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Identification of war victims of the 20th century from Poland through DNA testing from skeletal remains

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Nothing in life is to be feared, it is only to
be understood. Now is the time to
understand more, so that we may fear less.

— Marie Curie

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Abstract

Identification through forensic methods is crucial in crime investigations. The Polish Genetic Database of Victims of Totalitarianism (PBGOT) is allowing the positive identification of many victims of Communist and Nazi regimes which have remained unknown for a long time. Forensic science is advancing really fast and the application of Next-Generation Sequencing (NGS) techniques in the identification of victims has completely changed the procedures making them faster, more accurate and less propitious to contamination and manipulation errors. The main objective of this project is to present real-life cases of victims identified with the PBGOT as we continue to identify all the remaining victims. Bones such as teeth and femur were chosen for the analysis because DNA is better conserved in long bones and teeth. Samples were cleaned manually and chemically. Afterwards, bone powder was obtained using a freezer mill and it was kept at -20°C. DNA was extracted with PrepFiler BTA Forensic DNA Extraction Kit (Applied Biosystems) and analyzed with GenoProof 3.0.7. The identified victims presented here are Petr B., Tadeusz Stabrowski and Hieronim Dekutowski.

Introduction

Identification through forensic methods is crucial in crime investigations but it also plays an important role in anthropology, biology, dentistry, epidemiology, psychology, psychiatry and so on (1). The Polish Genetic Database of Victims of Totalitarianism (PBGOT) was created in 2012 by the Pomeranian Medical University in Szczecin together with the Institute of National Remembrance (IPN) and it is allowing the positive identification of many victims of Communist and Nazi totalitarian regimes which have remained unknown for a long time. The PBGOT (www.pbgot.pl) consists of three main elements: genetic information collected as reference material from living relatives, genetic information collected as evidence from burials, and historical and archaeological information (2).

The first attempt to isolate DNA from old skeletal remains was in 1990 (3) and the field of forensic genetics has advanced really fast since. Nowadays, the state-of-the-art laboratory kits and the application of Next-Generation Sequencing (NGS) techniques have completely changed the procedures for victim identification, making them faster, more accurate and less propitious to contamination and manipulation errors (4).

Facial recognition and odontology are traditional methods for identification of remains but in cases of natural disasters, mass graves, very old remains and even unrecognizable victims as result of fire or mutilation, DNA analysis is needed (5). However, the application of genetic methods in the identification of victims from World War II is complicated given the big amount of victims, the time that has passed and the little historical information left. A successful identification of such kind of victim is only possible in multidisciplinary environments in which historical, archival, anthropological, archaeological and genetic research come together (6). When bone is the only biological tissue available, dense cortical bones will be chosen for DNA analysis. When remains are exposed to adverse environmental conditions or several weeks have passed since death, bone and teeth samples, if available, will be the most reliable source of DNA. Long bones such as femur will be preferred, as well as molars without fillings, due to a better DNA conservation (7).

DNA analysis of ancient bones presents many difficulties given that genetic material is usually not very well preserved in old skeletal remains (8). Factors that suppose a big challenge to overcome are the presence of a small amount of genetic material, the geochemical properties of the soil, the presence of PCR inhibitors, risk of contamination, damage of the genetic material and finally, the absence of living relatives for comparison (9)(10). Besides, perpetrators destroyed many archives with information of the victims and the location and size of burials with the objective of hiding war crimes (2). Sometimes, the bodies from a mass grave were relocated during

the violent conflict or afterwards so we may find disassembled and crushed body parts (11). All these difficulties mean that there is no detailed historic data and that bones are seriously compromised, so it requires very neat protocols in order to preserve the gravesites and to avoid contamination of samples.

The European history of the last century has seen several violent conflicts that have left many victims behind. Only in Poland, an estimated number of 6 million 6 hundred thousand people died during World War II and the posterior soviet occupation (12). After the tsunami disaster in South-East Asia in 2004, awareness was raised amongst forensic scientists to create protocols with the objective of preserving the victim's remains as much as possible in order for posterior identification to be viable. The International Society for Forensic Genetics (ISFG) postulated 12 recommendations in 2007 that every process of Disaster Victim Identification (DVI) should follow. Factors such as proper labeling and chain of custody are taken into account. DVI is a multidisciplinary field addressed at a national and international level by the Interpol Guide to Disaster Victim Identification, the Pan American Health Organization disaster manual and the US National Institute of Justice mass fatalities report (7).

As stated before in violent conflicts, bone samples are collected for victim identification. The standard genetic markers used in DNA typing are autosomal Short Tandem Repeat (STR) loci. STRs are regions of DNA where sequences of 2 to 6bp are repeated several times. They can easily mutate even though they don't affect normal cellular functioning. This high variability between individuals makes them a good marker to identify family kinships and for this reason, they are used in human identification (13). The standard procedures employ a set of 15 STR loci. 13 are chosen by the Federal Bureau of Investigation (FBI) by the CoDIS system and the other 2 are from the extended European Standard set of loci. An amelogenin marker is also incorporated for sex determination. These loci are included in all commercially available kits and are: FGA, TH10, vWA, D1S1656, D2S441, D2S1338, D3S1358, D8S1179, D10S1248, D12S391, D16S539, D18S51, D19S433, D21S11, D22S1045 (8) (14).

In some cases, the sample is a mixture of different people and autosomal STRs don't provide enough information. In order to establish kinship, other types of genetic markers can be used, for example, lineage-linked markers such as Y-STRs, X-chromosome markers or mitochondrial DNA (mtDNA). The use of mitochondrial DNA has advantages because there are thousands of mtDNA molecules in one cell as opposed to nuclear DNA and it can confer a stronger evidence of maternal lineage (4).

Single Nucleotide Polymorphism (SNP) genotyping is also used since targeting shorter regions than STRs can be more informative when analyzing degraded DNA (15).

Nowadays, the technology of massive parallel sequencing, also called Next-Generation Sequencing (NGS) is also applied to Forensic Genetics. It allows to simultaneously target autosomal, X and Y STRs, as well as identity-informative SNPs and additional markers for phenotype and bioancestry. This big advancement enlarges the power of discrimination in DVI processes and increases the chances of obtaining information from fragmented genetic material. NGS technology allows us to know not only the size of alleles but also the sequence, which is informative on many features such as eye and hair color, even the skin tone. Libraries are prepared with ForenSeq DNA Signature Prep kit (Illumina), sequencing is performed with a two-step PCR using the MiSeqFGx Reagent kit (Illumina). Data analysis is carried out with the ForenSeq Universal Analysis Software (UAS) when a full SNP profile is obtained. If not, the online calculator HirisPlex-S is used. Recent results from a study in King's College show a concordance rate of no less than 99.98% between NGS and Capillary Electrophoresis (CE) based technologies (16).

NGS is not yet applied into routine casework because more evidence of compatibility needs to be obtained, regulations to be written, new frequency databases to be generated and there is still no proper nomenclature for all the new allelic variants that are being discovered. Nevertheless, these techniques are already in use for DVI and research purposes. Further investigation is essential to develop NGS methods that can be daily used in forensic laboratories (16).

Due to the fact that there still are many unidentified war victims in Poland as a result of the turbulent conflicts in the 20th century it is essential, not only for science but also for society, to identify the remaining victims with as many tools as possible. Every country uses its own national database and in the cases presented here, PBGOT was used.

Historical background

Before WWII

The Polish population had always been very plural. Such multicultural environment led to political instability since there were Ukrainians, Germans, Russians, Lithuanians, Yiddish speakers, Roman Catholics, Greek Catholics, Orthodox, Jews and Protestants among others, living together. Despite their differences, the general population felt sympathy for a political leader of big relevance during the Great War, General Piłsudski. In 1930, Piłsudski was elected First Minister of the Republic of

Poland and he applied the “*Sanacja*”, a series of measures to “cure” the country from corruption. In 1934 he signed a nonaggression treaty with Germany since the alliance Poland had with France during the Great War didn’t provide them with any advantage. After Piłsudski died in 1935, the government was left divided but it managed to stay stable until WWII broke out in 1939.

During the 1930’s there were many communist and fascist movements in Poland that Piłsudski crushed with military authoritarianism. It was a period of repression, revolutions and strikes from peasants and railway workers that would usually end up with blood. In 1937 Stalin assassinated all the members of the Polish Communist Party residents in the USSR and in 1938 dissolved its organization.

The burst of WWII

Hitler wanted Poland as an ally due to its background as an anti-Communist, anti-Russian and anti-Semitic country. However, Poland refused all of his proposals. France, England and Russia were the Allies of World War II and Poland wanted to join them, yet the conditions that Russia imposed were unacceptable. These conditions included the loss of their sovereignty and self-government, as well as the obligation to host soviet troops in Polish territory.

Stalin didn’t trust the intentions of his western allies so he made a nonaggression pact with Hitler on August 25th 1939. This pact also included splitting up Poland in favor of both powers. On September 1st 1939, Hitler attacked Poland to start the invasion and as a response, England and France declared war to Germany. Later on, when Poland was weakened by the Nazi troops, Russia started the invasion of “their part”. France had promised troops to Poland to defend themselves against Germany but these never arrived.

War refugees from Poland created an almost-parliamentary body in France while the soviets started executing prisoners, lawyers, civil servants, etc., and deporting Polish citizens to Siberia and Central Asia to subdue them to forced labor. The Nazis, however, had a stronger offensive and on June 22nd 1941 invaded Polish territory that had been “given” to the USSR.

During WWII

After the Nazi invasion of USSR territory, a British-soviet treaty was signed on July 13th 1941. In this treaty, Churchill pushed Poland to sign diplomatic relations with the USSR in the Sikorski-Maiski treaty, signed on July 30th 1941. Nevertheless, the tensions between Poland and Russia remained because the first wanted independence and the second was not interested in negotiations. The Sikorski-Maiski treaty was suspended

on April 25th 1943 after a mass grave with more than 4.000 Polish officers was discovered in Katyn (USSR territory) even though the soviet denied being the perpetrators of the crime.

In the soviet occupied Polish territory, the perception of a “free Poland” started rising among the population and by 1944 the political situation was more complex than ever.

There were the following elements:

- Clandestine non-communist state. It had the support of most of the Polish population as well as of the *ArmiaKrajowa*(AK), which means “the Country’s Army” and it was the largest resistance organization in the occupied Europe. They were loyal to the legitimate government, now exiled in London.
- Legitimate government in London. It was recognized by the Allies and had the support of most of the Polish population.
- Polish Committee for National Liberation (PKWN). It was a government that came up in Chelm and that sympathized with the soviets and counted with the recognition and support of the USSR.

On August 1st 1944, the first Polish rising against the Reich took place in Warsaw. The rebels intended to conquer the city back from the control of the Nazi before the soviet Red Army arrived. However, it resulted in a slaughter and as a consequence, the AK was neutralized. At the same time, the PKWN was gaining followers and they would fight against the Germans, side by side with the soviets until the end of the war. On December 31st 1944, the PKWN auto declared itself as the provisional government of the Republic of Poland, with the recognition of Stalin.

It is estimated that the total losses in Poland during the war were of 1/5th of the population. By 1944 the Nazi had killed 90% of the Jewish population of Poland and they were accounted to be 3 million people. Even so, there were organizations all over the territory that presented resistance. Examples are the AK and Żegota, an independent council created by the Polish resistance and that coordinately helped to save 45.000 Jews until 1944.

After WWII

The Potsdam conference held in 1945 established the new oriental border of Poland in Szczecin. The border had never been as far west, which propitiated an ethnic cleanse of the newly gained German territory as revenge. About 500.000 Poles preferred political exile, mainly to England, the USA and Australia. The ones that stayed lived a

transition period between one authoritarian regime and the next. However, survivors were relieved of the end of the war at the beginning.

Transition period

The new Poland was 20% smaller than the previous Republic and it had a different geography. From the 35 million inhabitants in 1939, there were 24 after the war. Even though being under soviet control, schools and universities recovered quickly and the regime didn't have time to control their contents. The same happened with the radio, the cinema and all other forms of culture. Churches proliferated quickly making Poland mainly catholic for the first time in history.

Soviet invasion

There were tensions between the Pro-Soviet Party (PPR) and the Anti-Communist Party (PSL), and during the first years of the soviet occupation anti-semitic attacks were common. There was violence against the Ukrainians and there was even a civil war. By the means of violence, intimidation and electoral fraud the PPR "won" the elections on January 19th 1947. The fraud consisted in eliminating the PSL by imprisoning its leaders and depriving the right of vote to those with different ideology than PPR. During 1947 and 1948 there was a strong suppression against the Socialist Party (PPS) which was relatively new to politics. In the end, the PPS joined the PPR and they formed a new political coalition, the PZPR. The PZPR governed Poland in a totalitarian manner from 1948 to 1989, under the control of the USSR. They applied policies very similar to those of "Big Brother" because there was only one party (The Party), one ideology and one very numerous police force that kept the population living in fear. All organizations were surveilled, the army was purged, death sentences were given indiscriminately and everything was directly controlled by Stalin.

The economy was based on industrialization and even though there was a decline in unemployment, it led to a drop of agrarian production, which led to collectivization, mandatory requisitions and food rationing.

The education was based on marxist indoctrination. Young and poor proletarians were encouraged to have higher education while it was forbidden for bourgeois. The use of russian language was mandatory, as well as belonging to a sovietic "scouts" group for young adults between ages of 14 to 25. Culture was strongly promoted but censorship was palpable.

The communist were atheists but religion was deeply rooted in Poland so they decided to unilaterally take care of the ecclesiastical nominations. The soviets imprisoned all

contrary bishops and clerks and prohibited some seminars and all religious demonstrations in schools.

On May 5th 1953 Stalin died and the regime fell in countries such as Hungary and Czechoslovakia, but not in Poland. The post-stalinist period in Poland would be slow. In 1956 Poland was “freed” from the soviet influence by a politician the population saw as a rebel, Gomułka. However, he became president of The same Party and his economic policies didn't change and neither did the quality of life of the citizens.

During the 1960s The Party split in different opinions and one of these promoted anti-Semitic attitudes and behaviors of Nationalism. These tensions resulted in a failed putsch or *coup d'état* that forced many Jews with a position to flee the country.

During the 1970s prices increased drastically, sometimes triplicated from one day to the next, and as a consequence, there were regular risings from workers. The ones in Gdansk and Gdynia stand out due to the massacres that perpetrated. As of this violence trade unions were created and were still necessary during the 1980s. In 1981 the government activated the state of war (martial law) and 6.000 activists had been imprisoned by 1983. The martial law was reinstated in 1988 after numerous strikes.

On April 5th 1989, democracy was partially restored and trade unions were legalized, such as Solidarność, headed by Wałęsa (Nobel Peace Prize winner in 1983). The “partially” is due to the fact that the population could vote but only for 35% of the seats. The remaining 65% was reserved for the PZPR. On December 29th 1989, the III Republic of Poland was proclaimed.

Post-Communist period

A deep economic reform was carried out, Germany recognized the western border of Poland (otherwise there would have been another conflict), the USSR apologized for the events in Katyn and dissolved into many new independent countries. In the early 1990s Solidarność and the PZPR scattered in smaller parties and in the first elections of the Republic, the left-wing parties clearly won (17).

Problem approach or hypothesis

More than 6 million people were killed in Poland during World War II and the posterior soviet occupation in the 20th century. Since there is not many historical data left due to its obliteration by the perpetrators, and the human remains are highly damaged because of the time that has passed, many victims are still not identified.

Objectives

The main objective of this project is to present real-life cases of victims identified with the PBGOT as the remaining victims continue to be identified. The aim is to show the complete process of identification of three victims; from the discovery of the burial to the positive match in the PBGOT.

Materials and Methods

Anthropological analysis

The age at the time of death was determined by a comprehensive and multi-factorial analysis of the morphological configuration of the individuals. Sex determination took into account the dimorphisms manifested at a larger degree. The state of preservation assessment was based on the conditions of the bones. Activity of third parties was evaluated by observation of injuries to the bones.

Sample preparation

The human remains were carefully removed from the grave, put into appropriate containers and transported to the specialized laboratory in the Department of Forensic Genetics of the *Pomorski Uniwersytet Medyczny* (PUM) following the ISFG recommendations (7). In a room that is specific and exclusive for bones, these were washed manually with water and a brush and let dry at room temperature. Once dry, femur fragments of 1cm² were cut with a mechanical chainsaw. Femur fragments and teeth were polished with a precision drill with spiral grinding and diamond grinding bits, respectively. Afterwards, they were disinfected chemically with a 15% sodium hypochlorite solution and physically with 30 minutes under UV light. Samples were introduced in a Freezer Mill 6770 (SpexSamplePrep) with liquid nitrogen and bone powder was obtained following manufacturer's instructions. Powdered samples were stored at -20°C in Falcon-type tubes.

Four buccal swabs were collected from every living relative after they provided informed consent. Samples were extracted with AutoMate Express DNA Extraction System (Applied Biosystems) and conserved at -20°C.

DNA extraction

DNA from the powdered samples was obtained using the PrepFiler BTA Forensic DNA Extraction Kit (Applied Biosystems) following manufacturer's instructions (18). DNA from the living relatives was obtained using the organic method (phenol/chloroform). Purification was performed with the QIAquick PCR Purification Kit (QiaGen) following manufacturer's instructions.

DNA quantification

A Real-Time PCR was performed to quantify the amount of DNA in each sample. The analysis was performed with an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems) and the Quantifiler Human DNA Quantification Kit (Applied Biosystems) following manufacturer's instructions.

Amplification

15 autosomal STR loci and an amelogenin gene marker for sex determination were amplified in a multiplex PCR with the GlobalFiler PCR Amplification Kit (Applied Biosystems) following the manufacturer's instructions. In some cases a Y-chromosome marker analysis was carried out and the AmpFISTR Y-Filer Plus PCR Amplification Kit (Applied Biosystems) was used. In other cases, mitochondrial DNA sequencing was performed for HV1 and HV2 analysis. The products obtained from the PCR were separated by capillary electrophoresis with ABI Prism 3500 Genetic Analyzer (Applied Biosystems).

Statistical Analysis

A comparative analysis was made between the evidence (exhumed remains) and reference (relatives) profiles. To establish a possible kinship between the samples, autosomal STR profiles were compared using the DVI module of the software Familias 3_2_2. For haploid markers analysis, two online tools were used. The frequencies of mtDNA haplotypes were obtained with EMPOP mtDNA database v3/R11. The Y chromosomal haplotypes were analysed with YHRD database. The final calculations were performed with GenoProof 3.0.7. The final Likelihood Ratio (LR) was based on the autosomal STRs combined with haploid marker, and for the final probability calculations 50% prior odds were used.

Databases

Historical information was obtained from many different sources such as the Communist Police archives, the Institute of National Remembrance (IPN), the OBD database by the Ministry of Defense of the Russian Federation, the Deutsche Dienststelle (WASt) in Berlin, testimonies of witnesses and their still-alive family members and the cemeteries' data, to name a few. The results obtained from the DNA typing, both from remains and living relatives, were introduced in the Polish Genetic Database of Victims of Totalitarianism (PBGOT) with the authorial program ŁowcaDusz v.1.0.

Results and Discussion

The identification of victims of totalitarian regimes is complex and every case requires a different strategy. Sources of information are specific of every DVI process and they depend on the kind of historical background and the biological material that we have at our disposal. There have been historical persons whose identities have been confirmed or questioned by DNA typing. Examples are the Romanov family, Napoleon, Josef Mengele and Christopher Columbus.

Three case studies of identified victims by the Department of Forensic Genetics of PUM in Szczecin, Poland, are presented below. First, Petr B. is presented, a soviet soldier that was captured by the German army in 1941 and subdued to forced labor in a farm. After, there is Tadeusz Stabrowski, a historical person in Poland. He was a pilot in the Polish Air Force that became a national hero after his deeds during WWII. Finally, there is the complex identification of Hieronim Dekutowski, a Polish soldier responsible for many attacks aimed at Communist organs of repression. He became a hero to Poland and is nowadays also considered a historical person.

Petr B.

Petr B. was a soviet soldier captured in 1941 and made a war prisoner. According to a witness, he was forced to work in a big farm in really poor conditions. Workers would frequently die from starvation and exhaustion and were consequently buried in the Linowno local Cemetery. There was historical data about a mass grave in this cemetery and exhumation works began in December 2011. Despite the contents of the archives, no mass grave was found, but single graves with bodies buried without coffins. This suggests that it was a war burial plot and during exhumations, ten

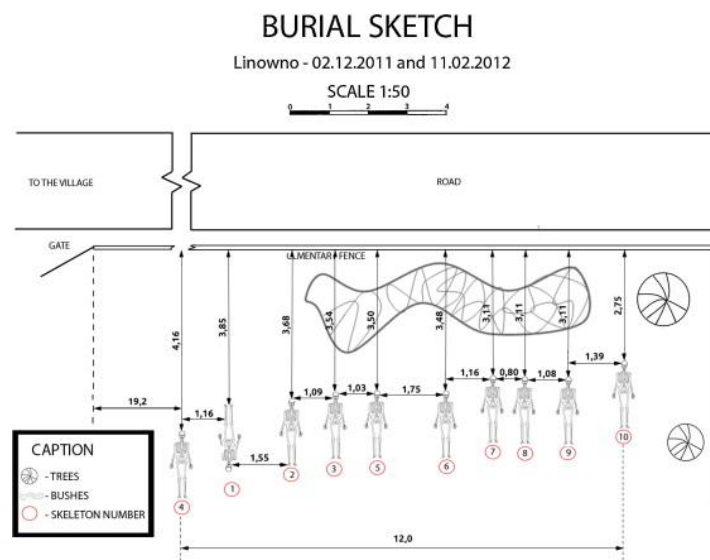


Fig. 1. Distribution of individuals in the grave.

prisoners of war were found. 8 out of the 10 had visible prisoner recognition marks, which allowed us to find their prisoner cards with their personal information.

Individual marked as #7 (Fig. 1), was estimated to be 19-23 years old and 167cm tall. The remains were put in the grave face up, with the head in the direction of the cemetery door and the palms on the pelvis. The inscription mark was “STALAG 315 15100”. Stalags were concentration camps for war prisoners during WWII. Prisoners were commonly sent away from Stalags for forced labour and only returned when were considered unable to work due to starvation, abuse or excessive work. The inscription of prisoner #7 led us to the prisoner card (Table 1 and Figs. 2 and 3).

Table 1. Archival query.

Father's name	Roman
Religion	Graeco-catholic
Place, date of birth	Jeral, 29/01/1920
Rank	Private
Unit	201 Rifle Regiment
Profession	Farm worker
Place, date of captivity	Kovno, 01/07/1941
Camp	Stalag II B, Hammerstein
Prisoner's number	15100
Height	161cm
Hair	Blond
Distinguished marks	None
Vaccinations	None
Condition of health	Healthy
Date and place of death	27/10/1941, Linowno/Woltersdorf

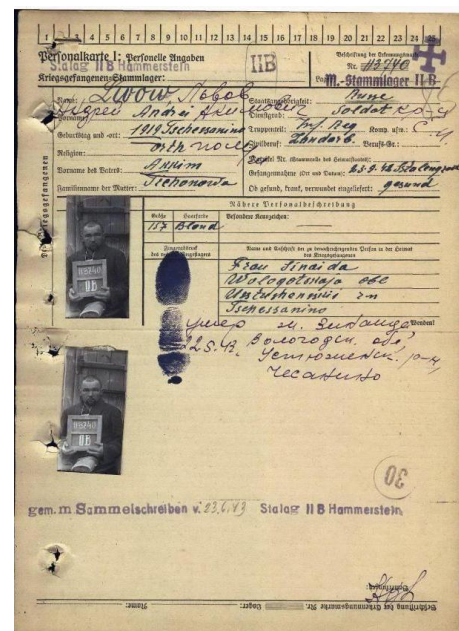


Fig. 2. Prisoner card.



Fig. 3. Inscription mark.

A thorough search for alive relatives was done and they were only found for two individuals, being #7 one of them. Anthropological analysis showed no perimortem traumas that revealed the cause of death. DNA typing was performed from teeth and phalanges and no contamination was found. Long bones were not chosen this time because the remains were not crushed and phalanges could be perfectly told apart. The putative son of Petr B. provided buccal swabs for comparison. Final biostatistical

calculations show a probability of 99,9999999994% that individual #7 shares a kinship of father-son with the person who provided the reference material, rather than the alternative hypothesis that both individuals are not related in any way.

Tadeusz Stabrowski

Tadeusz (Tadek) Stabrowski is a Polish historical person due to his deeds as a pilot in the Air Force during WWII (Fig. 4). He died in combat and for that he became a war hero. He was described by his friends as reckless and disrespectful on land but that when he was in the air, he became the most precise and courageous pilot of all. He was passionate about wine, women and planes, and he used to draw sketches of his interests. He also draw about everyday situations in the Air Force, like the pilots playing cards, and he would make caricatures. He was quite an artist and his emblem was a drunk angel, which he had drawn on the side of his Spitfire.



Fig. 4.Tadeusz Stabrowski in his Spitfire.

Tadeusz was son of Felicja and Władysław and was born on May 16th 1917, most probably in the industrial village of Lubimówka, near Aleksandrowka, even though the location of his birth is unclear. He never applied too much in school but felt a great fascination for aviation, as many kids of his generation. After junior High School, he graduated from the Dęblin Aviation Reserve Cadet School (SPRL) on September 21st 1936, and enlisted the Polish Air Force in 1940. He performed more than 140 operations in different locations of the UK and France during WWII, and was awarded two Cross of Valor medals during that time. He got married to Elizabeth Prescott and had a son with her, Andrzej William. On March 11th 1943, F/O Stabrowski fell on the English Channel during a mission and died, most probably of hypothermia.

On April 12th 1943, the body of a Polish pilot was found in a beach near Le Crotoy, in the North of France, a place called La Voie de Rue. He was buried in the local St. Firmin Cemetery in Le Crotoy, with a tombstone that said “Polish airman known only to God” (Figs. 5 – 7).

On September 28th 2017, exhumation works of this grave began in order to confirm that this unknown pilot is, in fact, Tadeusz Stabrowski.

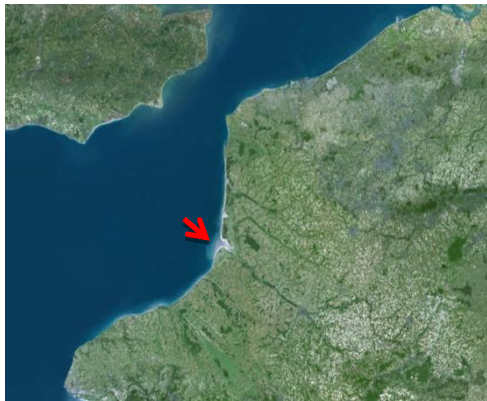


Fig. 5. Satellite image of Le Crotoy.



Fig. 6. St. Firmin Cemetery.



Fig. 7. Pilot's grave.

The body was found buried 75cm deep, originally in a wooden coffin, and in a non-anatomical arrangement, which suggested a high degree of decomposition at the time of the burial (Fig. 8). The skeleton was incomplete and only the 70% of the bones were found. The head, as well as some vertebrae, the sternum and phalanges among other bones, were missing. However, the present bones were moderately well preserved. Other objects were found with the skeletal remains: a belt plate from the Royal Air Force (RAF) pilot's uniform together with pieces from the life jacket from the RAF pilot's uniform (Fig. 9).



Fig. 8. Remains of Tadeusz Stabrowski.



Fig. 9. Beltplate.

Anthropological analysis of the remains indicated that the victim was of male sex, between 30 and 40 years old and 169-172 cm of height (Fig. 10). No pathologies or malformations were discovered but numerous signs of peri and post-mortem erosion were apparent in the bones.



Fig. 10. Exhumation of Tadeusz Stabrowski.

Genetic analysis of the skeleton was performed as evidence material. Andrzej Stabrowski, the son of Tadeusz Stabrowski, provided buccal swabs for genetic comparison. Analysis of autosomal STR markers and Y-STR markers was performed in order to establish kinship. Results confirmed the hypothesis that both samples share a father-son relationship, compared to the alternative hypothesis that these samples belong to two completely unrelated individuals, with a prior odds prediction of 50%. In other words, the unknown pilot from Le Crotoy and Andrzej Stabrowski are 672.709.120 times more likely to be father and son than to be unrelated. The probability of an existing relationship between these two people is of 99,9999998%.

Hieronim Dekutowski

Hieronim Dekutowski, also known as Zapora, is a martyr and a historical person in Poland due to his acts of rebellion not only against the Nazi during WWII but also against the Communists during the soviet occupation of Poland after the war (Fig. 11).

He was son of Maria and Jan and was born in Dzików on September 24th 1918 as the youngest of six brothers. He grew up in a patriotic environment considering that his father was member of the Polish Socialist Party (PPS) and a convinced follower of Piłsudski. During his childhood he belonged to a scouts group and was always implicated in organizations. He graduated High School in 1939 and since WWII started shortly after, he didn't get the chance to attend University. Instead, he volunteered for the Polish Army and fought the Battle of Lwów, actual Lviv in Ukraine. The battle ended in the surrender of the Poles, and as a consequence, the soviets arrested all Polish

Officers, some of which ended up being murdered in the massacre of Katyn (view historical background). Shortly before the battle ended, he crossed the border across Yugoslavia until France where he volunteered for the Polish Army in France and was assigned the 2nd Division of Infantry Rifles in 1940, later on, to a tank battalion in the UK, and then to the Polish 1st Independent Parachute Brigade. On April 24th 1942 he volunteered to go back to Poland and in 1944 became the commandant of an AK unit that conducted sabotage and propaganda against the Germans, the Kedyw, in the Puławy area. Armia Krajowa (AK) means “the Country’s Army” and it was the largest resistance organization in the occupied Europe. One of his most remarkable schemes was on May 24th 1944, when they attacked a German column of 16 trucks full of soldiers and SS. After the war, he decided to remain in Poland and started attacking the Communists. He had killed more than 400 soviet soldiers by 1946.



Fig. 11. Hieronim Dekutowski

In September 1947, Dekutowski and his subordinates were caught within the Polish city of Nysa and were imprisoned in Mokotów, Warsaw, a place of torture and execution of Poles and opponents to the regime. He was brutally investigated by the Ministry of Public Security in Warsaw and was sentenced to death. He was executed on March 7th 1949. Testimonies said that even though Dekutowski was only 29 years old at the time of his death, he looked like an older man.

In the summer of 2012, exhumations in the “Ł” section of the Powązki Military Cemetery in Warsaw started. This cemetery is known to be the most relevant place in Poland associated with Communist terror. Over 200 individuals were found and 50

people were successfully identified. A mass grave with 8 individuals was found and according to the available data, these could be Dekutowski and his subordinates.

The bodies were piled ones over the others, which caused erosion on the bones and samples needed special care. DNA typing was performed from the best preserved molars generating autosomal STR, X-STR and Y-STR profiles. Dekutowski didn't have any offspring; the only living relatives are three sisters and their descendants (Fig. 12). The comparison of the victim's DNA to those of their siblings doesn't provide enough robustness without a parent or an offspring. In other words, vertical kinship is required. For this reason, an unprecedented measure was taken: Dekutowski's parents were exhumed. However, they were buried in the first half of the 20th century and remains were highly eroded and severely compromised.

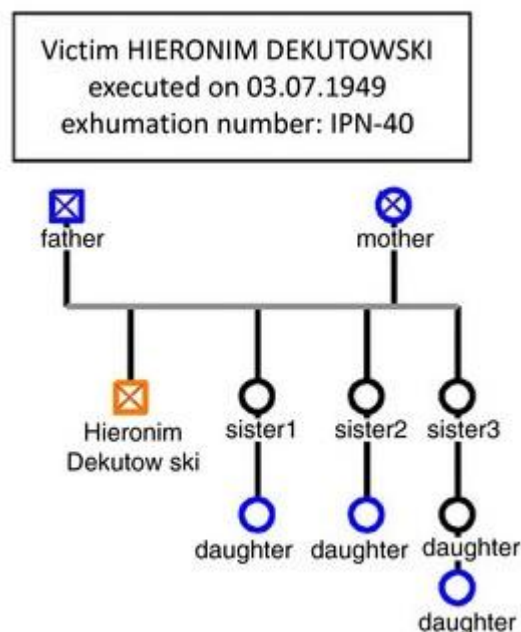


Fig. 12. Dekutowski'sfamilytree.

Genetic profiles showed a high heterozygosity and no contamination of the samples was observed. Results confirmed that the remains found in the Powązki Military Cemetery were those of Hieronim "Zapora" Dekutowski with a combined autosomal STR, X-STR and Y-STR LR of 928×10^{18} .

After the exhumation works were complete, "Zapora" was solemnly buried in the mausoleum of cursed soldiers in Warsaw Powązki.

Conclusions

The Polish Genetic Database of Victims of Totalitarianism (PBGOT) is an essential tool for Disaster Victim Identification (DVI) purposes and for National Remembrance.

Focusing on the identification of individuals allows us to identify other people buried with them. DNA typing of ancient skeletal remains presents many difficulties.

Detailed and accurate historical data made possible the positive identification of Tadeusz Stabrowski. Due to the positive identification of Petr B., through his son's reference sample, we were able to identify the rest of individuals in the grave. Exhumation of relatives was a key factor in Hieronim Dekutowski's identification and it's a method that can be applied in other cases.

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Conflict of interest

I declare that I have no conflict of interest.

Bibliography

1. Kuś M, Ossowski A, Zielińska G. Example of genetic identifications of victims of totalitarian regimes in Poland. *Forensic Sci Int Genet Suppl Ser.* 2015;e356-e357.
2. Ossowski A, Kuś M, Kupiec T, Bykowska M, Zielińska G, Jasiński ME, et al. The Polish Genetic Database of Victims of Totalitarianisms. *Forensic Sci Int.* Jan2016;258:41-49.
3. Cattaneo C, Smillie DM, Gelsthorpe K, Piccinini A, Gelsthorpe AR, Sokol RJ. A simple method for extracting DNA from old skeletal material. *Forensic Sci Int.* 28Jul1995;74(3):167–174.
4. Alvarez-Cubero MJ, Saiz M, Martinez-Gonzalez LJ, Alvarez JC, Eisenberg AJ, Budowle B, et al. Genetic identification of missing persons: DNA analysis of human remains and compromised samples. *Pathobiology.* 21Jun2012;79(5):228–238.
5. Westen AA, Gerretsen RRR, Maat GJR. Femur, rib, and tooth sample collection for DNA analysis in disaster victim identification (DVI) : A method to minimize contamination risk. *Forensic Sci Med Pathol.* 2008;4(1):15–21.
6. Ossowski A, Kuś M, Brzeziński P, Prüffer J, Piatek J, Zielińska G, et al. Example of human individual identification from World War II gravesite. *Forensic Sci Int.* 10Dec2013;233(1–3):179–192.
7. Prinz M, Carracedo A, Mayr WR, Morling N, Parsons TJ, Sajantila A, et al. DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI). *Forensic Sci Int Genet.* Mar2007;1(1):3–12.
8. Zupanič Pajnič I, Gornjak Pogorelc B, Balažic J, Zupanc T, Štefanič B. Highly efficient nuclear DNA typing of the World War II skeletal remains using three new autosomal short tandem repeat amplification kits with the extended European Standard Set of loci. *Croat Med J.* 15Feb2012;53(1):17–23.
9. Kuś M, Ossowski A, Zielińska G. Comparison of three different DNA extraction methods from a highly degraded biological material. *J Forensic Leg Med.* May2016;40:47–53.
10. Cooper A, Poinar HN. Ancient DNA: Do It Right or Not at All. *Science* 18Aug2000;289(5482):1139.
11. Sutlovic D, Boric I, Zulim T, Vucinovic A. Identification process of skeletal remains from mass graves: Our experience and proposal guidelines. *Leg Med (Tokyo)* Mar2015;17(2):102–108.

12. Ossowski A, Diepenbroek M, Zwolski M, Falis A, Wróbel M, Bykowska-Witowska M, et al. A case study of an unknown mass grave — Hostages killed 70 years ago by a Nazi firing squad identified thanks to genetics. *Forensic Sci Int*. Sep2017;278:173-176.
13. Butler JM. Short tandem repeat analysis for human identity testing. *Curr Protoc Hum Genet*. Sep2004;Chapter 14:Unit 14.8.
14. Butler JM, Buel E, Crivellente F, McCord BR. Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis*. Jun2004;25(1011):1397–1412.
15. Asari M, Watanabe S, Matsubara K, Shiono H, Shimizu K. Single nucleotide polymorphism genotyping by mini-primer allele-specific amplification with universal reporter primers for identification of degraded DNA. *Anal Biochem*. 1Mar2009;386(1):85–90.
16. Devesse L, Ballard D, Davenport L, Riethorst I, Mason-Buck G. Concordance of the ForenSeq system and characterisation of sequence-specific autosomal STR alleles across two major population groups. *Forensic Sci Int Genet*. 2018;34:57–61.
17. Lukowski J, Zawadzki H. *Historia de Polonia*. Madrid : Cambridge University Press; 2002.
18. Ossowski A, Diepenbroek M, Szargut M, Zielińska G, Jędrzejczyk M, Berent J, et al. Population analysis and forensic evaluation of 21 autosomal loci included in GlobalFiler™ PCR Kit in Poland. *Forensic Sci Int Genet*. Jul2017;29:e38–9.