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Genetic identification of a gunshot victim four years posthumously

Identyfikacja genetyczna ofiary postrzału po czterech latach od zgonu

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Praca przedstawia wyniki genetycznych badań identyfikacyjnych ofiary postrzału, której ciało zostało wydobyte po 4 latach od zgonu z dołu ziemnego wypełnionego wapnem. Badanie sekcyjne wykazało obecność zaawansowanych zmian gnilnych oraz częściowo przemiany tłuszczowo-woskowej. Ze zmian urazowych stwierdzono obecność dwóch ran postrzałowych głowy i klatki piersiowej, które doprowadziły do zgonu. Zarówno stan zwłok, jak i brak bliższych danych medycznych uniemożliwiały ustalenie tożsamości zwłok. Zabezpieczony do badań materiał, w postaci fragmentów tkanek miękkich, pozwolił na pełną identyfikację w oparciu o analizę genetyczną.

The paper presents a personal identification case of an unrecognized corpse, presumably belonging to a male missing for four years. The cadaver was buried in a ground ditch and covered with slaked lime and soil. During the investigation the burial place was indicated. The corpse was exhumed and afterwards transferred to the Department of Forensic Medicine, Medical University of Białystok. External examination and autopsy findings demonstrated adipocere formation and putrefaction, as well as two gunshot wounds in the thorax and the head assumed to be the cause of death. Personal identification procedure included skeletal and dental examination. As a source material for genetic typing, the femur, brain, lung, kidney and spleen samples were collected. DNA templates were extracted by a modified organic procedure and genotyped with the use of AmpFlSTR Identifiler Amplification Kit and PowerPlex Y System in an ABI 310 Genetic Analyzer (Applied Biosystems). All the soft tissue samples yielded sufficient quantity and quality of DNA to perform genetic profiling.

Słowa kluczowe: obrażenia postrzałowe, rozkład, identyfikacja osobnicza, DNA, AmpFlSTR Identifiler, PowerPlex Y
Key words: Gunshot wounds, decomposition, personal identification, DNA typing, AmpFlSTR Identifiler, PowerPlex Y

INTRODUCTION

Genetic profiling has been integrated with personal identification of unrecognized corpses and remains an element of the procedure owing to its discrimination power and potential typeability of decomposed biological material [1]. In consecutive stages of postmortem decomposition, human hard tissues, e.g. bones, and teeth have been the most suitable material for genetic identification [2, 3, 4, 5]; however,

their processing and DNA extraction is relatively costly and time-consuming. Taking into consideration papers reporting possible genotyping of decomposed human tissues [6, 7, 8, 9], the authors collected several soft tissue samples during the autopsy to verify their usefulness as a source of genetic profile.

CASE REPORT

In April 2006, an unrecognized male corpse was transferred to the Department of Forensic Medicine, Medical University of Białystok. The investigation data revealed that the victim was shot dead and his body was concealed in a ground ditch filled with slaked lime (calcium hydroxide, Ca(OH)2). Removal of the thick lime deposits from the cadaver surface prior to autopsy disclosed signs of putrefaction and adipocere formation (fig. 1). For identification purposes, two tattoo patterns revealed on the upper extremities were photographed. Based on the skeletal and dental findings, the victim's age was estimated at 30-35 years. During the autopsy, two gunshot wounds to the head and the chest were found. The track of the former wound led through the brain disclosing a presumptive cause of death. The character of the injuries, gunshot wound tracks and investigation findings were confirmatory of a homicide case. For genetic identification purposes, samples of brain, lung, kidney, spleen and femur were collected (fig. 2). DNA was extracted using a modified organic procedure: the specimens were placed in 1.5 ml Eppendorf tubes and incubated overnight at 56°C in 0.5 ml digestive buffer pH 7.5 (10mM Tris-HCl, 10mM EDTA, 50 mM NaCl, 2% SDS) with 0.3 mg/ml proteinase K (Sigma); the centrifuged pellets (Eppendorf, 16500 rpm, 1 min) were discarded and the aspirated supernatants were transferred to fresh tubes containing 0.5 ml phenol-chloroform-isoamyl alcohol mix (Sigma); after centrifugation at 16500 rpm for 5 min (Eppendorf), the resultant supernatants were transferred to fresh tubes; the latter step was repeated 2-3 times until the phenol phase became transparent; DNA preparations were concentrated and purified using the QIAquick PCR Purification Kit (Qiagen). The recovered DNA was quantitated fluorometrically [10, 11]. DNA quality was assessed by ethidium bromide 2% agarose gel electrophoresis. Polymorphic autosomal systems: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, AMG included in the AmpFlSTR Identifiler PCR Amplification Kit (Applied Biosystems) and Y-chromosomal systems: DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439 included in the PowerPlex Y System (Promega) were amplified following the manufacturers' instructions with the exception, that the all reaction reagents were reduced proportionally so that the volume of the reaction mix was 10μl. Electrophoresis and genotyping were performed in a ABI310 Genetic Analyzer (Applied Biosystems) using the GeneScan v.3.7 and Genotyper v3.7 software. All loci included in the AmpFlSTR Identifiler and PowerPlex Y System were amplified and compared with profiles of putative parents (table I).

Fig. 1. Gross appearance of the cadaver.
Ryc. 1. Ogólny wygląd zwłok.

Fig. 2. Gross appearance of the brain.
Ryc. 2. Ogólny wygląd mózgu.

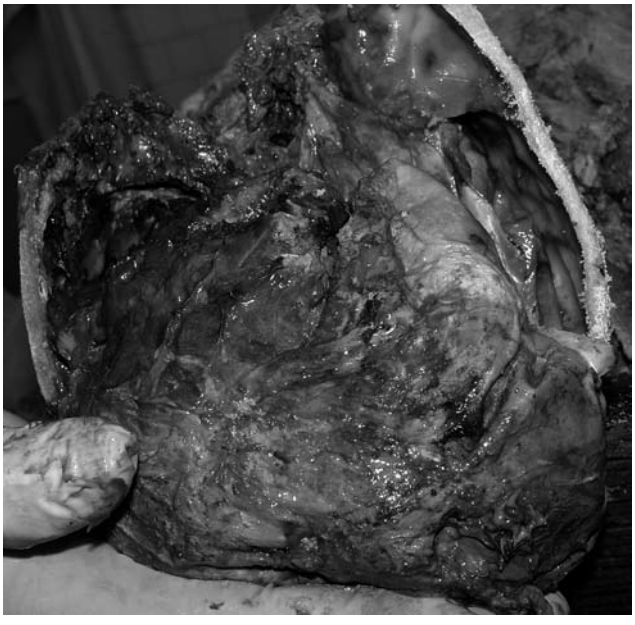

Table I. Genetic identification results.
Tabela I. Wyniki identyfikacji genetycznej.

Autosomal profiles Profile autosomalne				Y-chromosome profiles Profile chromosomu Y		
Locus	N/n corpse N/n zwłoki	Putative mother Domniemana matka	Putative father Domniemany ojciec	Locus	N/n corpse N/n zwłoki	Putative father Domniemany ojciec
D8S1179	11,12	12,13	11,13	DYS391	11	11
D21S11	29,32.2	28,32.2	28,29	DYS389I	14	14
D7S820	11,12	12	11,12	DYS439	12	12
CSF1PO	11	11	11	DYS389II	30	30
D3S1358	15,16	15,17	16,17	DYS438	12	12
TH01	6	6,9.3	6,9.3	DYS437	14	14
D13S317	11,12	11,12	8,11	DYS19	14	14
D16S539	11,12	12	11,12	DYS392	13	13
D2S1338	17,24	17,24	19,24	DYS393	13	13
D19S433	13,15.2	15,15.2	13,15	DYS390	24	24
vWA	17	16,17	16,17	DYS385	11,14	11,14
TPOX	8,11	10,11	8,11			
D18S51	18	15,18	12,18			
D5S818	11,12	11	11,12			
FGA	22.2,23	22.2,23	23			
AMG	XY	X	XY			
		MI = 578955,67	PI = 40625,31			PI = 1250

MI – maternity index (indeks macierzyństwa), PI – paternity index (indeks ojcostwa)

DISCUSSION

Genetic profiling is a potential method of choice in contemporary personal identification of unrecognized human corpses and remains [1, 3, 4, 5, 12]. In the presented case, the DNA source was represented by soft tissue samples. DNA was extracted using the organic method, commonly employed in genetic identification of mass disaster victims [13, 14]. The method was also reported as the most efficient in DNA extraction from aged blood specimens [15]. The usefulness of soft tissues in genetic typing was described by other authors [6, 7, 12], who successfully typed DNA profiles in cadavers within postmortem interval of 2 to 132 days. Extracted DNA yield ranged from 3 to 6 ng. The AmpFlSTR Identifiler kit was validated as highly specific and sensitive for human DNA and suitable in typing of degraded samples [16]. The authors of the present paper previously reported typeability of AmpFlSTR SGM Plus loci in specimens of human organs stored in selected soil environments [9]; however, the success rates were significantly lower than those observed in the present case. The cadaver under study was concealed immediately after death and exposed postmortem to the slaked lime environment for four years, what resulted in adipocere formation. The process involves conversion of body fat into solid white substances and is characterized by hydrolysis and hydrogenation of fatty tissue into a mixture of predominantly saturated fatty acids (myristic, palmitic, stearic). Unsaturated fatty acids (oleic and palmitoleic), calcium salts of fatty acids, hydroxyl and oxo-fatty acids have all been identified as constituents of adipocere. Their presence is of a special interest to forensic scientists as they have a potential to inhibit decomposition and thus to preserve the tissue material in a varying degree depending on the surrounding environment [8]. The optimum environment for adipocere formation described by many authors may be damp, warm, anaerobic conditions [17, 18, 19, 20]. We suggest that such factors acted on the corpse under study and resulted in preservation of DNA sufficient for successful genotyping of all loci of the AmpFlSTR Identifiler and PowerPlex Y System.

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