An archaeogenetic approach to identify the remains of the Hungarian Kings

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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

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The construction of the Royal Basilica in Székesfehérvár began during the reign of King Stephanus I of Hungary at the early years of the 11th century. During the succeeding periods it was rebuilt several times and expanded with new leans (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, Stephanus I, Colomanus, Prince Álmos, Béla II, Géza II, Ladislaus II, Stephanus 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).

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

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

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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 (Neparáczki *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.

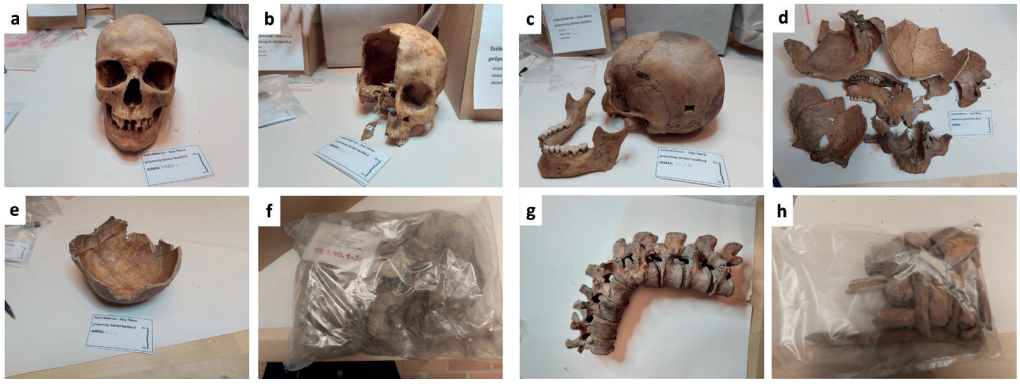

Figure 1. Examples of the bone material found in the charnel-house. a) complete skull, b) deficient skull, c) skull with trace of genetic sampling, d) fragmented skull, e) skull without Petrous bone and teeth (unsuitable for archaeogenetic analysis), f) sack of scattered skulls, g) abnormal vertebral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

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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 dinasty 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. Whit 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. ■

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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.

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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 azonosítatlanul fekszenek a bazilika területén kialakított osszáriumban. Az archeogenetika megjelenése és gyors fejlődése napjainkra immár lehetővé teszi a királyi csontvázak szétválogatását a bazilika maradványai 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