An archaeogenetic approach to identify the remains of the Hungarian Kings

Gergely I. B. Varga,1,* Kitti Maár,2 Alexandra Ginguta,2,3 Bence Kovács,2 Balázs Tihanyi,1,4 Luca Kis,1,4 Orsolya Váradi,1,4 Petra Kiss,2 Dávid Szokolóczki,2 Oszkár Schütz,2 Zoltán Maróti,1,5 Emil Nyerki,1,5 István Nagy,6,7 Dóra Latinovics,6,7 Tibor Török1,2 and Endre Neparáczki1,2

ABSTRACT

The Royal Basilica of Székesfehérvár was the burial place of fifteen Hungarian kings. Unfortunately, the anthropological findings excavated at the site of the Basilica were mixed up during the tumultuous centuries of Hungary, hence the royal remains still lie unidentified in a charnel-house. The appearance and rapid development of archaeogenetics now allows the personal identification of the royal skeletons from among the remains of the Basilica. The genetic information necessary for the identification of the Árpád dynasty members is accessible, while sequence data of the non-Árpádian kings’ relatives still need to be obtained by further genetic analysis. Here we provide an outline of the investigation for the identity of the royal skeletons: we sketch the process of sample preparation and DNA extraction, the steps of library preparation for next-generation sequencing (NGS) and give a brief report of the current progressions.

KEYWORDS: Archaeogenetics, next-generation sequencing, Árpád dynasty, Kingdom of Hungary, Hungarian history, personal identification

1 Department of Archaeogenetics, Institute of Hungarian Research, H-1014 Budapest, Hungary
2 Department of Genetics, University of Szeged, H-6726 Szeged, Hungary
3 Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeș-Bolyai University, 400006 Cluj-Napoca, Romania
4 Department of Biological Anthropology, University of Szeged, H-6726 Szeged, Hungary
5 Department of Pediatrics and Pediatric Health Center, University of Szeged, H-6725 Szeged, Hungary
6 SeqOmics Biotechnology Ltd., H-6782 Mórahalom, Hungary
7 Institute of Biochemistry, Biological Research Centre, H-6726 Szeged, Hungary
* Corresponding author, varga.ergely@mki.gov.hu
The construction of the Royal Basilica in Székesfehérvár began during the reign of King Stephenus I of Hungary at the early years of the 11th century. During the succeeding periods it was rebuilt several times and expanded with new lean (e.g., Szabó 2010; Szabó 2018; Buzás 2019). The Basilica was the coronation church of the Hungarian kings, but it also served as a burial place for many kings, royal family members and aristocratic dignities (Engel 1987): inter alia eight kings and two princes of the Árpád dynasty (Prince Emericus, Stephenus I, Colomanus, Prince Álmos, Béla II, Géza II, Ladislaus II, Stephenus IV, Béla III and Ladislaus III), and seven other kings of the Kingdom of Hungary (Charles Robert, Louis I the Great, Albert the Magnanimous, Matthias I Hunyadi, Vladislaus II Jagiellon, Louis II Jagiellon, Johannes I Szapolyai) were laid to rest in the church (Table 1). During the 16-17th centuries the city was occupied by the Ottomans and besieged in multiple cases by Christian troops, and the Basilica suffered lots of damage: the graves and crypts were looted, the walls were destroyed, and the stones were taken to reconstruct the city buildings (Hankó 2004; Éry 2008).

<table>
<thead>
<tr>
<th>Name</th>
<th>Dynasty</th>
<th>Date of birth</th>
<th>Time of reign</th>
<th>Date of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prince Emericus</td>
<td>Árpád dynasty</td>
<td>1000/1007</td>
<td>—</td>
<td>1031</td>
</tr>
<tr>
<td>King Stephanus I</td>
<td>Árpád dynasty</td>
<td>c. 980</td>
<td>997–1038</td>
<td>1038</td>
</tr>
<tr>
<td>King Colomanus</td>
<td>Árpád dynasty</td>
<td>c. 1070</td>
<td>1095–1116</td>
<td>1116</td>
</tr>
<tr>
<td>Prince Álmos</td>
<td>Árpád dynasty</td>
<td>c. 1071</td>
<td>—</td>
<td>1127</td>
</tr>
<tr>
<td>King Béla II</td>
<td>Árpád dynasty</td>
<td>c. 1108</td>
<td>1131–1141</td>
<td>1141</td>
</tr>
<tr>
<td>King Géza II</td>
<td>Árpád dynasty</td>
<td>c. 1130</td>
<td>1141–1162</td>
<td>1162</td>
</tr>
<tr>
<td>King Ladislaus II</td>
<td>Árpád dynasty</td>
<td>c. 1131</td>
<td>1162–1163</td>
<td>1163</td>
</tr>
<tr>
<td>King Stephanus IV</td>
<td>Árpád dynasty</td>
<td>c. 1133</td>
<td>1163</td>
<td>1165</td>
</tr>
<tr>
<td>King Béla III</td>
<td>Árpád dynasty</td>
<td>c. 1148</td>
<td>1172–1196</td>
<td>1196</td>
</tr>
<tr>
<td>King Ladislaus III</td>
<td>Árpád dynasty</td>
<td>c. 1200</td>
<td>1204–1205</td>
<td>1205</td>
</tr>
<tr>
<td>King Charles I</td>
<td>Anjou house</td>
<td>1288</td>
<td>1301/1308–1342</td>
<td>1342</td>
</tr>
<tr>
<td>King Louis I</td>
<td>Anjou house</td>
<td>1326</td>
<td>1342–1382</td>
<td>1382</td>
</tr>
<tr>
<td>King Albert</td>
<td>Habsburg house</td>
<td>1397</td>
<td>1437–1439</td>
<td>1439</td>
</tr>
<tr>
<td>King Matthias</td>
<td>Hunyadi house</td>
<td>1443</td>
<td>1458–1490</td>
<td>1490</td>
</tr>
<tr>
<td>King Vladislaus II</td>
<td>Jagiellon house</td>
<td>1456</td>
<td>1490–1516</td>
<td>1516</td>
</tr>
<tr>
<td>King Louis II</td>
<td>Jagiellon house</td>
<td>1506</td>
<td>1516–1526</td>
<td>1526</td>
</tr>
<tr>
<td>King Johannes I</td>
<td>Szapolyai house</td>
<td>1490/1491</td>
<td>1526–1540</td>
<td>1540</td>
</tr>
</tbody>
</table>

Table 1. Hungarian kings buried in the Royal Basilica of Székesfehérvár.

In 1848, astonishingly, undisturbed royal tombs were found during well-sinking in the courtyard of the Episcopal Palace, the original area of the Royal Basilica (Éry 2008). The skeletons found in the graves were identified as King Bela III and his wife, Queen Anna of Antioch. During the following centuries, further excavations were carried out (1848, 1862, 1874, 1936–37, 1967–2002), which resulted in the exploration of the remains of almost one thousand individuals and hundreds of unsorted skeletal fragments. Unfortunately, thanks
to the disturbance of the graves and the removal of the skeletons by the Ottoman and Christian soldiers, and mishaps during the excavations and the handling of the remains, the skeletons were mixed up. Later an anthropological effort was made to sort the bones of the different individuals, as there was not any scientific method available to identify the royal remains (Éry 2008). In 2002 most of the skeletons were placed into stainless steel caskets stored in a charnel-house in Székesfehérvár, excluding Béla III and seventeen other remains which were reburied in the Matthias Church, in Budapest (Hankó 2004; Éry 2008).

The solution to the problem of the identification is provided by the new discipline of archaeogenetics, which has evolved rapidly thanks to the development of fast and effective molecular biology methods and population genetic tools (Rizzi et al., 2012; Pickrell and Reich, 2014). The toolkit of this new discipline among others enables us to determine the origin and family relations of ancient individuals or peoples (Nepárczki et al., 2018, 2019; Maár et al., 2021). Now it is possible to describe the ancestry, admixture and migration of ancient or modern populations (Haak et al., 2015; Järve et al., 2019; Narasimhan et al., 2019); to identify maternal and paternal lineages (Csáky et al., 2020); or to reveal kin relations and to reconstruct family trees (Keller et al., 2015; Kuhn et al., 2018; O’Sullivan et al., 2018; Vai et al., 2020; Keyser et al., 2021). What is more important for us, there were several examples wherein archaeogenetic approach was applied successfully to identify the remains of famous deceased persons (Rogaev et al., 2009; King et al., 2014). These cases indicated that genomic sequence information from certain relatives was necessary to determine the exact personal identity of historical remains, so it is indispensable to obtain genetic data from relatives of Hungarian kings to identify their remains.

The genetic investigation of the royal remains of Székesfehérvár began in 2013 within the framework of the House of Árpád Program with the low-resolution examination of King Béla III and a couple of other skeletons placed in the Matthias Church. Based on Y chromosomal STR analysis it was established that Béla III and the House of Árpád belonged to the R1a paternal ancestry group (Haplogroup), thereby an additional Árpád dynasty member’s skeleton could be identified (Olasz et al., 2019). After the deep analysis of the Árpáds’ complete Y chromosome sequence, it turned out that the paternal lineage of the first Hungarian ruling dynasty belongs to the R1a-Z2125 sub-Haplogroup, which was originated in Northern Afghanistan in 2500 BC. The most similar sequences could be found among present-day Bashkirs of which lineage the Árpád Y-chromosomal lineage separated about 2000 years ago. Based on eight unique single nucleotide polymorphism (SNP) markers the Árpád family members define an exclusive sub-Haplogroup R-ARP (Nagy et al., 2021).

We planned to carry out the archaeogenetic investigation of the skeletons in the charnel-house of Székesfehérvár with next generation sequencing (NGS) techniques to identify the remains of the Hungarian kings. The key device to identify the Árpád dynasty members, namely the Y chromosome sequence of the Árpáds has become accessible (Olasz et al., 2019; Nagy et al., 2021), nevertheless, in the case of the other seven kings of the Kingdom of Hungary genetic analysis of certain remains of royal relatives is still required.
As a first step of this investigation, we obtained the required permissions from the Diocese of Székesfehérvár, the Museum of King Saint Stephan and the Municipality of Székesfehérvár, opened the charnel-house and sorted the proper bone samples. The best quality DNA for archaeogenetic analysis can be extracted from petrous bone or tooth cementum, so we concentrated on the available skulls (Hansen et al., 2017). The preservation status of the remains varied widely: complete or almost complete skulls could be found as well as deficient or fragmented ones. The traces of previous sampling for genetic analysis were detected on several skulls (Éry, 2008). On numerous skeletons we found signs of various illnesses (Fig. 1). In the case of remains without skulls we selected skeletal bones of different types. Surprisingly, notably higher number of bones were found than could be expected based on the previous anthropological work report (Éry, 2008). Altogether we selected skulls and skull fragments along with 34 skeletal bone samples of 633 separated remains and 1222 unsorted skull fragments.

Figure 1. Examples of the bone material found in the charnel-house. a) complete skull, b) deficient skull, c) skull with trace of genetic sampling, d) fragmented skull, e) skull without petrous bone and teeth (unsuitable for archaeogenetic analysis), f) sack of scattered skulls, g) abnormal vertebral column and h) sack of fragmented bones.

The next step of the process, the DNA extraction and library preparation for NGS is still in progress. All pre-PCR laboratory procedures are carried out with stringent clean-room conditions in the common ancient DNA laboratory of the Department of Archaeogenetics, Institute of Hungarian Research and the Department of Genetics, University of Szeged. When maxillary tooth is available, we extract DNA from tooth root by a minimally destructive protocol according to (Harney et al., 2021). Bone powder samples are taken and extracted according to the method described in (Neparáczki et al., 2017). We apply the double stranded library protocol of (Meyer and Kircher, 2010) with double indexing (Kircher et al., 2012). Libraries are generated from partial uracil-DNA glycosylase (UDG)-treated DNA extracts (Rohland et al., 2015) and are purified on MinElute columns (ThermoFischer).
Quantity and quality measurements were performed with the Qubit fluorometric quantification system (ThermoFischer) and the TapeStation automated electrophoresis system (Agilent). Additionally, the endogenous human DNA content of the libraries is estimated with shallow shotgun sequencing on iSeq 100 platform (Illumina). The biological sex of the individuals is determined based on the X/Y ratio of the reads gained from the shotgun sequencing (Skoglund et al., 2013). At the time of the submission of this manuscript 389 libraries were completed, and 198 of those were sent to whole genome sequencing on NovaSeq 6000 Sequencing System (Illumina).

After obtaining the whole genome sequences, those will be mapped to the Human genome, and the marker set, characteristic to each individual, will be determined. During this process, the mitochondrial-, Y chromosomal- and autosomal markers will be defined as well. The Y chromosomal sequences will be compared to the previously established Árpád dynasty sequences, and the members of the first Hungarian ruling family will be identified. Based on autosomal analysis the degree of kin relationships between the individuals will be determined and the place of each person on the family tree will be assigned, thus the personal identities will be determined. Mitochondrial data will help to verify the identities by comparing them to maternal relatives.

Due to the adverse history of the skeletons of the Basilica, it is unlikely that the remains of all the kings will be found. Nevertheless, we are taking the best scientific approach to identify additional Árpád dynasty kings in the charnel-house of Székesfehérvár if their remains are there. At the same time, to identify the kings and members of other dynasties and nobles, genomic data from their relatives is necessary. Thus, the search for such relatives has begun: the researchers of the Institute of Hungarian Research have gone to Lepoglava, Croatia, excavated the crypt of John and Christopher Corvinus, son and grandson of Matthias I, and they have taken samples from the human remains. With the help of the Y-chromosomal sequence of the Corvins, the skeleton of Matthias I can be selected with the same method as in the case of the Árpáds. Similar international cooperations are necessary to obtain genetic information from other Hungarian royal kins, to allow successful identification of the royal remains. This is a prerequisite for the establishment of a worthy memorial for our kings which is one of the principal goals of this effort.
FUNDING

This research was funded by grants from the National Research, Development and Innovation Office (TUDFO/5157-1/2019-ITM and TKP2020-NKA-23 to E.N.). Prepared with the professional support of the Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry of Innovation and Technology financed from the National Research, Development and Innovation Fund (KDP-2020, Grant No. 1020404 to L.K.) K.M. was supported by ÚNKP-20-3-SZTE-470 New National Excellence Program.

ACKNOWLEDGEMENT

We are grateful to the Diocese of Székesfehérvár, the Museum of King Saint Stephan and the Municipality of Székesfehérvár to enable the accession of the bone material. We thank to Ferenc Stilling and András Halász for their help during the sorting of the bones. We are also grateful to Miklós Kásler, Gábor Horváth–Lugossy and Tamás László Vizi for their support and encouragement and Szabolcs Tóth for his administrative work. We thank to Miklós Makoldi, Eszter Dudás, Adrienn Hornyik, Zsolt Tóth, Zoltán Szentirmay, Horolma Pamjav and Péter L. Nagy for their contribution.

CONFLICTS OF INTEREST

I.N. and D.L. at SeqOmics Biotechnology Ltd. had consulting positions during the time the study was conceived. SeqOmics Biotechnology Ltd. was not directly involved in the design and execution of the experiments or in the writing of the manuscript. This affiliation does not alter our adherence to Ephemeris Hungarologica policies on sharing data and materials. All other authors have no conflicts to declare.

REFERENCES


KIVONAT

A magyar királyi maradványok azonosításának archeogenetikai megközelítése

A székesfehérvári királyi bazilikát számos magyar király választotta végső nyughelyéül. Sajnos a bazilika területén feltárt antropológiai leletek összekeveredtek Magyarország zűrzavaros évszázadai alatt, ezért a királyi maradványok még mindig azonosítatlannak fekszenek a bazilika területén kialakított osszáriumban. Az archeogenetika megjelenése és gyors fejlődése napjainkra immár lehetővé teszi a királyi csontvázak szétválogatását a bazilika maradványait közül. Az Árpád-ház tagjainak azonosításához szükséges genetikai információk hozzáférhetők, azonban a vegyesházi királyaink rokonainak szekvenciaadatait további archeogenetikai vizsgálatokkal tudjuk megszerezni. Közleményünkben röviden beszámolunk a királyi csontvázak azonosításáról: ismertetjük a mintavétel, a DNS-kivonás, a szekvenálókönyvtár-építés és az újgenerációs szekvenálás folyamatát, valamint rövid jelentést adunk az aktuális fejleményekről.

KULCSSZAVAK: archeogenetika, újgenerációs szekvenálás, Árpád-ház, Magyar Királyság, magyar történelem, személyazonosítás