Phylogenetic analysis of mysterious burials revealed in the former penal labor camp Treblinka I*

Analiza filogenetyczna tajemniczych pochówków ujawnionych na terenie byłego karnego obozu pracy przymusowej Treblinka I*

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Abstract

The purpose of this paper is to formulate recommendations for the disclosure of biological traces in the laboratory and the handling of forensic evidence submitted for identification tests, recommended by the Polish Speaking Working Group of the International Society for Forensic Genetics. The paper organizes the knowledge of the most relevant stages of preliminary analysis of biological traces based on both literature sources and those resulting from years of research practice. Recommendations formulated in the course of multi-stage expert consultations contained in this study should be used in the development of laboratory procedures applied during the execution.

Keywords

ISFG-PL guidelines, biological traces, forensic evidence, disclosure and proceeding, preliminary studies, pre-genetic tests

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Streszczenie
Artykuł prezentuje interdyscyplinarne podejście do wyjaśnienia tajemnicy siedmiu pochówków odkrytych na terenie byłego karnego obozu pracy Treblinka I, podczas badań terenowych przeprowadzonych przez Polską Bazę Genetyczną Ofiar Totalitaryzmu (PBGOT). Oprócz szczątków odkryto także wiele artefaktów. Ze względu na lokalizację i charakter pochówków można przypuszczać, że szczątki mogły należeć do strażników obozu. Do weryfikacji tej hipotezy wykorzystano różne narzędzia genetyczne: analiza markerów STR i Y-STR, analiza DNA mitochondrialnego (HV1/HV2, sekwencjonowanie całego genomu mitochondrialnego) oraz przewidywanie haplogrup mtDNA i Y-DNA. Dodatkowo, ekshumowane szczątki poddano analizie antropologicznej. Stwierdzono, że wschodnioeuropejskie populacje mają rozkład haplogrup podobny do tego odkrytego w badaniu. Dlatego możemy wnioskować, że uzyskana analiza genetyczna nie przeczy zapisom historycznym. Badania zostały zlecone przez Prokuraturę Instytutu Pamięci Narodowej (Komisję Okręgową ds. Ścigania Zbrodni przeciwko Narodowi Polskiemu).

Słowa kluczowe
Treblinka I; Y-DNA; mtDNA; markery haploidalne; obóz nazistowski, strażnicy

1. Introduction

The penal labor camp Treblinka I functioned from 1941 to 1944 and was the last of the three camps that operated in the General Government (1).

Guard units began to be organized in September 1941, following Heinrich Himmler's decision. He authorized SS and police commanders to recruit citizens of the USSR and prisoners of war into SS auxiliary units (2). This part of the camp structure was primarily composed of individuals recruited from collaborationist formations during World War II. They were mainly Soviet prisoners of war who had aligned with the German side and underwent training in the SS camp in Trawniki. The selection process involved choosing those who appeared loyal, strong, healthy, and suitable for hard work.

In September 1945, the High Command of the Wehrmacht issued a statement releasing Red Army soldiers from prisoner-of-war camps if they were willing to switch to the German side (2). The training location was the camp in Trawniki, led by the SS- und Polizeiführer im Distrikt Lublin, at the Trawniki Training Camp (1,2).

According to the literature, approximately 5,000 guards passed through the SS-Wachmannschaften (guard units that operated during World War II, primarily in concentration camps and extermination camps). Originally, they were intended to serve in SS and police units in the occupied territories of the USSR. However, they were utilized in implementing the plan to exterminate the Jewish population in occupied Poland. With their assistance, as part of "Operation Reinhardt," ghettos were liquidated, mass executions were carried out, and they were sent to camps where they performed guard functions in the Nazi extermination camps (Bełżec, Sobibór, Treblinka). Following the conclusion of this operation, some were sent to the Adriatic coast, while others were recruited into the crews of concentration camps (1,2). In September 1943, Odilo Globocnik (co-founder of the extermination camps) assumed the position of Higher SS and Police Commander in the Operational Zone of the Adriatic Coast Region. His report indicates that when he left occupied Poland, approximately 3,700 guards served in the SS-Wachmannschaften. This number differed from the count of identification cards issued at that time (lower by 1,050 people), owing to desertions and deaths from infectious diseases. Alongside Globocnik, a group of his subordinates from the time of Operation Reinhardt was dispatched back to the Adriatic coast, including most of the SS men from the staff of Bełżec, Sobibór, and Treblinka. Their primary task in the new deployment was to conduct roundups of local Jews and engage in combat against Yugoslav and Italian partisans (1,2).

In mid-1942, the Germans constructed Treblinka II extermination camp nearby. The staff of this camp comprised approximately 40 Germans and Austrians, predominantly members of the SS and police, along with around 120 camp guards, the majority of whom were of Ukrainian origin. In the autumn of the same year, Poles were also recruited, but they constituted a very small group from the Podhale region. Various Jewish nationalities were also represented - notably, the watchman serving as the commandant of the labor camp in Treblinka was of Jewish origin (3,4).

The guards were provided with captured Polish and Belgian uniforms, which were subsequently dyed black. This is why

they were referred to as "blacks" among the prisoners. Their armament comprised captured Soviet rifles. Individuals fluent in German were assigned the roles of subunit commanders (Zugführer and Oberzugführer (5).

In 2019, as part of planned modernization works in the forest parking lot of the museum in Treblinka, archaeological surveys were conducted. During these surveys, one mass grave and seven individual burials were discovered (Photo 1). The research results were subsequently published in our team's work (6).

Photo 1. ***Place of exhumation, seven revealed burials*** *(source: Prof. Andrzej Ossowski, Department of Forensic Genetics, PUM)*

Photo 2. ***Revealed artifacts. The photo shows fragments of a metal wreath and flowers*** *(source: Prof. Andrzej Ossowski, Department of Forensic Genetics, PUM)*

The exhumed remains were retrieved from individual grave pits. Given that these burials were situated in the designated "place of executions" and their characteristics deviated from the burials of camp victims, our team hypothesized that they could have belonged to the camp guards of Treblinka camp. It is documented that the victims were interred in mass graves in this area. Furthermore, the individual burials yielded artifacts, including buttons from Wehrmacht jackets, an official whistle, combs, and underwear buttons (Photo 2).

Functional whistles served as a call to order and were also used to announce the so-called "Lagerruhe," signifying silence in the camp around 9 p.m. Metal wreaths were discovered on the lids of the coffins, symbolizing respect for the deceased.

The camp guard was a privileged unit utilized by the camp authorities to enforce internal camp policies. The study of World War II sites typically follows two directions. The first involves providing information to the IPN (Institute of National Remembrance) prosecutor's office as a result of ongoing proceedings, while the second aims to comprehend the painful and tragic period of our history. This provides an opportunity to obtain evidence or legal information regarding war crimes or other offenses committed during World War II. The IPN in Poland is involved, among other things, in prosecuting Nazi and communist crimes, and research on these sites from this perspective may aim to expose responsibility for crimes from that period. Contemporary analysis and research of such places focus on documenting and analyzing historical events, cultural traces, as well as understanding the consequences of war for the population and society. This aspect of research may aim at commemorating victims, historical education, or analyzing social and cultural consequences of the war.

In Photo 1, the exhumation site is visible after exploration. The outlines of seven regular burials are apparent. In Photo 2, one of the revealed burials is visible. In the grave pit, remnants of metal wreaths, adorned with red flowers, fragments of uniforms, and personal items, can be observed.

2. Objective of the work

The objective of this study is to demonstrate a multidisciplinary approach to the identification process. The research attempted to validate historical and anthropological hypotheses through the application of genetic methods. Mitochondrial DNA analysis, utilizing Sanger sequencing and NGS, along with STR markers profiling, was employed. Additionally, efforts were made to ascertain the biogeographic origin of the exhumed remains.

3. Material and methods

3.1. Material

In 2019, the team from the Polish Genetic Database of Victims of Totalitarianism, commissioned by the IPN prosecutor's office (Institute of National Remembrance, Branch Commission for the Prosecution of Crimes against the Polish Nation), con-

ducted the exhumation of human remains discovered in seven grave pits. The research received consent from the IPN prosecutor, and the study obtained approval from the Bioethics Committee. Maxillary molars were extracted from the exhumed remains for DNA analysis. The collected biological material posed challenges in processing and isolating DNA, attributed to the time elapsed between the moment of death and the discovery of the remains, as well as the environmental conditions in which they were stored.

3.2. Methods

Anthropological analysis

The biological profile assessment was conducted using methods appropriate for the studied population. Based on pubic symphysis morphology (7), auricular surface morphology (8), teeth abrasion and teeth root translucency (9,10), and skeleton ossification (11), the biological age-at-death was estimated. Biological sex was evaluated using morphological features (12) and metric methods (13,14). The stature was estimated using a formula based on the measurements of the long bones (15). This study used skull measurements and two computer software programs to estimate ancestry (16,17).

Genetic analysis

The work utilized the most common genetic tools, including the analysis of STR and Y-STR markers, examination of HV1/HV2 mtDNA hypervariable regions, and sequencing of the entire mtDNA genome through the NGS method. The determination of mtDNA haplogroups was conducted using the EMPOP database. (Launched on October 16, 2006, the EMPOP database has been available at http://www.empop.org).
The estimation of Y-DNA haplogroups was carried out using the NevGen tool (https://www.nevgen.org/). This tool not only determines haplogroups but also provides a percentage probability, allowing for adjustments based on the average value of one or more haplogroups.

Preparation of material for research

Three maxillary molars from each skeleton were utilized for DNA analysis.

The teeth underwent mechanical cleaning with diamond drills until the enamel was removed, eliminating cavities and bacteria that could potentially act as PCR reaction inhibitors. Subsequently, they underwent thorough chemical cleaning, rinsing in sterile water, and exposure to a UV lamp for 30 minutes on each side. Dry cleaning involved rinsing the samples in a ready-made Tween20 detergent (Sigma-Aldrich, USA) for 5 minutes. The material prepared in this manner was then ground in a cryogenic mill in the presence of liquid nitrogen, resulting in the complete powdering of the cleaned tooth.

DNA isolation

The DNA isolation of the biological material was performed using the PrepFiler® BTA Forensic DNA Extraction Kit (Thermo Fisher Scientific, USA), following the manufacturer's protocol (18). This kit is specifically designed for highly calcified tissues and includes a special buffer to facilitate DNA release from such tissues. Positive and negative controls were applied to exclude contamination. For DNA isolation, 50 mg of bone powder was collected.

Determination of DNA concentration, degree of DNA degradation, and the presence of PCR inhibitors.

DNA extracts were quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, USA) on a 7500 Real-Time PCR instrument (Thermo Fisher Scientific, USA) to assess the concentration of human DNA and human male DNA in the extracts, detect the presence of PCR inhibitors, and determine the level of DNA degradation. The degradation rate was assessed based on the ratio of long to short fragments.

The DNA concentration, the amount of DNA from the male and the degree of DNA degradation were calculated using the HID Real-Time PCR Analysis Software v1.2 (Thermo Fisher Scientific, USA). The kit utilizes target loci with multiple copies to improve detection sensitivity. There are three human-specific target loci: small autosomal, large autosomal, and the target on the Y chromosome. Each consists of multiple copies dispersed across different autosomal chromosomes (small autosomal and large autosomal) or multiple copies on the Y chromosome. (19,20).

Due to the nature of the research material, it is expected that DNA extracts may contain small amounts of template DNA.. All samples were tested in triplicate (three teeth independently taken from one skeleton), depending on data quality, and a consensus sequence was used for the final analysis according to literature guidelines (21).

Table 1. Total DNA concentration and degradation index (DI) of remains collected from grave pits.

Material	Sample	DNA concentration [ng/µl]	Degradation Index DI
Skeleton 1	teeth from the jaw	0.0215	3.1662
Skeleton 2	teeth from the jaw	0.0820	7.0152
Skeleton 3	teeth from the jaw	3.1964×10^{-4}	3.6491
Skeleton 4	teeth from the jaw	N/D*	N/D*
Skeleton 5	teeth from the jaw	5.2984×10^{-4}	2.9865
Skeleton 6	teeth from the jaw	0.0199	N/D*
Skeleton 7	teeth from the jaw	7.0161×10^{-4}	1.2263

*N/D – not determined

The results are presented in Table 1. The total concentration of human DNA was used in the study.

The average degradation index of the tested samples was 3.60. In samples from skeletons 4 and 6, the degradation index could not be determined because long DNA fragments were not amplified. However, the amplification of STR (Table 3) and Y-STR (Table 4) markers was obtained from these samples. Degradation index values of 1-10 (according to the manufacturer) indicate that the DNA is slightly or moderately degraded. Inhibition of the PCR reaction is also possible, but not enough to significantly suppress IPC (Internal Positive Control) amplification. Degradation index values <1 indicate that the DNA has not been degraded or inhibited. In cases where IPC amplification remains effective despite slight or moderate degradation of DNA, it suggests that the DNA analysis results are reliable, despite some loss (degradation) in the quality of genetic material.

Amplifying STR fragments in the Global Filer system

Autosomal STR markers were amplified using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific, USA), with a 29-cycle thermal profile, on a GeneAmp™ PCR System 9700 thermal cycler (Thermo Fisher Scientific, USA). The maximum amount of DNA isolate was used for the reaction, to the expected high degree of degradation of the material.

Detection of PCR products was performed on a 3500 Genetic Analyzer sequencer. The results were analyzed using GeneMapper® ID-X Software v1.1 (Thermo Fisher Scientific, USA).

During data analysis, the analytical threshold, cut-off point (AT) was set at 100 RFU, as internally validated by the laboratory. Alleles below this value were not considered.

Amplifying STR fragments in the Y Filer Plus system

The Y-Filer™ Plus PCR Amplification Kit (Applied Biosystems, USA) was used to analyze Y-STR markers. The kit is characterized by specific differentiation capacity thanks to highly discriminatory markers. Dedicated to degraded samples. Improved genotyping accuracy with an extended allelic ladder.

The identification of specific individuals based on the analysis of the Y chromosome in isolation is challenging because this type of analysis provides information only about the male line and does not allow for distinguishing between father and son, grandfather and grandson, etc.

Mitochondrial DNA analysis

Two reaction mixtures were prepared: one described as 56 using primers 15971 (N5; forward), 16410 (N6; reverse), and the other described as 78 using primers L15 (N7; forward), R429 (N8; reverse). Each mixture for one sample consisted of 0.5 µl forward primer, 0.5 µl reverse primer, 6 µl deionized water, and 10 µl HotStarTaq Master Mix. Prepared mixtures were to be distributed into 200 µl tubes at 17 µl each, labeled 56 or 78 depending on the primers used. The examined DNA was added to the mixtures 56 and 78 in a volume of 3 µl. Positive control (17 µl reaction mixture + 3 µl control DNA) and negative control (17 µl reaction mixture + 3 µl nuclease-free water) were also to be prepared. The samples were then placed in the Veriti™ 96-Well Fast Thermal Cycler (Thermo Fisher Scientific, USA).

The thermal profile of the reaction was as follows: 90°C – 90 min, 30 cycles of (60°C – 2 min, 72°C – 30 sec), 20°C – 10 min, ∞ – 4°C. The total volume of the reaction mixture was 20 µL.

Enzymatic purification:

The product of the amplification reaction served as a matrix for the next reaction. 10 µl of the products from the previous reaction was added to the new, sterile 200 µl tubes. Subsequently, 1 µl of ExoI enzyme (Thermo Fisher Scientific, USA) and 2 µl of Fast-AP enzyme (Thermo Fisher Scientific, USA) was added to all samples, respectively. The thermal profile of the reaction was the same as for the amplification reaction, and the total volume of the reaction mixture was 13 µL.

Cycle sequencing reaction:

The Exo-FastAP reaction product served as a matrix for the next stage. A reaction mixture was prepared, consisting of 4 µl BigDye® Terminator v3.1 RR-100, 2 µl BigDye® Terminator v1.1 (Thermo Fisher Scientific, USA) & v3.1 5X Sequencing Buffer (Thermo Fisher Scientific, USA), 2 µl of the respective primer, and 9 µl deionized water. Samples previously divided into 56 and 78 were separated so that each primer underwent the reaction in a separate tube (the 56 reaction product was a reagent for reactions: 5 and 6; N5 and N6 primers were added, respectively; similarly, the 78 reaction product was a reagent for reactions: 7 and 8; N7 and N8 primers were added, respectively), yielding 4 tubes of 200 µl per tested sample (and two tested products of the Exo-FastAP reaction). The tubes were labeled with the sample name and numbered sequentially 5, 6, 7, 8. 17 µl of the prepared reaction mixture was added to each tube. 3 µl of the 56 product was added to tubes labeled 5 and 6, and 3 µl of the 78 product was added to tubes labeled 7 and 8. The thermal profile of the reaction was the same as for the amplification reaction, and the total volume of the reaction mixture was 20 µL.

Purification of sequencing reaction products:

The product of the cycle sequencing reaction served as a matrix for the next step. This stage was conducted according to the manufacturer's instructions (Thermo Fisher Scientific, USA).

Capillary electrophoresis:

Capillary electrophoresis in the sequencing of mtDNA (HV1 and HV2) refers to a technique in which fragments of mtDNA (mitochondrial DNA) containing hypervariable region 1 (HV1) and hypervariable region 2 (HV2) are separated and analyzed using capillary electrophoresis. Capillary electrophoresis is a technique in which DNA fragments are introduced into a capillary and then separated based on the size and electric charge

under the influence of an electric field. After separation, detectors record fluorescent bands, which are then transformed into nucleotide sequences, enabling the reading of the unique mtDNA sequence for a given hypervariable region.

Sequences were subjected to capillary electrophoresis on the 3500 Genetic Analyzer 8-Capillary Array (Thermo Fisher Scientific, USA). The obtained sequences of tested samples, positive and negative controls, were compared with the corrected reference sequence Cambridge (rCRS) and evaluated using Sequencher DNA Sequence Analysis Software (22)

Primary data analysis:
Files generated by Data Collection Software were transferred to an external drive. After the initial analysis, the files were saved on the drive and ready to be uploaded to Sequencher 5.4.6 software. Data were analyzed using Thermo Fisher Scientific's Sequencing Analysis Software 7.

Secondary data analysis:
Subsequently, data were analyzed using Gene Codes' Sequencher 5.4.6 software.(23–26)Mitochondrial DNA haplogroups were analyzed using two phylogenetic tools: the EMPOP database (http://empop.online) (27), and the HaploGrep program (https://haplogrep.i-med.ac.at).

Sequencing of the entire mitochondrial genome
IonTorrent technology was used to sequence entire mitogenomes. Libraries were prepared using the Precision ID Library Kit and the Precision ID mtDNA Whole Genome Panel. Thanks to the innovative tiling approach in this panel, it is possible to obtain entire mitochondrial genomes from highly degraded material. The Precision ID mtDNA Whole Genome Panel contains 2 primer pools, each with 81 primers that allow sequencing of short, overlapping amplicons. Library preparation, including amplification of target sequences, partial amplicon digestion, adapter ligation, and library purification, was performed according to the manufacturer's instructions. Libraries were quantified using the Ion Library TaqMan® Quantitation Kit (Thermo Fisher Scientific) and the QuantStudio™ 5 Real-Time PCR System according to the manufacturer's instructions, diluted to the optimal concentration, and pooled.

Template preparation and chip loading were performed by the Ion Chef™ instrument using the Ion S5™ Precision ID Chef & Sequencing Kit and the Ion 530™ Chip. Sequencing was performed on an Ion S5™ HID sequencer. Sequencing data were analyzed by Torrent Suite 5.10.2, and mtDNA sequencing products were compared to the Cambridge Corrected Reference Sequence (rCRS) by Torrent Variant Caller software (all above: Thermo Fisher Scientific)..Next-generation sequencing was performed for 4 individuals: skeletons no. 1, 4, 5 and 7, due to material degradation.

Y-chromosome haplogroup prediction
Y-chromosome haplogroup estimation was performed using allele frequencies of 23 Y-STR loci (DYS576, DYS389I, DYS635, DYS389II, DYS460, DYS458, DYS19, YGATAH4, DYS448, DYS391, DYS456, DYS390, DYS438, DYS392, DYS570, DYS43 7, DYS385a/b, DYS449, DYS393, DYS439, DYS481, and DYS533) using the NevGen utility (https://www.nevgen.org/).

Currently, Y-STR markers are used to predict the haplogroup of the Y chromosome. These analyzes use databases containing genetic information from different populations, which allows comparison of results and determination of an individual's haplogroup in the context of global genetic diversity.

4. Results

4.1. Results of anthropological and medical analyses
The poor skeleton preservation did not allow for the full biological profile assessment of several skeletons. Skeletons 3 to 7 did not have skulls preserved, or the skull was in a fragmentary state. Anthropological analyses are presented in Table 2. Detailed results of anthropological, archaeological, and forensic medical studies have been presented in a separate publication. (28).

4.2. Genetic analysis results
As a result of genetic analysis, 6 male DNA profiles were obtained. No profile was obtained from skeleton 3. The reason may be the degradation of biological material and the presence of PCR inhibitors. The results are given in Table 3.

The profiles (skeleton 1, 2 ,4, 6 and 7) obtained as a result of the research will be suitable for individual identification if a reference material is available.

For skeletons 1, 2 and 6. mtDNA haplotypes were determined in the range of HV1/HV2 regions. In the case of skeleton 4, the haplotypes in the HV2 range and the incomplete HVR1 range (16024-16350) were determined due to the degradation of the biological material. The results are presented in Table 5.

Table 2. The results of anthropological analysis

Sample	Skeleton preservation	Trauma	Sex	Age	Stature [cm]	Ancestry
Skeleton 1	The skeleton is preserved in good condition, a few skeletal elements are missing, and there is little post-mortem damage to the skeleton.	no injuries	Male	34-44	169.22 +/- 3.27	European
Skeleton 2	The skeleton is preserved in good condition, and most skeletal elements are present.	Traces of gunshot wounds to the skull indicate at least three shots, possibly inflicted by a weapon of the same caliber (9 mm).	Male	34-49	173.03 +/- 3.27	European
Skeleton 3	The skeleton is preserved in a fragmentary state, many skeletal elements are missing, while the present parts of the skeleton are mostly with traces of post-mortem damage.	no injuries	Male	35-44	160.89 +/- 3.27	NA*
Skeleton 4	The skeleton is preserved in a fragmentary state, many skeletal elements are missing, while the present parts of the skeleton are mostly with traces of post-mortem damage.	Embedded shrapnel or bullet in the right kneecap. On the skull, injuries were inflicted with blunt-edged instruments.	Male	18-25	169.01 +/- 4.05	NA*
Skeleton 5	The skeleton is preserved in a fragmentary state, no element of the skeleton has survived without post-mortem damage, and many skeletal elements are missing.	no injuries	Male	18-25	168.51 +/- 3.27	NA*
Skeleton 6	The skeleton is preserved in poor condition, most of the preserved bones are damaged after post-mortem, and many skeletal elements are missing.	no injuries	Male	18-25	170.41 +/- 3.27	NA*
Skeleton 7	The skeleton is preserved in a very bad condition, no bone element is preserved without damage, and most of the bones are present as small bone fragments.	no injuries	Male	18-30	NA	NA*

* NA (not applicable)

Table 3. The results of DNA typing using the Global Filer™ system

Loci	Skeleton 1	Skeleton 2	Skeleton 3	Skeleton 4	Skeleton 5	Skeleton 6	Skeleton 7
D3S1358	14,15	15,18	-	16,17	15,16	14,17	15
vWA	15,17	14,16	-	14,17	13,15	15,17	15,18
D16S539	12,13	9,14	-	11	-	9,12	10,11
CSF1PO	12,13	9,10	-	11,12	-	12	-
TPOX	8,11	8	-	8	8	-	9
INS/DEL	2	2	-	2	2	2	2
AMELO	XY	XY	-	XY	XY	XY	XY
D8S1179	12	12,13	-	12,13	13,14	13,15	11,15
D21S11	30,30.2	29,31.2	-	28,32.2	29	28	29,30
D18S51	13,16	15,20	-	13,16	-	11,17	-
DYS391	11	11	-	10	-	10	-
D2S441	10,14	11	-	10,11	11	11,12	10,13
D19S433	14,15.2	13,14	-	12,14	13,15	12,13	14.2,15
TH01	6,7	6,9.3	-	6,8	9,9.3	9	8,9.3

Loci	Skeleton 1	Skeleton 2	Skeleton 3	Skeleton 4	Skeleton 5	Skeleton 6	Skeleton 7
FGA	21,22	19,22	-	22,24	23,25	21	25
D22S1045	11,14	11,16	-	16	14,15	15,16	11,15
D5S818	11	11,12	-	10,12	11	11,12	10,12
D13S317	8,12	9,13	-	11,14	12	10,12	11
D7S820	8,11	7,11	-	10,11	-	10	-
SE33	19,33.2	-	-	29.2,34.2	-	-	-
D10S1248	14,16	13,14	-	15,16	14	14,16	13,15
D1S1656	12,17.3	15,17	-	12,17.3	-	12,14	14
D12S391	18,24	18,23	-	18,21	-	18.3,21	21
D2S1338	16,18	-	-	17,23	19	-	17

Table 4. The results of DNA typing using the Y- Filer™ Plus system

Loci	Skeleton 1	Skeleton 2	Skeleton 3	Skeleton 4	Skeleton 5	Skeleton 6	Skeleton 7
DYS576	19	19	-	20	18	17	17
DYS389I	13	13	-	13	13	13	14
DYS635	23	23	-	24	-	24	23
DYS389II	29	31	-	30	30	29	-
DYS627	-	17	-	21	-	20	18
DYS460	11	11	-	11	12	11	11
DYS458	17	15	-	16	15	17	15
DYS19	14	17	-	14	-	15	16
Y GATAH4	12	-	-	13	-	12	-
DYS448	19	20	-	19	-	19	-
DYS391	11	11	-	10	-	10	-
DYS456	16	15	-	16	15	14	15
DYS390	24	25	-	24	-	23	25
DYS438	12	11	-	12	-	10	11
DYS392	13	11	-	13	11	14	-
DYS518	38	41	-	38	39	38	38
DYS570	20	18	-	17	20	21	19
DYS437	15	14	-	15	14	14	14
DYS385	11,14	11,15	-	11,15	-	11,14	11
DYS449	29	33	-	30	-	28	-
DYS393	13	13	-	13	13	14	14
DYS439	12	10	-	11	-	11	8
DYS481	22	23	-	22	-	20	-
DYF387S1	36,37	36,38	-	35,36	-	35	37,38
DYS533	11	13	-	12	-	11	-

* The loci DYS385 and DYF387S1 are multi-locus markers.

Table 5. The obtained results of mtDNA tests (regions HV1, HV2) and NGS were analyzed in the EMPOP database

Sample	HV1 (16024-16365)	HV2 (73-340)	WG (1-16569)	Haplogroup mtDNA	Haplogroup mtDNA (NGS)
Skeleton 1	16287T	93G 152C 263G 309.1C 315.1C	93G 152C 263G 309.1C 315.1C 750G 1438G 4769G 8609T 8860G 9071T 10394T 10828C 13651G 15326G 16287T 16519C	H	H16c
Skeleton 2	rCRS	263G 309.1C 315.1C	no result obtained	R0	R0
Skeleton 3	no result obtained	no result obtained	no result obtained	no result obtained	no result obtained
Skeleton 4	16256T 16270T 16294T The haplotype was incompletely determined 16024-16350	73G 152C 263G 315.1C	73G 152C 263G 315.1C 750G 1438G 1700C 2706G 3197C 4769G 5495C 7028T 8860G 9477A 11467G 11719A 11803C 12308G 12372A 13617C 14766T 14793G 15218G 15326G 15924G 16256T 16270T 16294T 16399G	U5a1a1h	U5a1a1h
Skeleton 5	no result obtained	no result obtained	73G 210G 228A 263G 295T 309.1C 315.1C 462T 482C 489C 750G 1438G 2706G 3010A 3394C 4216C 4769G 5773A 7028T 7055G 7184G 8860G 10398G 10463C 11251G 11719A 11918A 12612G 13020C 13708A 14766T 14798C 15326G 15452A 16069T 16126C	no result obtained	J1c1b1a
Skeleton 6	16114A 16192T 16256T 16270T 16294T	73G 146C 263G 309.1C 315.1C	no result obtained	U5a2a	U5a2a
Skeleton 7	no result obtained	no result obtained	186A 263G 309.1C 315.1C 750G 1438G 4688C 4769G 8715C 8860G 11191T 12085T 15326G 16221T 16519C	no result obtained	H28a

The high degree of DNA degradation and the presence of possible PCR inhibitors made it impossible to determine the DNA haplotype from a biological sample from skeleton 3.

For the samples secured from skeletons 1, 4, 5, and 7 mtDNA haplotypes were determined for the complete mitochondrial genome (Table 5). The high degree of degradation made it impossible to perform the determination in the sample taken from skeletons 2 and 6.

The analysis of mtDNA haplogroups of remains exhumed at the former Treblinka I penal labor camp, discovered in seven individual graves, supports the hypothesis that these remains may belong to guard units of the Treblinka camp due to the observed distribution of mtDNA haplogroups (Table 5).

The marked haplogroups are characteristic of Eastern European populations. They are also found in the Ukrainian and Russian populations.(29,30). Due to the nature of the exhumation

site and historical facts about what nationalities could have died in Treblinka, the frequency of mtDNA haplogroups in the examined skeletons was related to the frequency of mtDNA haplogroups in various populations: Roma (Table 6),, Poles (Table 7), Poles and Russians (Table 8), and Russians and Ukrainians (Table 9), included in the literature analysis. The results are presented in the tables below. This analysis was performed to distinguish the population structure.

Table 6. Comparison of the frequency of occurrence of mtDNA haplogroups in Roma populations and the study sample, and the results obtained in the course of this study (31)

Haplogroup mtDNA	Roma (total of 275 persons)	Skeleton 1,2,4,5,6,7 (total of 6 persons)
R0 (including HV and H)	35.6%	50.0%
U (without K)	26.5%	0.0%
J	12.8%	33.3%
M	9.1%	16.7%
X	7.6%	0.0%
I	2.2%	0.0%
N1b	1.8%	0.0%
T	1.8%	0.0%
K	1.4%	0.0%
W	1.1%	0.0%

Table 7. Comparison of the frequency of occurrence of mtDNA haplogroups in the Polish population and the study sample, and the results obtained in the course of this study (32)

Haplogroup mtDNA	Polish people (total of 5852 persons)	Skeleton 1,2,4,5,6,7 (total of 6 persons)
R0 (including HV and H)	48.0%	50.0%
U (without K)	20.1%	33.3%
J	9.8%	16.7%
T	9.1%	0.0%
K	4.1%	0.0%
W	2.4%	0.0%
I	1.8%	0.0%
V	1.2%	0.0%
N1a, N1b	1.1%	0.0%
M (including C, Z)	0.8%	0.0%
D	0.5%	0.0%
X	0.4%	0.0%
A	0.3%	0.0%
R1, R2, R3, R9	0.3%	0.0%
G	0.2%	0.0%
L	0.1%	0.0%
B	0.1%	0.0%

Table 8. Comparison of the frequency of mtDNA haplogroups in the Polish and Russian populations and the study sample, and the results obtained in the course of this study (33)

Haplogroup mtDNA	Poland (total of 413 persons)	Northwestern Russia (total of 157 persons)	Skeleton 1,2,4,5,6,7 (total of 6 persons)
R0 (including HV and H)	43.6%	46.5%	50.0%
U (without K)	21.8%	17.8%	33.3%
J	7.7%	7.0%	16.7%
V	4.8%	5.1%	0.0%
T	7.5%	7.6%	0.0%
K	4.4%	1.9%	0.0%
R (without R0)	0.5%	2.5%	0.0%
N1a, N1b, N1c, N*	1.5%	1.9%	0.0%
I	2.2%	1.9%	0.0%
W	3.6%	1.9%	0.0%
X	1.9%	0.6%	0.0%
C	0.2%	0.0%	0.0%
Z	0.0%	1.3%	0.0%
D	0.0%	1.3%	0.0%
G2a	0.2%	0.0%	0.0%
M1	0.0%	1.3%	0.0%
M10	0.0%	0.6%	0.0%
A	0.0%	0.6%	0.0%

*Table 9. **Comparison of the frequency of mtDNA haplogroups in the Ukrainian and Russian populations and the study sample, and the results obtained in the course of this study** (29)*

Haplogroup mtDNA	Russians (total of 50 persons)	Ukrainians (total of 18 persons)	Skeleton 1,2,4,5,6,7 (total of 6 persons)
R0 (including HV and H)	48.0%	44.4%	50.0%
U (without K)	12.0%	22.2%	33.3%
J	6.0%	5.6%	16.7%
I	2.0%	0.0%	0.0%
K	2.0%	5.6%	0.0%
Z	2.0%	0.0%	0.0%
T	18.0%	16.7%	0.0%
V	4.0%	0.0%	0.0%
W	2.0%	0.0%	0.0%
X	4.0%	5.6%	0.0%

Haplogroups obtained as a result of mtDNA analysis were as follows:
R0 (including H and HV) 50%, U (without K) 33.3%, J – 16.7%, and W - 10%, about the population of Jews, Roma, Russians, Ukrainians, and Poles. The group in which the haplogroup was determined comprised 6 people, which constitutes about 85%

Results of Y-DNA haplogroups

The results of Y-haplogroup prediction using the NevGen program are summarized in Table 10.

*Table 10. **Prediction of Y-DNA haplogroups of analyzed skeletons** (https://www,nevgenorg), accessed August 10, 2023)*

Sample	Y-DNA Haplogroup	Probability of occurrence [%]
Skeleton 1	R1b	100
Skeleton 2	R1a	100
Skeleton 3	undefined	Undefined
Skeleton 4	R1b	100
Skeleton 5	R1a	39.57
Skeleton 6	N1a1	99.89
Skeleton 7	R1a	99.80

Successfully assigned haplogroups were obtained for 6 Y-STR profiles. The haplogroup for skeleton 3 was not obtained. The reason is the degradation of the biological material from which the Y-STR profile was not obtained either.

5. Discussion

The research highlights the interdisciplinary nature of the project. Genetic evidence aligns with historical facts. An experienced team, along with the selection of appropriate laboratory procedures and research methods, enabled the acquisition of high-quality, repeatable DNA sequences from the collected material. In the case of degraded material, proper insulation methods play a crucial role. In cases involving human remains discovered at the scene, hard tissues (bones, teeth) are the primary materials used for genetic identification tests. The location for our exhumation work was chosen based on historical data, analysis of archival aerial photos from 1944, and prior non-invasive research conducted in this area (34,35). In the case of Treblinka, historical sources provide information that the camp guards were mostly of Ukrainian and Russian origin, with individuals of Polish and Jewish descent (1,2,36).

Analyzing the mtDNA haplogroups of the examined remains, we can observe that 50% of the recovered skeletons were assigned to the HV haplogroup. It is one of the four most numerous clusters of mtDNA haplogroups in Europe.(37). Some studies demonstrate the representation of a haplogroup in the mitochondrial gene pool of Russians. Other researchers indicate that about 40% of Poles have haplogroup H, which derives from the maternal line of haplogroup HV.(29,38) As mentioned earlier, Poles were also incorporated into the structures of guard units.

The haplogroup obtained in the study is haplogroup U (33.3%). This haplogroup has been observed by other researchers in the populations of Russians and Ukrainians (38). Other researchers indicate that the U5a1 and U5a1a subhaplogroups marked in our study (derived from haplogroup U) are also observed in the populations of Russians and Ukrainians. (38,39). We can say that the haplogroups and their subhaplogroups observed in our study can support historical hypotheses. There are also analyses determining the affiliation of the U5a2a haplogroup to the Volga-Ural region with a clear geographical expansion to the east. (33).

In the presented study, haplogroup J was also determined, which constituted 16.7%. This haplogroup is observed among Polish Roma (40,41). Other researchers indicate that the frequency of haplogroup J is highest in the Middle East at 12%, followed by Europe at 11%, the Caucasus at 8%, and northeastern Africa at 6% (42). As an additional tool, an attempt was made to determine the Y chromosome haplogroups (inherited in the paternal line). Haplogroup R, with its main sublineage R1a, was identified in 5 skeletons (skeletons 2, 5, and 7). Subhaplogroup R1b was also found in skeletons 1 and 4. Please note that the bio-origin prediction refers to a geographical area. The marked haplogroups are also observed in the populations of Eastern Europe and other Western Eurasian groups. (42).

In the Russian population, the Y-chromosomal haplogroup R1a is observed. Meanwhile, the Y-chromosomal haplogroup N is observed among Russians, and its wide geographical distribution has been noted in northern Eurasia (29).

In the case of analysis of Y chromosome markers, population homogeneity was demonstrated, Five of the six obtained haplogroups are haplogroup R1, and one skeleton (43) showed haplogroup N1a1. This is a haplogroup that is observed more often in Northern Europe and less in Siberia. It reaches about 60% in Finns and about 40% in Latvians, Lithuanians and 35% of Estonians (42). These are also haplogroups common in Eastern Europe (43). It should be emphasized that the study population was very small. Data obtained as a result of analyses of Y chromosome and mtDNA haplogroups, in most cases, suggest a similar geographical area, namely Eastern Europe. We can say that the genetic results obtained support historical and archaeological hypotheses that the revealed burials could have belonged to the guards of the Treblinka camp.

Conclusion

Genetic research has been successfully conducted on the remains exhumed from the former forced labor camp Treblinka I. Profiles were obtained for 6 out of 7 individuals under study. Mitochondrial DNA analyses were also conducted. Hyper-variable regions of mtDNA (HV1/HV2) and the entire mitochondrial genome were sequenced to determine the haplogroup affiliation of the exhumed remains. The obtained mtDNA haplogroups align with those commonly observed in Eastern European populations. Additionally, haplogroups of the Y chromosome were determined, producing similar results. The findings of our study complement archaeological and anthropological analyses, providing further support for historical hypotheses.

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