genome. At the half of the twentieth century, a real number of human chromosomes was found out as well as highlighted the structure of deoxyribonucleic acid. In the fifties and sixties, intense studies were conducted regarding the issue of the living organisms volatility. The experts, by using the rules of population biology, tried to establish the frequency of certain biological features in the population. By using serological markers, the information on the replicability of the selected features in a population consisting of a few hundred people was obtained provided that the samples contain rare markers. Still, the most important advantage of forensic biological examinations was the capability to eliminate a person as a source of the biological evidence found on a crime scene. The criminal justice system, however, required scientific methods with a much high information potential[12].

Forensic genetics

Together with the studies on the issues relating to genetic inheritance, an appropriate scientific methodology was being developed. From the perspective of forensic genetics, it was important to make progress in understanding the phenomenon of variability among living organisms and the reasons behind it. Those studies resulted in 1985 in the very first case, where forensic biology helped to solve a crime. Nowadays this moment is considered to be a decisive step towards a new era in forensic human identification. Before 1985 it was impossible to make human identification based on biological evidence left in the crime scene. The examinations allowed only to declare that this evidence probably originates from the person, whose biological samples had been sent to the experts.

The scientific group led by Aleca Jeffreys was using RFLP (restriction fragments length polymorphism) method. Desikribonucleic acid isolated from biological evidence was studied by applying the same electrophoretic base, usually by placing the samples near each other. If the fragments of VNTR in

11 Turowska, B. (1980). *Enzymy krwi ludzkiej w medycynie sądowej.*

12 Ramsland, K. Serology: It's in the blood: http://www.crimelibrary.com/criminal_nied/forensic/serology.

biological material from the crime scene and samples obtained from the suspect appeared to be similar, the identity of the culprit was considered to be established. It was possible to declare the conformity between crime scene evidence and those samples. This conformity of the results was treated as proof that the DNA profile originates from one person or two unrelated persons, who accidentally have similar DNA profiles. The determination of the possibility of DNA repetition in the population became a requirement. To address this issue, studies on the frequency of DNA profiles were being undertaken. It appeared that the probability of the existence of the same DNA profile among unrelated people was 6 x 10–14, which meant that it was extremely unlikely for the same profile to be found[13].

It is worth mentioning that the object of the study considering forensic genetics is certain fragments of the human genome. Those are direct studies based on DNA, different from the previous serological examinations based on proteins. Moreover, genetic markers are more resistant to sudden environmental changes compared to protein structures. Because of this, it is possible to determine the characteristics of deoxyribonucleic acid long after a human's death. Appropriate protection and storage of biological material such as blood, epidermal cells, hair or semen allow us to analyze those samples many years after the event[14]. First genetic examinations showed that the most important feature is the high variability of polymorphic regions of deoxyribonucleic acid between individuals belonging to the same group and the population. In practice, this means that the probability that the same set of features will repeat in two unrelated people is negligible. A large number of alleles per locus and many polymorphic loci that can be used in forensic examinations allow for the issuance of an opinion, supported by a statistical analysis, which confirms the agreement or lack thereof between the evidence and comparative material[15].

Due to these advantages, genetic laboratories relatively quickly started to apply genetic methodsin real-life criminal cases. In 1988, the FBI forensic laboratory implemented the method of cutting the DNA helix with restriction enzymes. It was preceded by an increase in the level of the sensitivity of the tests and the collection of the data on the frequency of the occurrence of different lengths regarding DNA fragments in the population. In Poland, the first

13 Gill, P., Jeffreys, A. & Werrett, D. (1985). Forensic application of DNA 'fingerprints'. *Nature,* 318, 577–579.

14 Pawłowski, R. (1997). *Medyczno – sądowe badanie śladów biologicznyc*, 130.

15 *Brief History of Forensic DNA, Typing: http://www.cstl.nist.gov/biotech/strbase/ppt/intro.pdf.*

forensic examination in this field was conducted in May 1989[16]. In the following years, more and more laboratories began to perform genetic testing, abandoning forensic serology.

An equally important problem to be solved was the legal admissibility of the research results obtained with the use of new techniques. In the beginning, forensic genetics aroused great enthusiasm. New opportunities were noticed by the judges and prosecutors. Genetic testing appeared to be a way of identifying the culprit almost immediately. The criminal process was supposed to become much faster and less circumstantial. Forensic genetics and the use of statistical analysis became subjects of controversy[17]. The allegations concerned the use of uncertain methodologyregarding the interpretation of DNA profiles in evidential material and comparative samples. The result of the analysis was expected to be the determination of compliance or non-compliance. At that time, forensic laboratories did not use standards for assessing obtained profiles, and the only criterion used was the expert's subjective opinion. Often obtained results were illegible and yet were considered as the basis for issuing the judgment on the perpetrator's identity[18].

During the initial period of forensic genetics, along with the introduction of new methods, there was no change in the procedures regarding biological evidence on the crime scenes as well as their further storage. The lack of appropriate regulations increased the risk of contamination, for example, from the policemen carrying out standard search activities at the crime scene. Opponents of introducing the technology into investigative practice strongly emphasized this problem in their speeches, often dismissing expert opinions during court hearings.

The most controversial aspect in the history of forensic genetics, however, turned out to be the issue of statistical interpretation of the obtained results. Opponents argued that establishing the consistency of the DNA pattern regarding the evidence and comparative samples is irrelevant if we could not precisely determine how rare such a pattern is in the population. When it seemed that this problem had been solved by introducing statistical tests, it turned out that those tests were based on the general laws of population genetics and did not

16 Słomski, R. (2010). Trudne początki badań DNA w Polsce. *In:* Kwiatkowska – Wójcikewicz, V. (red.) (2010). *Kryminalistyka dla prawa, prawo dla kryminalistyki.*

17 Thompson, W. C. (1993). Evaluating the Admissibility of New Genetic Identification Test: Lessons from the „DNA War". *The Journal of Criminal Law and Cryminology*, 84, (1), 22.

18 Thompson, W. C. (1993). Evaluating the Admissibility of New Genetic Identification Test: Lessons from the „DNA War". *The Journal of Criminal Law and Cryminology*, 84, (1), 22.

regard smaller groups of people. Failure to take into account the θ coefficient (relating to non-random crossing of individuals in small populations) resulted in the opinion that the results of statistical analysis were inadequate to the reality. In addition, forensic experts in the early 1990's used various statistical data. This led to a situation, eagerly used by opponents of forensic genetics, in which the same DNA profile had significantly different frequencies in the population. This weakened the credibility of the expert opinions. It was emphasized that when dealing with such incredibly low values, the probability that the suspect was the actual perpetrator is very high[19].

The above-described facts were the reason why the expert opinions were often rejected as evidence, right after the period of fascination with the new possibilities offered by forensic genetics. Even though the discussion between supporters and opponents of forensic genetics was provoking some strong emotions, it nevertheless led to the verification of the validity of the methodology, modification of some of the methods and the introduction of the validation in forensic examinations[20].

Another breakthrough in the history of forensic genetics was the development in 1985 by Karl Mulis of the polymerase chain reaction (PCR)[21]. This discovery revolutionized biological sciences as scientists found out how DNA molecules could be multiplied outside a living organism[22].

In 1986, for the first time, PCR was used for the needs of criminal justice[23]. The polymorphic HLA – DQA1 system was analyzed. This system consisted of seven allelic classes that were recognized by probes with complementary sequences. Then the isolated DNA was amplified by PCR. The amplified deoxyribonucleic acid molecules were applied to a membrane and treated with the solution with colourimetrically labelled probes. The DNA hybridizing was identified as blue dots. The obtained test results had a high identification value. Relatively quickly, this system started to be used in criminal proceedings, primarily to verify previously incorrectly and positively identified defendants[24].

19 *The Future of Forensic DNA Testing. Raport Komisji DNA Departamentu Sprawiedliwości Stanów Zjednoczonych 2000:* http://www.ojp.usdoj.gov/nij.

20 Tomaszewski, T. (1996). Genetyczne badania identyfikacyjne – przełom i wyzwanie dla kryminalistyki. In: *Problemy współczesnej kryminalistyki,* 127–141.

21 Mullis, K. B., Faloona, F. A. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymo*, 155, 335–350.

22 Brown, T. A. (2012). *Genomy,* 18–20.

23 Goodwin, W. et al. (2007). *An introduction to forensic genetics*, 5.

24 *The Future of Forensic DNA Testing. Raport Komisji DNA Departamentu Sprawiedliwości Stanów Zjednoczonych 2000:* http://www.ojp.usdoj.gov/nij.

In 1993, another five polymorphic genetic markers were introduced into practice and the Polymarker DQ & kit was created. This system became widespread, especially in private forensic laboratories, and the results obtained on its basis were broadly accepted by the courts in the United States. The introduction of additional genetic systems, with two or three alleles at the locus, greatly enhanced the discriminatory power of genetic research. However, the level of statistical uniqueness of the DNA profile was not achieved as it was in the cases of the analysis using the RFLP method. The probability of a random match was approximately 1 in 4,000 with the polymarker system. The size of this parameter was so small that it was only possible to perform group individualization based on the characteristics of the DNA isolated from biological evidence[25]. Therefore, the search for further genetic markers that could completely replace previously used technologies based on the use of restriction enzymes, was still going on. Population geneticists noted the D1S80 locus[26]. It is a sequence repeated in tandem by 16 bp of the VNTR type, but it is also small enough so its analysis must be preceded by deoxyribonucleic acid amplification. After the PCR reaction, the amplified DNA fragments could be separated on a vertical polyacrylamide gel, and the bands were revealed by silver staining. The Amelogenin gene DNA was amplified simultaneously with the D1S80 locus. After performing one PCR reaction, the forensic expert might obtaininformation about allele polymorphism at the locus and the person's sex[27]. After the gel was stained, the position of the alleles was analyzed by comparing them with the allelic ladder – the set of the alleles present in the population. It was necessary to meet the condition that the ladder and the analyzed samples were subjected to the electrophoresis process on the same carrier. This genetic system quickly became accepted by the judiciary. Nothing stood in the way of presenting in the opinions the results obtained with the Polymarker + DQ & set and the results of the analysis of the D1S80 locus. It is now believed that the use of the D1S80 kit was a bridge between the VNTR polymorphism and organism variability based on short repeat units. Moreover, the basis for the development of multiplex technologies in genetic forensic research, consisting in obtaining

25 *The Future of Forensic DNA Testing. Raport Komisji DNA Departamentu Sprawiedliwości Stanów Zjednoczonych 2000:* http://www.ojp.usdoj.gov/nij.

26 Baechtel, F. S., Presley, K. W., Smerick, J. B. (1995). D1S80 typing of DNA from simulated forensic specimens. *Journal of Forensic Science*, 40, (4), 536–545.

27 Pawłowski, R. (1993). *Zastosowanie reakcji łańcuchowej polimerazy (metoda PCR) do ustalania płci osobnika na podstawie badania DNA izolowanego ze śladów biologicznych. Rozprawa habilitacyjna, 53.*

results from many genetic systems after performing one polymerization reaction, was introduced.

This stage in the history of forensic genetics ended up with the development and verification of methodological principles relating to the nature of the variability of large polymorphic fragments[28]. A new research methodology (PCR, polyacrylamide gel electrophoresis, silver staining) was implemented in practice, which could be useful during the analysis of polymorphism of much smaller DNA fragments[29]. The foundations for the uniform statistical analysis of the testing results were also laid down[30]. Studies focused on the establishing of the statistical indicators, such as, for example, the random match of DNA profiles[31].

Currently used research methods

The development of the human genome and the improvement of research technologies resulted in the discovery of further polymorphic structures. It turned out that there are many types of repeating sequences, built up of a small repetitive unit of 3 to 5 base pairs. Their tandem nature gave them a name – short tandem repeats (STR). Short repetition units are somewhat similar to VNTR. Their total size is a maximum of 500 base pairs. Smaller sizes mean that to be able to analyze this variability, based on a different number of repeat units in different individuals at the same place on the chromosome, it is necessary to use PCR technology[32]. A lower concentration of DNA is also needed to amplify the molecules, i.e. less than 1 ng / μl[33]. These were the reasons for the dynamic development of new research methods. What so far could not be analyzed due to the degree of degradation or the negligible amount of biological material, became the subject of genetic examinations. The introduction of STR allowed for crossing another threshold – it eliminated the barrier, a few years earlier considered impossible to overcome. The analyzes of deoxyribonucleic acid particles left on e. g. the sticky side of postage stamps, on the mouth pieces of

28 *The Future of Forensic DNA Testing. Raport Komisji DNA Departamentu Sprawiedliwości Stanów Zjednoczonych 2000:* http://www.ojp.usdoj.gov/nij.

29 Brown, T. A. (2012). *Genomy,* 20.

30 Mastana, S., Papiha, S. (2001). D1S80 distribution in world populations with new data from the UK and the Indian sub-continent. *Annals of Human Biology*, 28, (3), 308–318.

31 Falcone, E., Spadafora, P., de Luca, M., Ruffolo, R., Brancati, C., de Benedictis, G. (1995). DYS19, D12S67, and D1S80 polymorphisms in population samples from southern Italy and Greece. *Human biology*, 67, 689–710.

32 Brown, T. A. (2012). *Genomy,* 21.

33 Bal J. (red.) (2001). *Biologia molekularna w medycynie*, 84–86.

cigarette butts became routine. Moreover, experts were able to obtain sample substances from the part of the biological footprint and not the entire footprint as had been the case before. As a result, it became possible to perform biological analyzes of the genetic material that remained on the secured objects. The wide application of the methodology based on short repeated units polymorphism resulted mainly from the following research capabilities:

- a highly degraded DNA sample can be used for forensic analysis; short units in chromosomes are resistant to destructive processes,
- thanks to the PCR reaction, small concentrations of deoxyribonucleic acid can be analyzed,
- a potential number of loci that can be analyzed is very large, which is particularly important in the case ofhuman identification,
- the examination itself becomesless time-consuming – it is possible to obtain test results within two days,
- it is possible to amplify multiple STR loci simultaneously in a multiplex PCR reaction.

In the late 1990's, scientists startedthe research regarding the use of mitochondrial DNA and polymorphic fragments located in the Y chromosome. The analysis of the male haplotype turned out to be a useful tool in rape cases. By studying polymorphic genetic systems located on the Y chromosome, it was possible to determine variable characteristics of the male DNA, while the use of the standard procedure (based on the variability of DNA fragments located on autosomal chromosomes) did not always allow to obtain reliable results[34].

These main features were the reason for the dynamic development of new examination techniques. What so far could not be analyzed due to the degree of degradation or the negligible amount of biological material, was now the subject of genetic research. Introducing STR to practice allowed for crossing another threshhold, it eliminated the barrier, a few years earlier considered impossible to cross. This is because analyzes of deoxyribonucleic acid particles left on e. g. the sticky side of postage stamps, on the mouth pieces of cigarette butts have become a standard. Moreover, the experts were able to sample substances from the biological footprint and not the entire footprint as before. As a result, it became possible to perform biological analyzes in the future, as part of the genetic material remained on the secured object. The wide application of the methodology based on short repeated units polymorphism in forensic genetic

[34] Prinz, M., Sansone, M. Y. (2001). Chromosome specific Short Tandem Repeats in Forensic casework. *Croatian Medical Journal*, 42, (3), 288–291.

research resulted mainly from the following advantages:

- a DNA sample with a high degree of degradation can be used for forensic analysis; short units arranged on many chromosomes are resistant to destructive processes,
- thanks to the PCR reaction, small concentrations of deoxyribonucleic acid can be analyzed,
- the potential number of loci that can be analyzed is very large, this is of particular importance in the process of identifying a person based on kinship,
- research technology is not time-consuming – it is possible to obtain test results within two days,
- the biological basis shows that it is possible to amplify multiple STR loci simultaneously in a multiplex PCR reaction.

In the middle of the first decade of the twenty-first century, an analysis of polymorphic genetic systems located on the X chromosome was performed and new genetic markers were searched for, characterized by a high degree of polymorphism, useful in forensic examinations[35].

In the case of biological material with a high degree of degradation, where the chance of determining the nuclear DNA profile was minimal, the analysis of the circular, and therefore more resistant to degradation processes, DNA molecule contained in the mitochondria allowed to obtain results based on which it was possible to eliminate the person as a donor of the material genetic[36].

Due to the method of inheritance and the nature of the DNA molecule, the discrimination power of Y chromosome polymorphic systems and mitochondrial DNA is significantly smaller compared to polymorphic genetic systems located on autosomal chromosomes, which enables group identification[37]. Nevertheless, the introduction of these laboratory methods into forensic practise made it possible to analyze biological material that could not be studied so far.

A spectacular example of the use of mtDNA for identification purposes is the analysis of the nucleotide sequence of samples taken from the remains of the Romanov family. It was only in 1991 that the search for the burial place of the tsarist family and their exhumation began. In the first stage of the identification research, anthropological methods were used, based on which it was

35 Spólnicka, M. et al. (2006). Polimorfizm STR niekodującego regionu genu ludzkiego hormonu wzrostu (HUMGHCSA) i jego wykorzystanie w identyfikacji osobniczej – doniesienie wstępne. *Archiwum Medycyny Sądowej i Kryminologii*, 2, 95.

36 Butler, J. M. (2005). *Forensic DNA typing. Biology, technology and genetics of STR markers*, 241.

37 Goodwin, W. et al. (2007). *An introduction to forensic genetics*, 125–132.

established that eleven people were buried in the grave. The skeletons of four men and five women were reconstructed. The next step in the identification process was to compare the mtDNA sequence of the putative Empress Alexandra with samples taken from the Duke of Edinburgh, a contemporary maternal relative. Reference material for the alleged remains of Tsar Nicholas was collected from distant maternal relatives living today. The analysis of the nucleotide sequence made it possible to identify the remains of the tsarist family[38]. Nevertheless, the introduction of these laboratory methods into forensic practise made it possible to analyze biological material that could not be studied so far.

The skull of the Polish astronomer, Nicolaus Copernicus, was also identified using the research technique described above[39].

The future of forensic biology

The future of forensic biology is connected, on the one hand, with the increased availability of research equipment, its progressive miniaturization and the possibility of its application in non-laboratory conditions. On the other hand, there is increasing use of modern sequencing technologies such as NGS (Next Generation Sequencing) in profiling the perpetrators of criminal events. Currently, forensic laboratories can perform simultaneous testing of several dozen STR markers and several hundred SNP markers within one reaction. This makes it possible to obtain a greater capability of discrimination.

NGS technology is also used in mtDNA analysis, especially in the case of small amounts of material or degraded DNA. Examination of the entire genome of mitochondrial DNA increases discriminatory power, which is crucial, for example, in identifying NN human remains.

Besides, by using this method, experts perform the so-called DNA phenotyping (FDP)[40]. The currently used technique of DNA identification requires each time a template and comparative material. Oral swabs from the suspects may be a reference material concerning the secured evidence being the genetic

38 Ivanov P. L. et al. (1996). Mitochondrial DNA sequence hetroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the Romains of Tsar Nicholas II. *National Genetics*, , 12, (4), 417–420.

39 Bogdanowicz, W., Allen, M., Branicki, W., Lembring, M., Gajewska, M., Kupeic, T. (2009). Genetics identifikaction of putative remains of the famous astronomer Nicolaus Copernicus. In: Proceeding of the National Academy of sciece of United States of America, 106, (30), 12279–12282.

40 Kayser, M., Schneider, M. P. (2009). DNA-based prediction of human externally visible characteristics in forensics: motivations, scientific challenges and ethical considerations. *Forensic Science International: Genetics*, 3, 154–161.

material. In the case of NN human remains and the search for a missing person, reference material can be obtained ante mortem from, for example, the person's tooth brush, hair brush or razor. Nowadays, it is possible to conduct comparative analysis even without access to the missing person's close relatives. The material may be obtained from some distant relatives. However, there are cases where a DNA sample from a crime scene does not match in terms of DNA profile to any known suspect or any profile present in the DNA database. An important role in such situations might be played by DNA phenotyping, which allows for the recreation of the person's external appearance based on the DNA sample. This is an interesting and new method that does not require comparative material. It is possible to recreate a person's appearance based on the genetic test of coding DNA fragments regarding, for example, eye colour, hair colour, height, facial bone structure or biogeographic origin[41].

It should be noted that there is an increasing amount of studies focused on the analysis of animal DNA. Expertises are performed both in cases of traffic accidents involving animals as well as to identify protected and endangered species being the subject of illegal trade. Illegal trade concerns both live animals and a variety of products and objects, animals, and various types of products and items made of them. In the case of such products and objects, identification based on morphological features is not always possible, which is the reason behind the current increase in the number of genetic tests[42].

Despite the continuous improvement of the human identification methods, there are, however, situations where the use of forensic genetics alone might dispel doubts arising in the course of the investigation. Studies published in recent years indicate that sometimes answers can be found by using DNA microbiome testing. Based on the microbiome, it is possible to determine the origin of the evidence regarding not only specific tissues or body fluids but also body areas, e. g. it is possible to determine whether the source is the person's mouth or vagina. Thanks to those tests, it is also possible to locate an evidence sample of unknown origin and compare it with the reference material samples. This type of evidence can be used to prove the suspect's presence in a certain place or allow the investigators to follow the route the suspect used.

Promising results can be obtained by the genetic analysis of body fluids

41 Woźniak, A., Boroń, M., Zbieć-Piekarska, R., Spólnicka M. (2019). Zastosowanie wysokoprzepustowego sekwencjonowania DNA w genetyce sądowej. *Problemy Kryminalistyki*, 304, (2), 5–14.

42 Malewski, T., Dzikowski, A., Sołtyszewski, I. (2021). Molecular methods of animal species identification. *Polish Journal Natural Sciece*, 36, (1), 79–94.

(urine, blood, saliva) using mRNA and miRNA marker analysis. The advantage of this method is, above all, its high sensitivity, specificity and the possibility of simultaneous extraction of RNA and DNA, which is particularly important when DNA analysis is a priority and the amount of evidential material is limited[43].

There is an area in forensic biology that is still waiting for an effective solution. This area is the determination of the time of the formation of biological evidence, especially considering bloodstains. So far, none of the published research methods has been implemented into the day-to-day laboratory practice. It should be noted that finding an optimal and reliable marker of the complex ageing process of bloodstains is an extremely difficult task. The main reason is the fact that the ageing process is influenced by many different factors. The interrelationships between these factors have not been not fully understood. The key point is that the ageing process in many cases is not linear. Currently, available experimental techniques differ in accuracy and sensitivity, as well as the level of their invasiveness, which determines the degree of the wear considering tested evidential material. Comparing methods presented in scientific publications, such as HPLC (High-Performance Liquid Chromatography, EPR (Electron Paramagnetic Resonance), FTIR (Fourier Transform Infrared) or mobile technologies, it can be concluded that each of them is reliable only within a certain range of parameters. The deviations, however, increase along with ageing, so these methods are difficult to use in laboratory practice[44].

Conclusions

That said, forensic biology is a dynamically developing field of forensic sciences which over recent years has become in a certain way a "golden standard" of modern criminalistics. It opens up unique possibilities compared to forensic serology: the possibility of human identifications based on microscopic amounts of biological material, predicting some features of external appearance, the state of health and so on. It is worth noticing, however, that there is also an element of deep scientific reflection: forensic biologists constantly improve their methodology, adopt new theories and use new scientific knowledge to effectively solve crimes and bring justice to "cold" criminal cases.

43 Sijen, T. (2015). Molecular approaches for forensic cell type identification: on mRNA, miRNA, DNA methylation and microbial markers. *Forensic Science International: Genetics*, 18, 21–32.

44 Bremmer, R. H., de Bruin, K. G., van Gemert., M. J., van Leeuwen, T. G., Aalders M. C. (2012). Forensic quest for age determination of bloodstains. *Forensic Science International*, 216, 1–11.

KRIMINALISTINĖ BIOLOGIJA: ISTORIJA, DABARTIS IR ATEITIS

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Santrauka

Teismo biologijos istorija yra glaudžiai susijusi su dviem vardais – Karoliu Landsteineriu, kuris 1901 metais atrado kraujo grupes ABO sistemoje. Kitas yra Alecas Jeffreysas, kuris 1985 metais atrado polimorfines minipalydovų sekas ir išrado „DNR pirštų atspaudų" techniką. Šie du nutolę laike įvykiai leidžia atsekti teismo medicinos kaip savarankiškos teismo ekspertizės srities vystymosi kelią bei pripažinti ją disciplina, būtina pažeidėjams nustatyti. Jau daugelį metų biologiniams įrodymams tirti buvo naudojami serologiniai tyrimai ir fermentų polimorfizmo analizė. Tačiau daugeliu atvejų, pavyzdžiui, kai pokyčius lėmė natūralūs irimo procesai, fiziniai ar cheminiai veiksniai, nusikaltimo vietoje paliktų biologinių pėdsakų „amžius", nurodyti metodai teigiamų rezultatų nedavė. Tačiau šie apribojimai netaikomi genetiniams tyrimams. Kraujas, sperma, seilės yra labai geri DNR šaltiniai. Mažesnį DNR kiekį plaukuose, dantyse ar kauluose kompensuoja didesnis atsparumas nepalankiems išorės veiksniams. Įvedus DNR analizę, teismo medicinos ekspertizėse buvo galima naudoti viršutinio odos sluoksnio (epidermio) ląsteles. Anksčiau taikyti serologijos metodai tokių galimybių nesuteikė. Galima naudoti ir skrodimo metu gautus mėginius. Tačiau verta atsiminti, kad suardytos genetinės medžiagos kiekis auga proporcingai pomirtinio laiko ilgaamžiškumui. Kai kuriais atvejais DNR buvo galima rasti audiniuose, konservuotuose ir padengtuose parafinu. Pabaigoje verta paminėti, kad esant pažangioms technologijoms ir galimybei taikyti skirtingus tyrimo metodus, galima išanalizuoti kiekvieno tipo biologinius pėdsakus. Visus šiuos teismo medicinos DNR metodo raidos etapus aptaria pateikiamas straipsnis.

Raktiniai žodžiai: teismo biologija, biologinė medžiaga, kraujo dėmės, serologija, DNR analizė, teismo fenotipų nustatymas, teismo medicina.