Identification of World War II bone remains found in Ukraine using classical anthropological and mitochondrial DNA results

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Received: 25 October 2018 / Accepted: 14 February 2019 / Published online: 13 March 2019
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Abstract
Gyula Ágner was a Royal Hungarian First Lieutenant (1st Lt.) during the World War II and died at 30 years old due to a mine shrapnel injury on 27 April 1944 in Luczky, Ukraine. In October 2014, the Hungarian Ministry of Defence exhumated the remains then transported them to Budapest in Hungary. Classical anthropological methods were used to determine morphological gender, height and age at death; furthermore, metrical and pathological characters were also analysed. Determination of maternal lineage was the only solution to examine the possible relationship of the bone fragments. Gyula Ágner did not have direct descendants, thus the living niece of the deceased (his sister's daughter) served as the reference person during the investigations. Hypervariable regions of the mtDNA control region (HV1, HV2 and HV3) were amplified by Qiagen® Multiplex PCR Kit in different monoplex reactions. The results of the anthropological and genetical analysis supported the hypothesis that the bone remains belong to Gyula Ágner.

Keywords Forensic science • Forensic genetics • Mitochondrial DNA • Forensic anthropology • Second World War • Identification

Introduction
Gyula Ágner was born on 31 May 1914 in Budapest, Hungary (Supplementary Material, Fig. S1). After graduating from high school, he joined the Hungarian Royal Army. He died a heroic death on 27 April 1944 due to a mine shrapnel injury close to Luczky, Ukraine, where his body was buried in the churchyard. Gyula Ágner received the highest Hungarian military honour, the Hungarian officer’s gold medal for bravery. In 2014, the Hungarian Ministry of Defence made an investigation in Luczky, Ukraine, and they found the putative remains of Gyula Ágner. The former Institute of Forensic Medicine, Network of Forensic Science Institutes (now Hungarian Institute for Forensic Sciences) made several identifications before [1, 2], therefore the Hungarian Ministry of Defence asked the Institute to identify of the bone remains. Anthropological and mitochondrial DNA analyses were then made on the remains. First Lieutenant Gyula Ágner did not have any direct descendants. The reference DNA sample (buccal swab) was collected from Gyula Ágner’s living niece (his sister’s daughter) to examine the maternal relatedness.

Materials and methods

Anthropological methods
First, we separated, cleaned and ordered the bones anatomically (Supplementary Material, Fig. S2). Afterwards, we
determined the biological profile. We carried out sex determina-
tion based on Éry et al. [3], age at death based on Meindl
and Lovejoy [4] and Nemeskéri et al. [5], furthermore, height
based on Sjøvold [6] and craniometrical analysis based on
Martin and Saller [7] methods. We recorded the dental status
using Ubelaker’s [8] and Huszár’s [9] method.

Fortunately, there were period photos of the late Gyula
Ágner so we could do a comparative morphological analysis
between the photos and the skull (Fig. 1).

Genetic methods

Sampling and DNA isolation

We used a DNA extraction method of bone samples devel-
oped by us. We cut the bone sample from the diaphysis of the
leftward femur (Supplementary Material, Fig. S3). The cut
particle of the bone was cleaned with dental polisher. The
polished particle was decontaminated one time for 2 min using
the chemical (alkaline-hypochlorite, 14 g/l) impact. The re-
 mains of the chemical material were removed from the surface
of the particle by using water wash three times and once with
96% ethanol flush. Then the particle was grinded and pow-
dered with Retsch® MM 200 Mill. DNA was isolated from
350 mg bone powder using EZ1 DNA Investigator Kit and
EZ1 Advanced Instrument (Qiagen®). The buccal swab of the
reference sample was collected with Whatman EasiCollect®
and DNA was isolated using EZ1 DNA Investigator Kit and
EZ1 Advanced robot system (Qiagen®).

PCR amplification and sequencing

The mtDNA hypervariable regions (HV1, HV2 and HV3)
were amplified in different monoplex reactions using
Qiagen® Multiplex PCR Kit. For BigDye sequencing PCR,
F15971/R16401 primers for the HV1, the L48/H408 primers
for HV2 and the F403/R599 primers for the HV3 were used.

To sequence the fragments, we used an ABI 3130 capillary
electrophoresis instrument. The following regions of mtDNA
hypervariable regions were sequenced: HV1, 16024–16,375;
HV2, 70–375; HV3, 451–546. To align the sequences, we
used Sequencing Analysis v.5.4 and SeqScape v2.7 software
and the sequences compared to the revised Cambridge
Reference Sequence (rCRS, GenBank: NC_012920) [10].
We followed the ISFG recommendations for the haplotype
nomenclature [11].

Results and discussion

Anthropological results and discussion

Based on the investigation, the remains belonged to an
approx. 30–35 years old male who was approx. 175 cm tall.
There were many dental treatments (inlays, crowns and bridges)
during his life but the abrasion of the teeth belonged to the
abrasio superficialis I group. This means that based on the
dental status, the remains belonged to a young person (approx.
30 years). We did not find any pre- and perimortem injuries on
the bones. All the injuries were postmortem. The comparative
morphological analysis between the period photos and the
skull was limited because most of the face skull was damaged
but those details which were suitable for the analysis showed
similar characteristics with the photos. These similar charac-
teristics were:

Table 1 mtDNA sequence differences from the rCRS sequence in the reference and the bone samples. Sequenced regions: HV1, 16024–
16,375; HV2, 70–375; HV3, 451–546

| Samples     | mtDNA sequence differences from the rCRS sequence |
|-------------|--------------------------------------------------|
| Reference person | A263G, −315.1C, −315.2C, T16093C | |
| Bone sample  | A263G, −315.1C, −315.2C, T16093C | |

Fig. 1 The comparative morphological analysis between a
photo of the late Gyula Ágner and the skull
Based on our results, the anthropological features did not exclude the possibility that the bone remains were those of 1st Lt. Gyula Ágner.

**Genetic results and discussion**

Based on the results of the mtDNA sequencing, we repeatedly detected the same haplotype between the bone sample and the living reference person. The detected mtDNA haplotype can be found in Table 1.

The detected haplotype belongs to haplogroup H based on EMPOP search [12]. The HV1-HV3 haplotype did not give any match among metapopulations of the EMPOP database according to the search on 4 January 2019. The unbiased estimation of the frequency of the haplotype in the European population reached the value at 5.06 × 10⁻⁴ using confidence limit from zero proportion [13]. The detected haplotype can be considered as a very rare one both in European and worldwide context. According to the genetic result, it is highly supportable that the two samples/persons are closely related within the maternal lineage.

**Conclusion**

Both the anthropological and genetic results support the hypothesis that the bone remains, which were exhumed from the putative grave of Gyula Ágner, really belong to 1st Lt. Gyula Ágner who died a heroic death on 27 April 1944. The reburial was carried out with military honours on 12 of May 2016 in Fiume Road National Graveyard, Budapest, Hungary.

**Acknowledgements** Open access funding provided by University of Pannonia (PE). This work was supported by the former Network of Forensic Science Institutes, Ministry of Justice. We would like to say special thanks to the assistants of the laboratory: Ildikó Antalné Éles, Jánosné Lesznóczki, Ágnes Fábián, Gézáné Kígyósi and Dezsőné Lakatos and Morgan Murchison for English editing.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.