

Optimization of Genetic Identification Procedures for Unidentified Human Remains

This study presents a comprehensive approach to the identification of unknown human remains, using the example of victims of mass executions during the Great Terror in Adjara (Georgia), conducted within the framework of the international Georgian Recovery, Documentation and Identification Project. The aim of the study was to develop and verify an analytical scheme enabling effective genetic identification of historical victims. In such cases, the problem is not only the degree of preservation of the biological material of the remains, but also the limited availability of reference samples from the victims' relatives, which poses a challenge for experts conducting identification analyses.

The research material included 28 male skeletons exhumed in 2021 from the site of a former military base in Adjara, as well as biological samples collected from 58 individuals representing 20 families searching for their missing relatives. Laboratory analyses were performed using commercially available reagent kits for the amplification and detection of autosomal STR markers (GlobalFilerTM) as well as markers located on the Y chromosome (YfilerTM Plus). The study also employed a kit for amplifying iiSNP markers and sequencing them using NGS (Precision ID Identity). All commercial reagents were manufactured by Thermo Fisher Scientific. In addition, a non-commercial kit (KinFinder) developed by the Molecular Diagnostics Laboratory GenMed sp. j. was used. Statistical analyses were carried out using Familias, FamLink2, and KinBN, as well as the pedbuildr package in the R environment, which allows for kinship simulations and the assessment of the strength of evidence for complex genealogical structures.

The research scheme used in this study enabled conclusive identification of all 17 families searching for relatives listed on the execution list associated with the analysed burial pit.

IBD simulations and kinship analyses confirmed family relationships in most cases and revealed incorrect genealogical assumptions in several families. In addition, biogeographical ancestry analysis was performed, which confirmed the genetic compatibility of the study group with the Georgian population.

Integration of classical and modern genetic methods, supported by biostatistical analysis, allows for reliable identification results even in conditions of limited quantity and quality of biological material. The use of statistical software that take into account genetic linkage, combined with simulation tools such as pedbuildr, enabled a more accurate representation of actual family

relationships, which significantly increased the evidential value of the analyses. The proposed approach can be successfully implemented in forensic identification cases.

The research shows that the developed integrated analytical model, combining STR, Y-STR and SNP analyses with kinship simulations, effectively overcomes the limitations of traditional identification methods and allows for reliable results even with low DNA quantities and in cases of distant kinship. Genealogical simulations in the R environment proved to be an efficient tool supporting data interpretation. The use of multiple independent statistical programs confirmed the consistency and reproducibility of the obtained LR values. The results of the study prove that effective identification of historical victims is possible despite significant postmortem intervals, provided that a comprehensive, multi-step analytical approach is employed. The developed model can serve as a basis for a standardized workflow for future projects concerning the identification of unknown human remains.