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DNA analysis of lineage markers from skeletons from a mass grave related to the Battle of Reichenberg in 1757

Jitka Votrubova¹, Hana Brzobohata², Petr Brestovansky³, Petr Tomasek⁴, Daniel Vanek^{1,4,5}

¹ Forensic DNA service, Budinova 2, 180 81 Prague 8, Czech Republic

² Department of Prehistoric Archaeology, Institute of Archaeology of the Academy of Sciences, Prague, Czech Republic

³ North Bohemian Museum Liberec, Czech Republic

⁴ 2nd Faculty of Medicine of Charles University, V Uvalu 84, 150 06 Prague 5, Czech Republic

⁵ Institute of Legal Medicine, Bulovka Hospital, Prague, Czech Republic

*Corresponding author: Daniel Vanek, Forensic DNA Service, Janovskeho 18, 170 00 Prague 7, Czech Republic, Tel.: +420 603 979 915; E-mail: daniel.vanek@fdnas.cz

Abstract

During a rescue excavation in October 2011, archaeologists discovered a mass grave containing the skeletons of 10 male individuals. The skeletons are expected to belong to victims of the Battle of Reichenberg between the Austrian and Prussian armies on April 21, 1757. We managed to obtain Y-chromosome and mtDNA haplotypes from all tested individuals. The resulting haplotypes were compared to the contemporary population from the vicinity of the excavation site as well as to the publicly available databases.

Keywords

Y-chromosome; mtDNA; DNA identification; bone samples

Introduction

DNA typing of lineage markers (Y-chromosome, mtDNA, SNPs) can provide useful information leading to the identification of skeletal remains [1-4]. The results of Y-chromosome and mtDNA can also be used for the prediction of haplogroups [5, 6] and comparison with available databases [7].

Material and methods

DNA extraction from bone samples

Several bones of skeletons from the mass grave were covered with a blue-colored encrustation. We found that this encrustation inhibited DNA analysis. Chemical analysis of the bluish encrustation indicated the presence of the iron phosphate mineral *vivianite* ($\text{Fe}_3(\text{PO}_4)_2 \cdot (\text{H}_2\text{O})_8$). DNA extraction and inhibitor removal were performed according to the procedure described previously [8].

DNA quantitation

Extracted DNA was quantified by real-time PCR with primers targeting the sequence of the ALU transposable element with an amplicon size of 63 bp [9]. The qPCR was performed in duplicate reactions with a Mastercycler ep Realplex Thermocycler (Eppendorf AG, Germany) with Human DNA Quantitation Standard 2372 (NIST, USA).

DNA typing

Mitochondrial DNA (mtDNA) regions – HVR1 and HVR2 – were amplified with primers designed for mtDNA miniamplicons [10] in a reaction volume of 50 μL with AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific, USA). Y-chromosomal STR typing was done using an AmpFISTR Yfiler PCR Amplification kit (Thermo Fisher Scientific, USA). Y-STR PCR was performed in accordance with

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