


EDITORS:
MIKLÓS KÁSLER AND ZOLTÁN SZENTIRMAY



IDENTIFYING THE ÁRPÁD
DYNASTY SKELETONS INTERRED
IN THE MATTHIAS CHURCH

Applying data from historical, archaeological, anthropological, radiological, morphological, radiocarbon dating and genetic research



MAGYARSÁGKUTATÓ
INTÉZET

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Cover: Marble sarcophagi of Béla III and Anne of Antioch in the Matthias Church, Buda Castle (photograph by László Bárdossy).

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FOREWORD

On our present and future, which started in ancient times

When I was a child, I failed to understand the duality between stories I was told by my parents, which I read in novels, legends and traditions, on the one hand, and the history taught at school on the other, which either denied most of the stories I was told or read, or explained them from a different perspective. In the years gone by I have discovered many contradictions and deficiencies which could not be resolved by this duality.

Thinking about the origins of my family, which I can trace back 500 years, I found it strange that the Árpád dynasty, one of Europe's most significant, most gifted and most powerful dynasties, did not know its origins, roots and ancestors, or was ill-informed. For 800 years, nobody challenged the dynasty descending from Attila and the Scythian, Hun origin of the Hungarian people, the Magyars. Neither did I understand why, in a Christian world, the Dynasty of Holy Kings refers to a pagan ancestor, unless this pagan ancestor gave something special to his people or the peoples under his rule, possibly to Europe or even mankind. I did not receive any adequate explanation about why Attila was called “malleus orbis”, Hammer of the World, and “flagellum Dei”, Scourge of God. I also entertained the idea that the songs of bards and word of mouth might not have been the only way for the kings from the Árpád dynasty to learn the

history of their own family, because runic writing existed to record all of this. Nor did I understand why the Hungarian Conquest was completed without any bloodshed, without any major battles fought. I did not understand why the most important battle in Hungarian history, the Battle of Pozsony ensuring our survival, is taught as part of the curriculum at the military academies in West Point and Saint-Cyr and not in Hungarian primary schools, nor did I understand why we learn only about the Battle of Merseburg and Augsburg from the at least 48 sieges and battles fought during the “expeditions”. I did not understand why the Frankish Empire did not attack the Carpathian Basin and spread Christianity after the Battle of Lechfeld. This is what Otto did to every defeated country and people if his victory was really decisive, since in that era, the power vacuum, if any, was filled by the victorious power as a rule.

I did not understand what it meant according to the Greater Legend of Hartvic that the title of apostolic king was conferred on Saint István (known in English as Saint Stephen) by the successor of Saint Peter, whereas the Pope himself remained apostolic. I did not understand exactly why the offer made by Saint István was necessary. The offer is not about the Virgin Mary being *Patrona Hungariae*, but about her being *Regina Hungariae*, i.e. Queen of Heaven, and also, or by virtue of this, Queen of Hungary. I viewed the evolution of the concept of the Holy Crown as mystical; it was something barely heard of. I did not understand why we do not talk about the concept of the Holy Crown, the essence of which is the unprecedented linkage forged between the spiritual and the earthly world, the proportion of the division of power, unknown in the period, and the ideological foundation of the special Hungarian development of law.

I did not understand on what basis the Hungarian king, the apostolic king, convened an ecclesiastical synod, and how he could adopt ecclesiastical laws as Saint László did (known in English as King St. Ladislaus I of Hungary) at the synod of Pannonhalma and Szabolcs, and Sigismund of Luxemburg between 1414 and 1418 in Constance between three living popes.

It was not entirely clear how it is possible that in *Chronicon Pictum*, the *Illuminated Chronicle*, under the illumination depicting Vazul being blinded, Saint István lying in bed blesses the sons of Vazul. Neither was it clear how it is possible that the pagan insurgents call back the three sons of Vazul, two of whom were known to have had religious education, entered into Christian marriages, practised their religion, then after returning to their home country they consolidated the country without any major battles. Was it a pagan revolt? Did they avenge the tyranny of the “Italians”? Or both? Why and how Saint Imre (known in English as Saint Emeric) passed away was not clear for me either, who through his mother Gisella was a descendant of the extinct Saxon dynasty, just like Conrad II of the Salian dynasty, who rose to the throne. It is an eerie coincidence that Conrad attacks the country in 1030, and the Hungarians claim a decisive victory, presumably led by Saint Imre, who then passes away immediately after this. Was he murdered?

I did not understand the official explanation why the Mongols left the territory of the Kingdom of Hungary. The explanation was that Batu Khan returned to Karakorum for the election of the Great Khan. This argument is not solid enough, since we know from *The Secret History of the Mongols* that there were doubts as to the origin of Jochi, the eldest son of Genghis Khan, so his son Batu could not

be a candidate for the position of the Great Khan. We know that the kurultai, the council electing the Great Khan, which Batu wanted to attend and therefore left Hungary in 1242, was actually only held eight years later. He could have reached home multiple times in eight years.

At the same time, for eight months the Mongols could not cross the Danube, they could not occupy several fortresses, because the Hungarian forces prevented them from doing so. Interestingly, after the Mongols retreated in 1242, during his castle-building programme King Béla IV did not have most of the castles built in the East to defend the country against the assumed Tatar threat, they were largely built along the western border. Also peculiar is how the king of the “completely destroyed” Kingdom of Hungary, King Béla IV, was able to regain the stolen western counties from the last Babenberg. We did not learn too much about Kun László (King Ladislaus IV of Hungary) defeating the Mongolian armies during the second Mongol invasion in 1285 close to the ridge of the Carpathian Mountains, and similarly, the story of Endre Lackfi brutally defeating the third wave of the Mongol invasion in 1345 has also disappeared from history textbooks. Later I learned that the Mongols believe the western expansion was halted in the Kingdom of Hungary, which reversed the fate of the Mongolian nation in the making.

I did not understand how, for 800 years, everybody, our Holy Kings, the female line of the Árpád dynasty, then the Habsburgs, West-European and Italian books from the 1600s, but before that the contemporary Arabs and Byzantines all knew without exception – as did the Hungarian chronicles – that the Hungarians had descended from Scythian-Hun-Turkic-Avar ancestry, and so had the dynasty.

This is how the greatest Hungarians, King Matthias, Zrínyi, Mihály Vörösmarty, János Arany and nearly everybody knew and wrote about it, and yet after 1850, a dual perspective of our origins emerged. One side addresses the kinship with Finno-Ugric languages, which is also used to derive the ethnic kinship, while the other focuses on the Turkic kinship. The two were debated for 160 years yet completely ignored the Scythian-Hun origin. I did not understand how disciplines in a position to formulate a substantive opinion on the issue, such as history, linguistics, chronicles, folklore, folklore motives, folk music, anthropology and archaeology ignore each other's findings, and instead of complementing one another, they tend to underestimate and even often discredit each other's findings. At the same time, I did not understand why our history reflecting the Hungarian mentality and our insatiable desire for freedom was reinterpreted and frequently rewritten. I took the opportunity of conferences I attended to visit the coronation and burial sites – St. Denis, Reims Cathedral, El Escorial, the Capuchin Crypt in Vienna, Wawel Royal Castle and many more – where the members of more fortunate nations may go to pay tribute to outstanding figures of their history. I felt immense sorrow that we cannot pay such visits to the tombs of our own glorious kings and dynasties, because their burial places were destroyed by history in many cases. I was downhearted to see the current state of the Saint Stephen Basilica in Székesfehérvár and its sad fate, and I have always desired to have a national place of worship erected, a place of pilgrimage where we can pay tribute and express our gratitude.

Over the years I have devoted myself to medical sciences, more specifically to the most complex group of diseases: tumours. It was

a fortunate coincidence that molecular pathology – which examines DNA transmission to identify the changes in DNA leading to serious tumorous diseases – appeared in oncological diagnostics. Equally a special gift of fate, the first molecular pathology research profile in Central and Eastern Europe was established in the National Institute of Oncology, which I was the director of. As a result of this research of international significance, we identified and described several types of gene polymorphism in the DNA of tumours. By the beginning of the 2010s the number of molecular analyses reached several thousand per year. Pursuing this brand-new science required a genuinely innovative approach, as solutions had to be found to an extremely large number of problems. In this situation, and full of these recurring emotions, together with Professor Szentirmay I was listening to a lecture of Professor István Raskovits in Kolozsvár (Cluj Napoca in Romania) on his archaeogenetic analyses covering the period of the Hungarian Conquest. During these analyses they even examined the DNA of Hungarian horses used in those times to find the horse breeds whose DNA is closest. It was there that Professor Raskó said that the Turkmen horses he called “the Rolls-Royce of that age” were the closest. It was also fortunate that I listened to Professor Raskó because I could have done something different, but because I was in a student association at the Institute of Microbiology of the University of Szeged, and assistant lecturer Raskó was one of my mentors, I listened to his lecture out of respect. His lecture triggered a new idea. Namely, that given the competence of the institute, we should make an attempt to analyse the DNA of the bones found in Székesfehérvár and determine and identify our kings buried there, one by one. Given that King Béla III was the only king who could have

been identified with high probability, the solution was simple: let us try to extract the DNA from his skeleton and determine the remains of all the males belonging to the Árpád dynasty, and if possible, the individual persons. If we succeed in determining the DNA of King Béla III, we are able to specify the DNA of all the other kings of the Árpád dynasty, and perhaps the specific persons as well, based on the DNA section of the Y chromosome that passes from father to son. But in this phase I was already thinking of ways to identify all the other kings. In cooperation with Margit Földesi, then György Szabados, we started to compile the genealogy of the Árpád dynasty, its female lines, and the genealogy of the Hunyadi and Szapolyai families. Special assistance was provided by Balázs Holczmann, who was dealing with the same topic completely independently from us; he elaborated the genealogy of the kings in minute detail, then informed me by email. I was really glad to welcome him into our emerging team, made up of colleagues driven by the same emotions and joining forces of their free will to accomplish the same goal, in the interest of more noble objectives. The next step in this process was to see if we were even capable of extracting and examining DNA from ancient bones. Professor Szentirmay had already succeeded here on bones from the Medieval Period, so we took the next step confident that, in all probability, the seemingly hopeless mission might be accomplished. After this I submitted an application to the Ministry of Interior in charge of archaeology, requesting financial assistance to start our examinations. The HUF 20 million granted by Minister of Interior Sándor Pintér ensured we could start. I should note here that this funding was sufficient to pay specialist company Reneszánsz Ltd. to open and restore the

crypts in Matthias Church, and it also covered the costs of foreign researchers joining the team. To date, the Hungarian participants have neither requested nor received any financial consideration for their work. I endeavoured to gather together all the people who were motivated. This is how I invited Ms Piroska Biczó, archaeologist, and the Archaeology working group of the Hungarian Academy of Sciences led by Professor Elek Benkő, to whom I hereby express my gratitude for the work he has done. In the project he assigned Balázs Mende to participate in the genetics work. Professor Béla Meleg joined the team, who participated in lifting and sampling the bones, while he also invited the foreign participants. Specifically, he invited the internationally renowned archaeogenetics department associated with the University of Göttingen, another major research centre from Germany, and after identifying the DNA of the ancient bones it was he who involved Péter Nagy, a US-based geneticist of Hungarian origin and motivated by Hungarian sentiments, who after initial examinations joined the DNA sequencing process. Having been granted financial support, I contacted Cardinal Péter Erdő, who with an extremely generous gesture and motivated by his deep commitment to science immediately assured us of his support for the research. He consented to the lifting of the skeleton currently located in Matthias Church – transported there from Székesfehérvár in the 19th century and laid under appropriate circumstances – and to the sampling of the bones. After this, Erzsébet Csernok, a student of Professor Szentirmay, joined with great enthusiasm from the National Institute of Oncology, as did my colleague Judit Olasz, who participated in this project in her free time with the permission of her superior, Orsolya Csuka.

In the first step we had discussions with archaeologists who were knowledgeable about the bones in Székesfehérvár, the bones in Matthias Church, and all the other bones in the Ossuary. Two of the three archaeologists in this discussion opined that our project was completely impossible and hopeless, as such attempts had already been made involving prestigious researchers under international cooperation but had failed to come to fruition.

The crypts were opened in Matthias Church at night, after the masses had finished. Reneszánsz Ltd. opened the crypts of Anne de Châtillon and her husband King Béla III in a professional manner. We removed the skeletons from the metal containers under the same aseptic conditions as in operating theatres, loaded them into the sterilised transport vehicle of the National Institute of Oncology, and transported them to the isolated operating theatre prepared for this specific purpose at the National Institute of Oncology. The sampling was conducted in the aseptic operating theatre with an oscillating saw to avoid the warming up usually caused by bone drills and thus further degradation of any ancient DNA. It goes without saying that we cleaned the bones with disinfectant used for washing before surgery and with hydrogen peroxide. During the sampling a kind colleague of mine, Éva Csorba, took on the role of the surgical nurse, while Professors Béla Meleg and Zoltán Szentirmay assisted with the task. We repeated the same procedure on all the skeletons and bones located in the crypt of Matthias Church. After sampling, we replaced the skeletons in approximately the same anatomic position before transporting them to the diagnostic imaging centre of the institute, where we made CT images of each and every bone. In the course of the sampling we divided the samples, which were 4-5 cm long, into

four groups. One was given to the Institute of Archaeology of the Hungarian Academy of Sciences, two were given to Professor Béla Meleg to pass them on to our foreign partners, and one remained at the National Institute of Oncology. We coded every sample of course, so in the subsequent phases of the work nobody knew which code corresponded to which individual skeleton sample. We conducted the research in several institutes to avoid the criticism that this does not fit the profile of the National Institute of Oncology, or that the institute is not geared up for such work, not to mention that the institute might be accused of falsifying the results. Knowing the circumstances in Hungary, this was a possibility. Two of the four samples were successfully examined. One at the archaeogenetics department based in Göttingen and the other one at the National Institute of Oncology. The ancient DNA was successfully extracted in both places, and the appropriate markers were also examined. The findings of the two institutions were practically identical. This meant the credibility and significance of the research findings were beyond all doubt, enabling us to publish our findings in a prestigious European journal. This did not signal the end of this work, as Péter Nagy, who had joined us in the meantime, continued sequencing the samples applying another modern technology, naturally with the same team members who had participated in the work until then. I was delighted to learn that parallel to our work, and without us knowing about each other, Endre Neparáczki and Tibor Török had completed population genetics examinations on male and female skeletons from the Avar period and the era of the Hungarian Conquest. As their work progressed, they regularly published their findings in prestigious journals, based first on matrilineal then on patrilineal descent.

At this time my situation changed, and I had the opportunity to try and coordinate the researchers and efforts in the interests of the original objective. On my initiative, the Government of Hungary set up the Institute for Hungarian Studies (*Magyarságkutató Intézet, MKI*), whose programme existed before its official establishment as this prompted the Government's decision to set it up. This Institute coordinates all the disciplines which are able to provide substantive data concerning the origin as well as early and later history of the Hungarian people.

I asked Gábor Horváth-Lugossy to head the Institute. With immeasurable dedication, accuracy and a large degree of intuition he organised the eleven research institutes which are able to manage, research and synthesise the activities of various relevant scientific disciplines from historical science, folk music, ecclesiastical history, classical philology through to archaeology, anthropology and archaeogenetics.

The initial idea of inviting capable and competent researchers to accomplish one particular objective was implemented when the MKI was established. My responsibility is now to support its survival and operational capacity, select research topics and provide the multiple conditions needed for the research.

In the last few years of the 2010s, a promising examination was launched into the genetics of the Szekler (*Székely*) and Csango populations. Professor Attila Miseta and Professor Béla Meleg are leading this research. After favourable negotiations this research was also included in the profile of the Institute for Hungarian Studies when Endre Neparáczki joined the organisation.

We might conclude that the Institute for Hungarian Studies met one of the conditions for its establishment by encompassing and

standardising archaeogenetic research on the origins and geographic location of the Hungarian people and finding its place in the chronology.

Another reason for establishing the Institute for Hungarian Studies was to coordinate work in the fields of related sciences. Within just one and a half years, it became possible to analyse the skeletons of all Hungarian kings buried in Székesfehérvár and extend our classical philology research to include sources. Finding sources in Armenian monasteries, Mongolian and Chinese written sources as well as critical revisions of the translations of Arab, Latin and Greek sources are all on the agenda, together with extending archaeological excavations in Hungary and in areas where the ancestors of the Árpád dynasty and the population around them lived in the past 4500 years.

It seemed obvious to me, and this is why we completed the examination of King Béla III, that the dynasty was a reference point, which the population followed and adjusted to in various fields of their everyday activities. Another task for the Institute for Hungarian Studies is to interpret and monitor the stability and changes in the history of ideas in the course of Hungarian history. A separate priority area is analysing early Christianity in Hungary, the Byzantine and Roman impact, and Hungarian traditions.

Almost all researchers working at the Institute for Hungarian Studies hold scientific degrees. They are required to work without preconceptions, guided strictly by scientific principles, and to publish their findings in a language spoken by academicians and ordinary people alike, in Hungarian and in world languages. During their migration, Hungarians clearly encountered Finno-Ugric peoples, but they also met Turkic peoples. However, the most recent research

findings emphasise the Scythian-Hun-Avar-Magyar line for the main political, military and cultural descent. To determine the most probable of all the possible ideas by applying scientific methods will pose a major challenge, not only for the Institute for Hungarian Studies but for Hungarian science as a whole. The Hungarian Government has provided not only funding to support these endeavours, but also diplomatic support through the ministries of culture and research institutes of the governments in the countries concerned.

“In the beginning was the word”, the idea. The research into the origins of Hungarians was also born and developed from ideas, knowing for sure it is impossible to find the answer to every unanswered question.

This book is about one of the first steps following that initial idea, but it goes far beyond that. The idea has developed and expanded. The idea is to make progress in exploring the unexplored past with scientific accuracy and a synthesis of the scientific disciplines concerned. The know-how acquired in this way will strengthen our knowledge, our information base and self-identity. We will gain a better understanding of our views on life, our traditions, our history and our culture. Who we are, and why. From the National Curriculum to university departments.

Soli Deo Gloria!

Miklós Kásler

EDITORS' PREFACE

In this book, we provide a detailed description of the joint work conducted between 2012 and 2017, with the goal of genetically identifying the Kings of the Árpád Dynasty. The primary purpose of our research was to identify the persons whose skeletons were originally buried at the Basilica of the Assumption of the Blessed Virgin Mary in Székesfehérvár and are currently entombed in the crypt of the Matthias Church in Budapest. The end result was the identification of a skeleton of a previously unidentified king from the Árpád Dynasty, which in turn led us to investigate the origins of the Árpáds. The task we undertook – like all research, generally speaking – was not straightforward; we ran into many obstacles and setbacks, and had to start over on several occasions.

We had to be persistent, with uncompromising belief that our objectives were achievable. We had to endure systematic criticism and disagreements, and accept constructive remarks. We expected that there would be criticisms and attacks, which is why we had decided to involve in our investigations a foreign institution whose competence is beyond any doubt: the department of Historical Anthropology and Human Ecology of the Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology (University of Göttingen, Germany); their results often paved the way for us. Chief among the criticisms was the view that the genetic analysis

of the royal bones should be performed by a dedicated institution, whereas we conducted this in the National Institute of Oncology in Budapest, which has a very different profile. Several people, including Kinga Éry, expressed serious concerns about whether or not we were even capable of carrying out this task. Her doubts were especially great with regard to the fact that there had already been an attempt to identify the particular royal bones with foreign help, but it yielded no results at all. Others doubted that a team of researchers primarily composed of doctors could even distinguish one human skeleton from another. Others still gave advice on how to begin such a task. An example of the latter is Balázs Mende's study *Hogyan ne azonosítsuk az Árpád-házi királyokat?* [*How not to identify Kings of the Árpád Dynasty?*], in which he suggests using relics as controls. However, we did not want to use relics even if we were able to, not only for reasons of piety, but also because there was no pressing need to do so, seeing that we could rely beyond doubt on the genetic data provided by the skeleton identified as belonging to King Béla III.

We needed to learn new things along the way, a process which was facilitated by constant communication. Gábor Tusnányi, member of the Hungarian Academy of Sciences (MTA), provided us with some particularly useful insights: his stern, but well-intended constructive criticism helped us to repeatedly re-evaluate the data from different perspectives.

We needed to be able to connect and interpret data distant from each other, and should the need arise, to make the necessary adjustments to achieve a clear result. We developed this method of problem solving while performing modern diagnostics of tumours.

It was Dr Miklós Kásler who proposed the idea of performing genetic studies on the kings of the Árpád Dynasty in 2012 at a meeting of medical professionals in Szeged, after a presentation on the genetic analysis of bones extracted from graves in Hungary by Professor Dr István Raskó. At that point, we believed that this idea could be realized at the National Institute of Oncology (NIO) for the following reasons: (a) The tools necessary for genetic analysis were already available there; (b) The DNA isolated from the bones would obviously be fragmented, but the NIO Tumour Pathology Centre has a great deal of experience analyzing fragmented formalin-fixed, paraffin-embedded molecular DNA; (c) We are familiar with complex diagnostic problems and solving them as clearly as possible (in the interests of successful patient care), even when we do not have all of the necessary information. In such cases, we would return to the problem at hand once we had acquired new clinical information, researched new literature, or implemented new processes. This practice has often led to clear and useful diagnoses. Our work on this project benefited greatly from this ability.

The basic requirement for conducting the planned research was to reopen the sarcophagi, since this is where the skeletons of King Béla III and Queen Anne of Antioch are kept, in sarcophagi located in a separate chapel on the ground floor. Using the genetic analysis of the bone samples obtained from the royal couple, it was possible to individually identify the rest of the skeletons held in the sarcophagi of the crypt, which were thought to belong to Kings of the Árpád Dynasty or their family members. In order to confirm their possible Árpád Dynasty origins, it is important to note that each skeleton was taken to the Matthias Church from the Basilica

of the Assumption of the Blessed Virgin Mary in Székesfehérvár. Dr Miklós Kásler, head of the research project, was able to obtain permission from Cardinal Péter Erdő, Archbishop of Esztergom-Budapest, and secured one-time government funding for the research expenses.

We opened the sarcophagi in 2014 and created quite a large amount of photographic and video documentation when taking samples from the bones. We also generated computed tomographic (CT) images and used several genetic, mathematical and special morphological methods in the analyses. We then aligned the resulting genetic data with the results of the historical, archaeological, anthropological and radiocarbon research. The logical ordering of the evidence pertaining to particular results, the clarification and articulation of correlations, and the publication of the vast amounts of image documentation supporting those correlations are – with the exception of publications submitted during the process – only possible in book form. We are aware that others may interpret the data differently, but as far as we are concerned we remained grounded within the framework of scientific methodology and ethics. Although we tried our best to be as clear as possible, the specialized genetic data and many other kinds of data can be hard to understand. We have tried to mitigate this by including a glossary, as well as a summary at the end of each chapter.

Having mentioned all of the above, we heartily recommend this book for all who wish to know more about the brightest era in Hungarian history and Hungary's most important kings, to those wishing to pay homage to their recently identified remains in a heavenly pantheon, and on this earth, at the site where their eternal

slumber has been disturbed by history. We also recommend our work to anyone wanting to peek into the workings of modern genetics.

Budapest, August 2019

Miklós Kásler and Zoltán Szentirmay

RESEARCHERS CONDUCTING THE STUDIES AND THEIR ACTIVITIES

1. Dr Miklós Kásler, director-in-chief, MTA member, professor, head of department, National Institute of Oncology (NIO) – The project's initiator, organizer and head
2. Dr Béla Melegh, professor, Scientific University of Pécs, Genetics Institute – International relations
3. Dr Mária Gödény, radiologist, professor, head of department, National Institute of Oncology, Department of Radiological Diagnostics – CT imaging of the royal bones
4. Dr Gábor Tusnády, academic, MTA member, Alfréd Rényi Mathematics Research Institution, Budapest – Statistical analysis
5. Dr László Józsa,† MTA member, pathologist-palaeopathologist – Macroscopic palaeopathological description of the skeletons of Béla III and Anne of Antioch, as well as skeletons I/3 G5 and I/4 H6
6. Dr László Módis, anatomist, professor, University of Debrecen, Institute of Anatomy, Histology and Embryology – Histological and two-photon and polarized light microscopy analysis of the royal bones

7. Dr György Szabados, historian, director of the MKI Gyula László Research Centre and Archive (Budapest), historian and consultant at the King Saint István Museum (Székesfehérvár), historian at the Gyula Siklósi Urbanism Research Center (Székesfehérvár) – Historical summary, genealogical outline of the Árpád Dynasty, research history overview of King Béla III's identification
8. Dr Piroska Biczó, archaeologist, Hungarian National Museum – Summary of archaeological data pertaining to the royal graves, locating the royal graves on the schematics of the Basilica of Székesfehérvár
9. Dr Elek Benkő, academic, historian, director of the MTA Institute of Archaeology – Radiocarbon dating
10. Piroska Rácz, anthropologist, Saint István Museum, and Balázs Gusztáv Mende, MTA Institute of Archaeology, Laboratory of Archaeogenetics – Anthropological study of the royal bones and numerical comparison to the data from Kinga Éry's book
11. Dr Judit Olasz, biologist, NIO Pathogenetics Department – Study of the royal bones' Y-chromosome and autosomal STR markers and mtDNA analyses
12. Dr Erzsébet Csernák, biologist, NIO Tumour Pathology Centre – Sequencing of the royal bones using the next generation sequencing (NGS) method, A-STR, Y-STR and mtDNA analyses
13. Dr Verena Seidenberg and Dr Susanne Hummel, Historical Anthropology and Human Ecology, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, University of Göttingen, Göttingen, Germany – Investigation of the royal bones' Y-chromosome and autosomal A-STR markers

14. Dr Margit Földesi,† historian, habilitated associate professor, Péter Pázmány Catholic University, Gáspár Károli Reformed University – Family tree of the Árpád Dynasty kings with added biographical data; editing the map of Hungary in the age of the Árpáds and their burial locations
15. János Molnár, biologist, MTA Enzyme Research Centre – Evaluation of next generation sequencing data
16. Sándor Komáromi, National Institute of Oncology, and József Nagy-Bozsoki, Jr., director of photography, Duna Television – Photographic and video documentation of the sampling of the ancient bones interred at the Matthias Church
17. Dr Zoltán Szentirmay, doctor, specialist of cytopathology and molecular genetic diagnostics, professor, former director of the NIO Tumour Pathology Centre – Summary analysis of the DNA sequencing results and other data; photographing and editing the images of most skeletons in this book, creating the tables and figures

CHAPTER ONE

ZOLTÁN SZENTIRMAY

OPENING AND RE-SEALING THE SARCOPHAGI AT THE MATTHIAS CHURCH

The sarcophagi at the Matthias Church were opened by Reneszánsz Kft., under the supervision of Ms Csilla Bánhidi, with the approval of Cardinal Péter Erdő.



Figure 1. A: King Béla III and Queen Anne of Antioch depicted on the sarcophagus in the chapel.

B: Opening the sarcophagus by sliding the lid off.



Figure 2. A: Metal caskets of King Béla III and Queen Anne of Antioch.

B: The caskets were opened by József Prim, professional metal restorer.

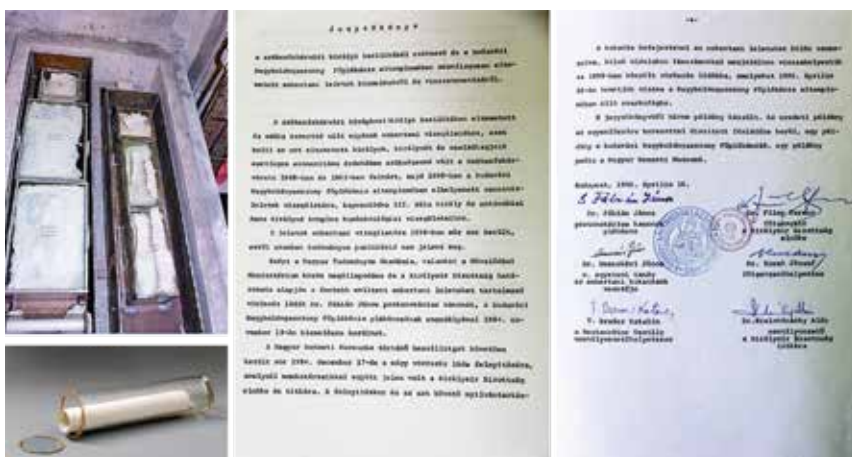


Figure 3. Skeletal remains of Béla III and Anne of Antioch wrapped in canvas in a wooden box after the opening of their copper caskets. The glass cylinder contains the records made on September 29, 1893 and April 16, 1886, describing the interment of the royal couple, as well as a poem entitled “Cipruság” [Cyprus Branch] written by the Order of Cistercians on the interment of Béla III in the Matthias Church. Our records describing the objectives of the genetic analysis of the Hungarian Kings were also placed in the glass cylinder. Depicted are the records from April 16, 1886.



Figure 4. A: The skull of Béla III in a glass container.
B: Opening of the glass container.
C: Removal of the skull from the glass container under sterile conditions.



Figure 5. Blessing the royal couple before re-sealing the sarcophagus.



Figure 6. A: The sarcophagus in the crypt in its original state.

B: The skeletal remains from the crypt in metal containers, before sampling.

C: “Reges Hungariae” inscribed in front of the sarcophagus by the pillars.



Figure 7. Before re-sealing the sarcophagus in the crypt.

SUMMARY: When opening the sarcophagi and removing the skeletal remains, special attention was paid to two things: (1) we operated with the utmost piety; (2) we extracted the skeletons wearing surgical attire, covering our heads and wearing masks and rubber gloves to avoid contamination (from our own DNA). Contamination is a real danger, because fresh epithelial DNA strands are much better preserved than the fractured DNA material from the bones. As a result, later DNA amplification by PCR multiplies contaminant DNA much more effectively than the ancient DNA template strands that are to be examined, leading to skewed results. After this point, Judit Olasz determined the Y-STR and A-STR markers of Miklós Kásler and Zoltán Szentirmay, and compared them with those of the bone samples. There were no matches, and thus no DNA contamination occurred (see Chapter 7).

CHAPTER TWO

MIKLÓS KÁSLER, GYÖRGY SZABADOS
(WITH THE ASSISTANCE OF BERNADETT SELLYEY
AND MARGIT FÖLDI)

HISTORICAL BACKGROUND

1. From the Turul Dynasty to the Dynasty Of Holy Kings

Having ruled for five and a half centuries between the mid-9th century and 1301, this dynasty played a role of great importance in medieval Europe: its historical legacy includes several talented grand princes, kings and a uniquely large number of saints. The dynasty believed they were the descendants of Attila the Hun (434-453). Among the names attributed to this dynasty, “Turul Dynasty” was recorded by master chronicler Simon Kézai (around 1285), referring to the hawk-like creature, which, according to the myth, revealed to the mother of Álmos that he was destined for greatness. His descendants from the 13th century did not refer to themselves as such, however, because after the canonization of King István I and Prince Imre in 1083, and King László I in 1192, they bore the title “The Dynasty Of

Holy Kings”. This dynasty was only called the “House of Árpád” by historians after 1779.

The founder of the dynasty, Grand Prince Álmos, organized the monarchical form of government around 850, when the Hungarians were in the Etelköz, a northern region of the Black Sea. The governmental-political entity which he created should be referred to as the Principality of Hungary considering that several foreign contemporary sources used terms that translate to “Grand Prince” (*megas arkhon* in Greek and *senior magnus* in Latin) to refer to the sovereign. The Principality of Álmos and his descendants was in every way in accordance with the criteria of statehood of his age, since a given territory was governed by an institutional, sovereign authority that could exert its political will (Pohl 2003; Szabados 2011).

Between 862 and 895, the Hungarians systematically conquered the Carpathian basin under the leadership of Álmos and his son, Grand Prince Árpád. The Hungarians, relatives of the steppe peoples and Hunnic-Turkic in culture, quickly and peacefully integrated the population of the Carpathian basin, while launching numerous offensive campaigns against Western and Southeastern European countries out of state interests. The Principality of Hungary represented the model of Eurasian steppe empires from 862 to 1000 in Central Europe (Szabados 2011; Szőke 2014; Szabados 2018).

The fifth descendant of Grand Prince Álmos, István reorganized the Hungarian state in terms of both domestic and foreign policy. On the one hand, he wanted to preserve power in the hands of the dynasty’s Christian line, while on the other hand he wanted his country to be accepted into Christendom. István I, later canonized as Saint István, was the last Hungarian Grand Prince (977-1000) and

the first Hungarian King (1000-1038), having received his crown from the Pope in Rome, earning his power widespread legitimacy abroad. This meant that the country was now an official member of the Western European community of states politically and culturally. At the same time, István also maintained good relations with the Byzantine Empire (Makk 1996; Szabados 2011).

The change in statehood was more than just a formal act: it resulted in deep, systemic changes in society. New titles and new institutions were created. It was István who created the system of counties, a form of territorial, secular public administration. István was the author of the first work concerning Hungarian state theory, the “*Intelmek*” [*Admonitions*] to his son. It is an important factor that when Saint István created the Hungarian Kingdom, he followed Roman traditions of governance, but in his own way. One of the biggest differences was in the hierarchy or lack thereof among the vassals. While western lords were first granted lands, for which they owed service, in the Hungarian Kingdom nobles first performed services and were granted estates based on their merit, which they could be found unworthy of and lose, along with their titles and rank. In Western Europe, the authority of lords over their vassals had priority over loyalty to the state, while in the Hungarian Kingdom, state power prevented feudal relations from forming. So great was the power of the dynastic central authority that the sharing of power between the members of the ruling family (*ducatatus*) could not function continuously, since the secular administrative bodies (the counties) could not become hereditary earldoms, as the heads of the counties could be deposed or transferred to different counties at the king’s discretion (Hóman 1931, Szabados 2011).

István also established a tradition when he had himself crowned in the provostry church of the Virgin Mary (Basilica) in Székesfehérvár, which was constructed under his reign. All of his successors followed this example up until the Turkish occupation of the city in 1543. Székesfehérvár therefore became the coronation capital of the Hungarian Kingdom where 38 Hungarian kings were crowned between 1000 and 1527. István also displayed his conscious royal sovereignty by choosing not to appoint a bishop or archbishop at Székesfehérvár. He was so fond of the Virgin Mary Basilica that Bishop Hartvik remarked “the King considered this remarkably beautiful church to be his own personal chapel, giving it such liberties that no bishop could exercise authority over it” (Kristó 1999). He did not allow the coronation church to become a part of the church hierarchy, giving it a privileged provost status to serve him and his successors.

The first Hungarian King, Saint István, died on August 15, 1038. His legacy was the restructuring of the Hungarian state: he put the Hungarian Kingdom in place of the Hungarian Principality and made it an autonomous and respectable member of the community of Christian monarchies in Europe. He offered up his country to the Virgin Mary, and it is symbolic that he died on the day of the Virgin Mary’s death and assumption into heaven. He had himself buried in the Basilica of Székesfehérvár built under his reign. His personal tragedy was that he was not the first of the house of the Árpád to be buried in the Basilica: in the autumn of 1031, his only son to reach adulthood, Prince Imre, who would later be canonized alongside him, was placed in his grave there.

It took some time for the burials at Székesfehérvár to become a real tradition. In the 11th century, the mortal remains of Hungary’s Kings

were laid to rest at various locations, usually where they had founded (or funded) a church. One has to wonder, however, after 1038, why it took until 1116 for a royal burial to occur at Székesfehérvár?

We know that after István's death, his two maternal nephews succeeded him on the Hungarian throne between 1038 and 1046: Péter Orseolo was buried in 1046 at Pécs and Sámuel Aba was buried in 1044, initially at Feldebrő, then later at Abasár. Each was laid to rest at a church they founded (or funded). As for explaining the cases after 1046, a new factor must be considered. From 1046 to 1301, when the dynasty came to an end, the male line of the Árpáds held the throne. They were all descendants of István's nephew, Vazul (István and Vazul's common grandfather was Taksony, Grand Prince of Hungary). Prince Vazul had been blinded by István himself and had exiled Vazul's sons, Levente, András and Béla. He did this because after Imre's death, having no other sons, he had designated his maternal nephew, Péter as his official successor, which understandably prompted Vazul – the paternal nephew – to supposedly plan an assassination plot against István. In any case, Péter Orseolo governed the kingdom so unsuccessfully that he was driven away twice: his second reign was swept away by a pagan rebellion, which restored the paternal succession of the Árpáds, and Vazul's sons finally returned. Of those sons, András I (1046-1060) and Béla I (1060-1063) became kings, and their sons succeeded them: András' eldest son, Salamon (1063-1074), Béla's two eldest sons Géza (Magnus) I (1074-1077) and (Saint) László I (1077-1095). Out of those listed however, neither planned to be laid to rest near Saint István: András I was buried at Tihany, Béla I at Szekszárd, Géza I at Vác, and Saint László at Nagyvárad (today: Oradea, Romania).

Burial in their own churches may have been motivated by them wanting to distance themselves from István. The controversial nature of their relationship with the first king revealed itself: while it is true that as Christian kings they were his successors (and thus did not allow a pagan restoration of any kind), on a family and personal level they could not forget that they had only suffered losses at István's hands, and indeed András and Béla had to endure their father's mutilation and their own exile. It took some time until the family would remember the first king more fondly. Reconciliation came from a political angle. A first sign of this was that Vazul's grandson, László I, declared István I a saint. László's successor continued this trend of reconciliation on a family level.

King Kálmán the Learned (1095-1116), son of Géza I, belonged to another generation, the first to be buried next to István. It is a mystery why his son, István II (1116-1131) did not follow him, choosing instead to be laid to rest near László at Nagyvárád. This is peculiar, because Kálmán – in order to secure the throne for his only son – had Prince Álmos and his innocent child Béla blinded. His case, however, mirrored István's fate: it was not the ruler who ordered the blindings, but rather the blinded themselves who became the patriarchs of future monarchs.

The dynastic burial at Székesfehérvár took place after a change in the line of succession: The son of Prince Álmos, Béla II the Blind (1131-1141), ruled at the time. In 1137, he had the remains of his father Álmos brought back from the Byzantine Empire where he had died in exile and buried him at Székesfehérvár, at the Virgin Mary Basilica. It is unlikely that his deed was to represent a *post factum* brotherly reconciliation between his uncle and his father, Kálmán and Álmos;

it is more likely that by burying Álmos at Székesfehérvár, he elevated him to the level of Kálmán the Learned, making in fact a sort of self-legitimizing gesture, which he further reinforced by designating the Basilica as his final resting place, where the ill-fated 32-year old blind king was buried not long after, at the end of the winter of 1141.

Béla II had to express the legitimacy of his own rule by every possible means, being the first Hungarian ruler who – though by no fault of his own – had ascended to the throne without being fit for actual governance. Furthermore, it was not entirely clear that he should wear the royal crown. The childless István II designated his maternal brother-in-law, Saul (the son of Kálmán the Learned's daughter, Princess Sophia) to be his successor, but by 1129 he was informed that the blind Prince Béla was hiding in Pécsvárad. István II had Béla brought to his court and arranged for him to marry the Grand Prince of Serbia's daughter, Helena. He did this in order to try to reconcile the Kálmán-line and the Álmos-line. The blind prince's marriage was fertile, and of his six children one was born before his ascension to the throne: Géza, later King Géza II (1141-1162), was born in 1130. László was born in the first half of 1131 (during the changing of kings) and would later become László II (1162-1163). Next in turn was István, who later became the pretender István IV (1163). While Saul would not have been the first ruler who was related to the Árpáds through a maternal line, his claim to the throne was not strong enough against a rival related through a paternal line, and thus, Béla II was crowned in April 1131 at Székesfehérvár. After his ascension to the throne, Queen Helena had the 68 nobles on whose advice Álmos and Béla had been blinded executed and their fortune distributed among the churches (Figure 8).



Figure 8. *Left: Execution of the noblemen responsible for the blinding of Béla II the Blind. Right: Depiction of King Béla II the blind (both illustrations from the Chronicon Pictum [Illuminated Chronicle]).*

Nevertheless, it took over a year to solidify the blind king's reign. Kálmán's supporters still had enough influence to summon Boris to Hungary, against Béla the Blind. Boris was the son of Kálmán the Learned's second wife, but his lineage was disputed, since Kálmán had sent his new wife, Euphemia, back to Kiev precisely because he had caught her in adultery: Boris was born in the court of his maternal grandfather, the Grand Prince of Kiev, Vladimir II Monomakh (1113-1125). It is worth noting that after Saul, Boris was the second capable man who was unable to wrest the throne from Béla. Béla II is an example of dynastic legitimacy in Hungarian political thought: a blind man prevails, thanks to his unquestionable Árpád bloodline over his capable opponents, who either do not belong to the dynasty through a paternal line (Saul) or this could hardly be believed about them (Boris) (Kristó–Makk 1995). This phenomenon plays an important positive role from the standpoint of ancient history when

we look at the results of the genetic examination of Béla III's skeletal remains.

Regardless of this, Béla II's lifestyle and especially his reign required the support of others: during his rule he relied on his wife Helena, her brother Belos, and a royal council composed of nobles loyal to them. Béla the Blind's reign and family life should both be considered successful, but he could not overcome his personal tragedy, his blinding as a child, which resulted in his descent into alcoholism, which clearly contributed to king's death at the age of 32.

It is a strange fact of history that all three of Béla II's sons who later became kings – Géza II, László II and István IV – died around the age of 32. As was the case with their father, a chronicler could write "his body lays at Fehérvár": it seems the blind king started a family tradition of burial in the Virgin Mary Basilica. (We should add to this that Béla the Blind had only one marriage, so the three brothers were from the same mother, Helena, which would make it extremely difficult for archaeogeneticists to identify their persons, if the royal skeletal remains from the mid-12th century were to be found.) The cause of Géza II's death (1162) is unknown. His firstborn son, István III (1162-1172), however, was quickly sidelined due to the Byzantine Empire's support for his uncles. A contemporary English source describes his final times in an interesting account: due to his taking the throne, the king found himself in opposition to the Archbishop of Esztergom, who, on Christmas eve of 1162, issued a curse-like prophecy of the king's imminent death, which came true in January 1163 (we would not be surprised if it was revealed that humans helped guide the hand of divine providence). László II was followed by his younger brother, István IV, but his reign only lasted half a year,

as István III drove him away. István IV lived in the Byzantine Empire until he was poisoned by his own former official while staying at the castle of Zimony (today: Zemun, Serbia) in the spring of 1165. His body lay below the castle for a while and he only received his final honours later: his decomposing remains were transported from the southern borderlands to Székesfehérvár. The reason for István III's death is as murky as István IV's is obvious. By 1171, István III had also come into conflict with Archbishop Lukács, and according to another prophecy by the strict bishop, István III would die within a year: this came to pass in March 1172 and thus the King died in his 25th year in Esztergom. We have conflicting information on István III's final resting place. The last Árpád Dynasty burials of the 12th century in Székesfehérvár are attributed to a married couple. Béla III, the second son of Géza II, lost his first wife, Ágnes of Châtillon, otherwise known as Anne of Antioch, in 1184/85. When Béla III accompanied his seven children on the final journey of their mother, he had already designated his final resting place to be next to Anne, since – as we shall see – he had the tomb built in such a manner in the first place. When Béla III died on April 23, 1196, his final wishes were honoured by his firstborn son and successor, King Imre (1196-1204), who had him placed in the grave on the right side of Anne. As an epilogue to the burial of the Árpáds at Székesfehérvár, it should be noted, that Imre did not follow the example of his predecessors, as he was laid to rest at Eger. The resting place of his son, King László II (1204-1205), who died at age five, is also disputed: the 14th century chroniclers designate Székesfehérvár and Eger. We only know for sure – and this is important in regards to further scientific personal identifications – that from this point forward, not a single Prince or

King was buried at Székesfehérvár from the House of Árpád. The next ruler to be buried in the Virgin Mary Basilica was Charles I (1301-1342), (Figure 9; see Chapter 11, Section 2).



Figure 9. *The Árpád Dynasty's places of burial (compiled by János Jeney, based on Biczó 2016, 21).*

Hungarian Kings

Name	Born	Reign	Time of death	Burial
(Saint) István I	After 980	997–†1038	1038	Székesfehérvár
King Péter Orseolo	1010	1038–1041	1146	Pécs
1044–†1046	1146	Pécs	1144	Abasár
King Sámuel Aba	1010	1041–†1044	1144	Abasár
King András I	1015	1046–†1060	1160	Tihany
King Béla I	After 1015	1060–†1063	1163	Szekszárd
King Salamon	1053	1063–1074	1087?	?
King Géza I	1040	1074–†1077	1077	Vác
King (Saint) László I	Ca. 1040	1077–†1095	1095	Nagyvárad
King Kálmán the Learned	1070	1095–†1116	1116	Székesfehérvár
King István II	1101	1116–†1131	1131	Nagyvárad (Oradea)
King Béla II the Blind	1109	1131–†1141	1141	Székesfehérvár
King Géza II	1130	1141–†1162	1162	Székesfehérvár
King László II	1131	1162–†1163	1163	Székesfehérvár
King István IV	1133	1163, †1165	1165	Székesfehérvár
King István III	1147	1162–†1172	1172	Esztergom or Székesfehérvár
King Béla III	1148	1172–†1196	1196	Székesfehérvár
King Imre	Ca. 1171	1196–†1204	1204	Eger
King András II	After 1171	1205–†1235	1235	Egres
King László III	Ca. 1200	1204–†1205	1205	Székesfehérvár vagy Eger
King Béla IV	1206	1235–†1270	1270	Esztergom
King Kálmán of Galicia	1208	1214–1221	1241	Iváncs
King István V	1239	1270–†1272	1272	Margit-sziget
King László IV (Kun)	1262	1272–†1290	1290	Csanád
King András III	Ca. 1265	1290–†1301	1301	Buda
King Charles Robert	1288	1301–†1342	1342	Székesfehérvár

Table 1. Birth dates of Kings of the Árpád Dynasty, with their birthdate, reign, time of death and place of burial (compiled by Dr György Szabados, based on Kristó–Makk 1995).

Princes of the House of Árpád

Name	Heritage	Born	Died	Burial
Prince Vazul	Son of Mihály, uncle of István I	Before 990	After †1031	?
Prince László Szár	Son of Mihály, uncle of István I	Before 990	?	?
Prince Szent Imre	Saint István's son	1007	†1031	Székesfehérvár
Prince Ottó	Saint István's son	Ca. 1007	Before †1031	?
Prince Levente	Vazul's son	Before 1015	†1046	?
Prince Bonuszló	László Szár's son	After 1015	?	?
Prince Dávid	Son of András I	After 1053	After †1090	After 1090
Prince Lampert	Son of Béla I	Ca. 1050	Ca. †1095	?
Álmos, Prince of Hungary, King of Croatia	Son of Géza I	After 1070	†1127	Székesfehérvár
Prince László	Son of Kálmán the Learned	1101	†1112	?
Prince Álmos	Son of Béla II	Ca. 1133	?	?
Prince Géza	Son of Géza II	Ca. 1150	Before †1210	?
Prince Árpád	Son of Géza II	Ca. 1150	?	?
Prince Salamon	Son of Béla III	After 1172	Ca. †1198?	?
Prince István	Son of Béla III	After 1172	Ca. †1198?	?
Prince András	Son of András II	Ca. 1210	†1234	?
István the Posthumous	Son of András II	1236	†1272	Velence
Prince Béla	Son of Béla IV	Ca. 1243	†1269	Esztergom
Prince András	Son of István V	1268	†1278	?

Table 2. Birth dates of the Princes of the Árpád Dynasty with their birth date, time of death, and place of burial (compiled by Dr György Szabados, based on Kristó–Makk 1995).

The Hungarian monarchy was a formidable European power during the age of the Árpáds. Its kings (not counting the German and Byzantine suzerainties in 1045/46 and 1163, respectively) maintained the sovereignty of Hungary from the Holy Roman Empire and the Byzantine Empire, and even from the Holy See.

From a foreign policy standpoint, the Hungarian Kingdom's ancient period from Saint István to Béla III can be divided into two sections. The first period lasting up until the reign of Saint László already saw a vigorous establishment of ties, but without permanent territorial gains. Saint István maintained good strong connections to both Empires for decades. Relations started deteriorate with the Holy Roman Empire first, because after the end of the Saxon Ottonian Dynasty with the death of Henry II, the Salian Dynasty which took its place represented a new, more expansive political line. The belligerent Hungarian-German relations lasting over a quarter of a century posed a significant challenge to the young Hungarian Kingdom, alternating between conflicts and "cold war" periods. Struggles for the throne and pagan rebellions indicated that Hungary was undergoing a deep crisis. Troubles inside and outside the Kingdom threatened Hungarian statehood, but the fact that the Hungarian state quickly overcame this dual crisis is a testament to its vitality.

By the age of Saint László (1077-1095), the Hungarian Kingdom's positions had been solidified both internally and externally. The canonization of István and Imre in 1083 was a powerful sign of recognition of Hungarian statehood. In 1091, the Hungarian Kingdom embarked an expansive campaign in the North Balkans, reaching its full extent during the 12th century.

In 1091, László took advantage of the internal struggles in Croatia to take over the country and crown his younger nephew, Prince Álmos, as king. László's direct successor, Hungarian King Kálmán the Learned (1095-1116), had himself crowned King of Croatia at Tengerfehérvár (today: Biograd na more, Croatia), thus creating the Hungarian-Croatian personal union which lasted until 1918. The list of titles was extended in 1137, after a change in the Dynasty's lineage, under the reign of Béla II, with the conquest of "Ráma" (Bosnia). During the time of the blind King's firstborn son, Géza II, Hungary became one of the most active actors in Europe. The fact that the Kingdom of Hungary had the strength to wage war on two fronts, against the Kievan Rus' between 1148 and 1152, and the Byzantine Empire between 1149 and 1155 shows that Hungary had taken on the role of a great power. On Russian soil, it supported an allied principality, while in the North Balkans, it vied for supremacy with Byzantine Emperor Manuel I (1143-1180), supporting his rival, Andronikos Komnenos. Warlike and active, much like his rival, Géza II, the Emperor took advantage of the rival claimants László and Prince István, and pitted them against István III, inheritor of his father's throne. The two pretenders however, did not prove effective, so Manuel devised a new method to incorporate Hungary to his sphere of influence.

Manuel I and István III made peace in 1163, by betrothing István's younger brother, Béla, to the Emperor's daughter, Maria. With Béla in his court, Manuel now controlled Croatia, Dalmatia and Syrmia, as they were Béla's paternal inheritance. As we know, Manuel was Saint László's maternal grandson, while Béla's great-great grandfather was King Saint László's older brother, King Géza

I (1074-1077), so both descended from Géza and László's father, King Béla I (1060-1063). In 1165, Béla (known as Alexios in the Byzantine Empire) was officially designated as the next Emperor. In the fall of 1169, however, the Emperor was gifted a male child by his second wife. As a result, Béla was stripped of his princely status and his betrothal to Maria was undone, for which he was compensated with the Empress's half-sister: Ágnes (Anne) of Châtillon, Princess of Antioch, the daughter of crusader knight Raynald of Châtillon, became Béla's wife in the spring of 1170.

István III died on March 4, 1172, at the age of 25. When his brother, Béla, who had a mixed upbringing, returned to Hungary, he was able to start his reign with the proper preparation, but he faced formidable opposition, as the Queen Mother Euphrosyne and Lukács, Archbishop of Esztergom, wanted Prince Géza to be king: Béla, who had been away for a long time, seemed to "alien" and too "Greek" to them. They were wrong: Béla III remained firmly Catholic and acted as an independent Hungarian King. Opportunities in foreign policy were favourable for Hungary's domestic security. After the death of Manuel, internal struggles – such as the Serbian and Bulgarian separatist uprisings – weakened the eastern empire, providing a prime opportunity for Hungarian conquest. This began with the quick recapture of the Adriatic coastal region, Béla's erstwhile "dowry". By 1181, the Hungarian Kingdom's territorial unity had been restored. After this, the King continued to expand at the expense of the Byzantine Empire. His new gains were returned only when his daughter, Margit (Maria) and Byzantine Emperor Isaac II Angelos (1185-1195 and 1203-1204) had married, as a "wedding present" at the end of 1185 (Makk 1989, 1996).

King Béla III enjoyed widespread “international” recognition. Written proof of this is found in several sources. “In the 1118th [1187th] year of our Lord’s incarnation, Pope Urban III wore the papal tiara; a German King, Friedrich, was Emperor in Rome, and Isaac in Constantinople; in France, Philippe, son of Louis, was King; in England Henry II, in Sicily William, in Hungary King Béla, in Palestine Guido; in this year, Saladin, Prince of Egypt and Damascus, having been victorious by the inscrutable, but never unjust will of God, occupied the Kingdom of Jerusalem” (Gombos 1937). Thus, Archdeacon Giraldus de Barri, a chronicler from Wales, court chaplain of English King Henry II (1154-1189), listed Hungary and its King, Béla III, alongside the known world’s elite. During this time, Richard, a prebendary from London, travelled to the Holy Land via Hungary in 1189 and wrote the following of Béla: “This man received many gifts from nature. His stature is tall, his face is noble, and if by nothing other, through the eminence of his regal gaze alone he could be deemed most worthy of the Kingdom. He received the soldiers of Christ with hospitality” (Kristó–Makk 1995).

Béla left a deep impression not only on foreign chroniclers, but on his successors as well. His grandson, King Béla IV (1235-1270) chose him as a role model. In 1237, he declared his programme to “restore our country to the state in which it existed under our beloved King Béla”. Although this endeavour was not successful, his respect for his grandfather was undiminished, referring to him as “King Béla the Great” in 1265 (Kristó– Makk 1995). We ought to view King Béla III (the Great) as a ruler, whose country could – without exaggeration – be called the “Hungarian Empire” and as an outstanding member of an outstanding dynasty.

In 1192, King Béla III celebrated the greatest holiday in the history of both his country and family: Pope Celestine III (1191-1198) canonized King László I. Part of the recognition was directed toward his descendant who requested it. It is no coincidence that László's canonization was initiated by Béla: it was he who merged the cult found in the Árpád family tradition with the Byzantine reverence passed on from Piroska (Saint Irene), who had been cultivating her father's memory, to her son, Manuel, and finally to him. And thus, the Árpád Dynasty came to be known as the "Dynasty of Holy Kings". Based on all of the above, it is quite clear how vast the Árpád Dynasty's dynastic connections were. The Árpáds' goal with their conscious dynasty-building was to build family ties as closely knit as possible, to gain powerful allies. They were successful in their efforts and shortly after taking up Christianity, they had become one of the most influential royal families with far-reaching family connections, securing a powerful position for the Kingdom of Hungary within Europe (Figure 10).

Links of the Árpád Dynasty with Byzantium and Kyivan Rus'

Compiled by György Szabados (based on Kristó-Makk 1995)

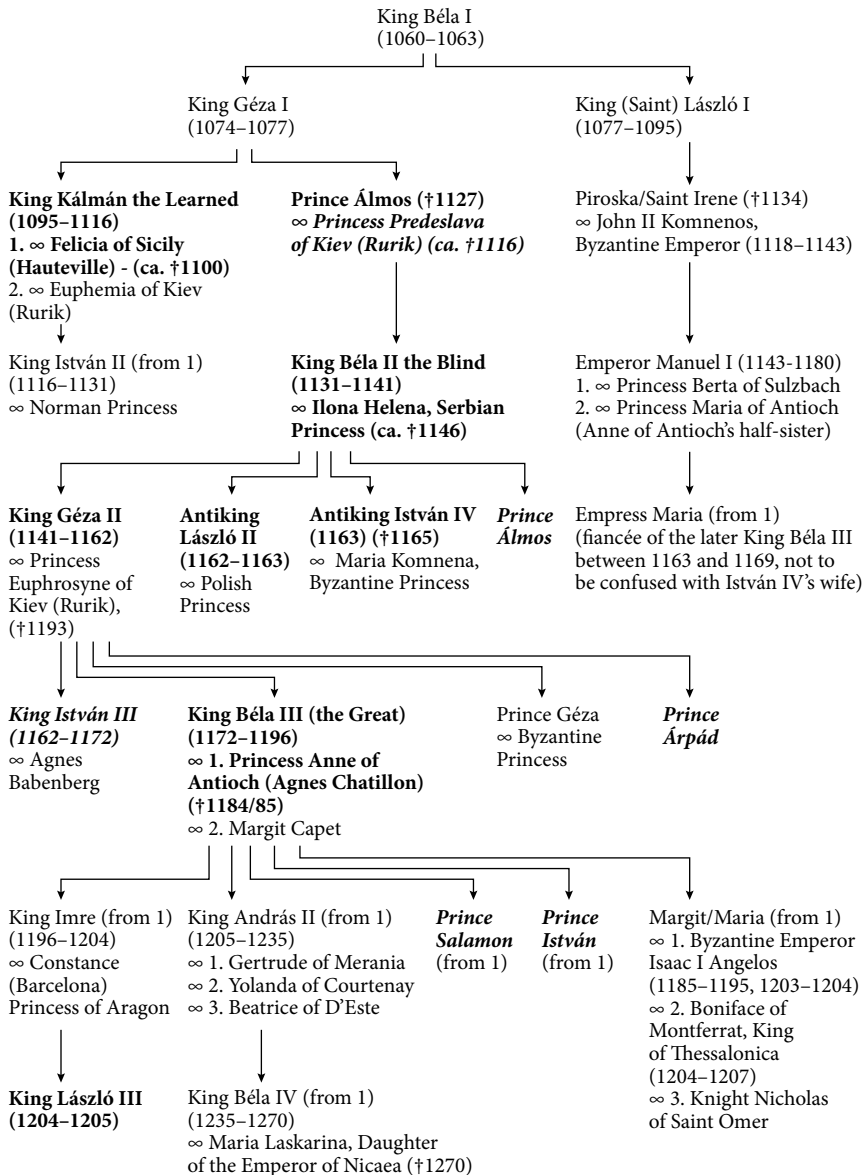


Figure 10. Family tree of the Kings of the Árpád Dynasty (compiled by Dr György Szabados, based on Kristó-Makk 1995).

SUMMARY: The Grand Princes and Kings descended from Álmos through a paternal line ruled for over 450 years, and many talented rulers came from the Árpád Dynasty. The Hungarian Monarchy's power stemmed from the religious reverence of the Dynasty. The birth of Álmos was foretold in divine prophecy. Beginning from Saint István's reign, this ancient sacral foundation served as a basis for "*dei gratia*", the Christian doctrine of ruling by the grace of God. However, this sacrality proved so powerful that it lived on in a new form. It is outstanding how many Christian saints stemmed from this Dynasty. King István I and his son, Prince Imre, King László I, András II's daughter, Erzsébet, and Béla IV's two daughters, Margit and Kinga all became Catholic saints, while King László I's daughter, Byzantine Empress Irene (Piroska), became an Orthodox saint. Erzsébet, the daughter of the Dynasty's last King, András III, was beatified (and in recent times, King István I was recognized as a saint in the Orthodox Church). The canonizations reflect the judgement of religious posterity. The political legacy of the Árpád Dynasty is that the Hungarian people have survived through history and still have their own state in Central Europe.

CHAPTER THREE

PIROSKA BICZÓ, MÁRIA GÖDÉNY,
GYÖRGY SZABADOS, ZOLTÁN SZENTIRMAY

ARCHAEOLOGICAL, ANTHROPOLOGICAL AND RADIOLOGICAL DATA

We have reliable data on the discovery of King Béla III and Queen Anne of Antioch. In 1849, János Pauer, a priest and teacher in the town of Székesfehérvár, and János Érdy, an archaeologist, were among the first to describe the events (Pauer 1849; Érdy 1853). At the János Érdy memorial meeting in 1998, Dr Zsófia Demeter (1999), director of the King Saint István Museum at the time, gave a perfect, detailed summary, using the minutes of the City Council of Székesfehérvár. Some details from these are quoted directly in the following:

“Székesfehérvár is good at drinking water, and in its poor inner city, digging or drilling wells was always a top priority. For this reason, the City Council declared that a new well should be drilled outside the walls of the Bishopric, starting from February 14, 1847. In early September, during the earthworks around the well, the walls of an old structure were found. The earthworks continued around the well in 1848: on May 5, the engineer Kállinger reported that the stones

had been taken out, but ‘in one of the corners of the rectangular hole that had been dug out, a casket could be seen, which could only be excavated after the well’s completion.’ (City Council minutes 1848, 1596; Pauer 1849, 2). Item 4509 from the City Council minutes from December 5, 1848 describes the discovery and opening of the first marble sarcophagus ‘20 and 30 feet away from the artesian well in a southeasterly direction’. The minutes describe the discovery of the ‘fragile bones of a human body’, which ‘according to the doctors’ analysis, was found to be the remains of a 40-year-old woman, next to her were ‘a simple silver crown weighing 15 carats’ and ‘fragments of a gold-threaded silk veil’. According to the resolution passed on this case, the circumstances point towards Árpád Dynasty remains. Notary Ede Eischl was tasked with bringing the items to the National Museum (the voucher number was 4573, the recipient was Ágoston Kubinyi, who took the findings as a gift). Eischl brought a letter to János Luczenbacher (János Érdy), secretary of the Academy, and the Defence Committee on ‘this interesting find’ and the fact that there seemed to be another grave next to the one that had been unearthed. The ‘nationally renowned expert on artifacts’ (János Érdy) was invited to the excavation of the other grave. Meanwhile, at the request of Mayor Hadhalmi, doctors in the city examined the first skeleton on December 5, at 2 pm in the afternoon. Mihály Marbik, Károly Hellensteiner, Ferenc Say, Ferenc Hanekker, János Schealler and József Aschner described a ‘woman of advanced age’, who had been buried ‘six hundred years prior’ (City Council Minutes 1848, 4510). Érdy entered the jewellery brought to Pest into the record of acquisitions on December 6, under Number 61. Starting from December 12, the records document the work of János Érdy and his

companion, engineer János Varsányi, who arrived on December 8. According to these, at 9 am, the second sarcophagus was opened before a 'large audience'; it contained the king's bones and jewellery. (City Council minutes 1848, 4613)."

János Pauer recalls the following about the events: "Back during the summer months, at the right wing of the bishop's residence facing the east, where the enormous basilica built by Saint István once stood, during the repair of the artesian well, while digging, the workers found pillar-fragments and huge stone tablets. After the work around the well was finished and the digging continued, they at last found a marble sarcophagus, which, after it was opened on December 5, 1848, in the morning hours, was found to contain the remains of a queen; on her head there was a crown, on her finger a golden ring. Time had wasted away everything on her, and only the skeleton remained. This event attracted great attention and was reported to the city's leaders; the digging was halted, until such time that the men of science sent by the National Museum appeared. After that, the digging resumed once again, and after the earth and Gothic stone fragments had been removed from the marble sarcophagus, it was opened at 8 am in the morning on December 12. We felt holy fear in our hearts, as the grave was opened before us and we saw the skeleton of the former King of the Hungarian Nation, and among the bones, the royal jewels lying inertly before us, as besides these, whatever time could consume, it had consumed." (Pauer 1849).

Érdy also recalled the discovery of the royal couple's grave in 1853: "a strong man's skeleton was revealed to us, sprinkled over it were the ashes of bygone centuries, the wind touching them for the first time since. 6 foot 2 inch tall, undisturbed silver jewels were splashed with

a reddish grey colour, which later turned darker. This beautiful sight brought a loud mass of people to the normally quiet square. They jumped down into the pit in droves, and the inspector could not order them back anymore. Old ladies prayed before the crumbled ark: while some marvelled and reflected on the dead image of a life once so vibrant, others were guessing or wondering: to which of our kings does this strong skeleton belong?” (Érdy 1853).

The skeleton was unusually tall, possibly about 188-190 centimetres. Its tall stature suggests a Nordic type, since the Árpáds and the Ruriks were blood relatives, as the rulers of the Kievan Rus’ were of Scandinavian origin. Béla III’s great-grandfather Prince Álmos married Predslava from the Rurik dynasty, and their grandchild Géza II (1141-1162) also married a wife from Kiev, with his marriage to Euphrosyne of the Rurik dynasty leading to the birth of Béla III (Szabados 2016).

Gyula László, acclaimed professor of archaeology, pointed out that the facial reconstruction of Béla III’s skull resembles the herm of Saint László stored in Győr, which is not surprising, considering the fact that Béla III was the one who had King László I canonized in 1192, and thus Béla III could have been the only living royal model for the herm (László 1965, Figure 11).



Figure 11. A: *Saint László's herm held in Győr.*
B: *Béla III's facial reconstruction (King Saint István Museum, Székesfehérvár, by Gyula Skultéty).*

An entry in the council meeting minutes from December 14 mentions other graves that were found without grave goods, and the fact that the artifacts collected would be brought to the National Museum at the expense of the Hungarian State (City Council minutes 1848, 4619).

Public display of the royal graves attracted great interest both in the city and in nearby villages. Discovery of the royal graves became national news and inspired the poet János Garay (1812-1853) to compose his work “*Síri hang az élőkhöz*” [*Voice from the grave to the living*]. The 10th verse of the poem is quoted by Demeter (1999). From this poem, we quote the first, second and tenth verses:

Székesfehérvárt a nép ásókkal sürg, forog,
Ástára felbukkannak ős százados sírok –
A sírokból királyok kelnek ki, ős apák,
Kik ottan a halálnak álmát rég aluvák.

Még csak helyét se tudták sok századéven át
A sírnek, mely fedezte királyaink porát;
S most, oh csodás jelenség! önkényt megnyílt a föld,
S mohos sírjából egy-egy Árpád-király kikölt!

...

Nem, nem! Apáid sírja azért nyílt meg neked,
Hogy a holtnak láttára fellobbanjon szíved!
Nem mert talán király volt, de oly kornak fia,
Melyben dicső, szabad volt s független e haza!

(In Székesfehérvár all bustling with spades,
While digging, appear ancient graves –
Kings emerge from graves, ancestors, so old,
There, in deep sleep of death for long.

The grave, hidden in ground for centuries,
Wholly covered the dust of our kings;
And now, amazingly! land opened up,
With Árpád kings from mossy graves coming up.

...

Oh, no! Ancient father's grave opened for you to see
That sight of dead will make your heart leap.
Not for being a king, but a son of an age,
When this land had glorious and independent days.)

The identities of King Béla III and his first wife, Queen Anne, were confirmed both by János Pauer and János Érdy in December 1848. Upon opening the grave, a silver funeral crown with four identical crosses was found on Béla III's head, and next to him in the grave there was a sceptre, a sword, a cross, a breast cross, a ring and a spur. There was a crown with four crosses on the Queen's head as well, along with a ring and some lacy textile fragments (Figure 12).

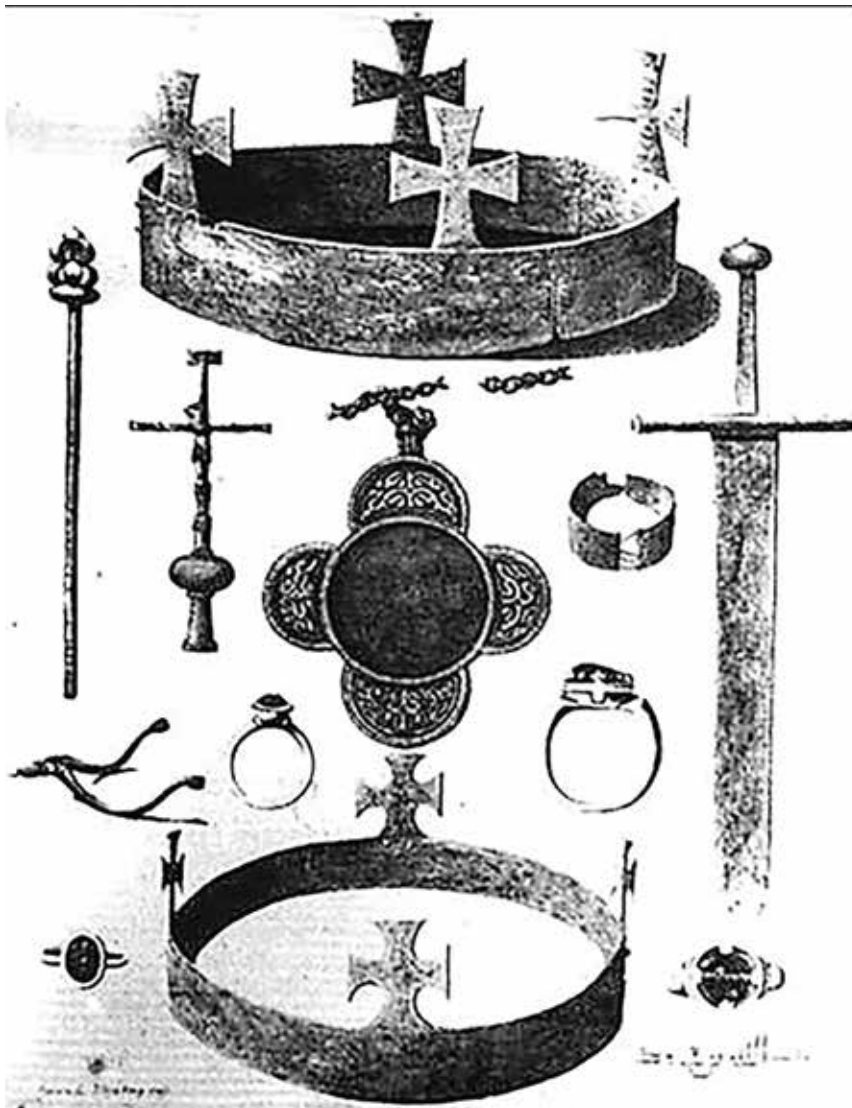


Figure 12. István Gróh, teacher at the Royal Hungarian National School of Applied Arts, created this drawing before the royal couple was interred at the Matthias Church. The drawing contains the objects found in Béla III's grave, as well as Anne of Antioch's funeral crown and ring.

Before the royal couple was interred at the Matthias Church, an anthropological examination took place. The following was written about the remains found in the first grave, that of Queen Anne of Antioch: “judging from the position of the collarbones, thorax, pelvis and thigh bones, it is a female skeleton. And since the cranial sutures are all clearly visible, the teeth are all there in good condition, and are not very worn, the woman’s age may have been between 30 and 40.” As for Béla III, resting in the second grave, his skull’s “sutures, with the exception of the lambda, which had started to agglutinate, can be seen clearly; the teeth are all there, but the enamel had worn off the top of the crowns, pointing to an age of around 50.”

Besides the royal graves, Érdy excavated three additional graves. The third grave was located in the vicinity of the royal grave, to the south, and was about 40 centimetres deeper (earlier burial). Érdy wrote the following about this in the inventory book of the National Museum for the year 1848, under Number 64: (Éry 2008, 17): “Below the ground, we found a tomb made of carved sandstone... its bottom was missing, since it was placed on carved stones anyway, stones which were put on piles or poles that were stuck into the black, marshy soil.” A “skeleton muddled on the left lay” in the grave, which, according to the examination was “a male skeleton, according to the above criteria, over the age of 30” (current skeleton number II/52_3). The grave had already been disturbed and contained no grave goods that would suggest a king, so these may have been taken out during the Árpád era. If it was a royal grave, it may have been one of Hungary’s Kings from the 12th century.

The fourth grave unearthed by Érdy may have been around the same age as that of Béla III: it was situated in the vicinity, contained

the metal remains of a wooden coffin, and the skeletons of a female and a fetus. The female skeleton buried in the coffin was lost, but the fetus is accounted for (its unused number is II/52_4). With the help of a genetic analysis of the fetus, we determined that the person found in the fourth grave was Princess Predslava of Kiev (Chapter 10, Point 3). Directly next to the fourth grave, a fifth grave was found with a wooden coffin and brick walls, but the skeleton it contained was so fragmented that it was not kept. We have certain knowledge that the remains of Princess Predslava's husband, Prince Álmos of the Árpád Dynasty, who died in 1127 in exile in Constantinople were repatriated by Béla II (the Blind) and buried in 1137 in the royal Basilica of Székesfehérvár (Biczó 2016). These facts help us to determine where Prince Álmos of the Árpád Dynasty may have been buried. Before the bones of Álmos were placed in their final resting place, they were neglected for 10 years, which explains their poor condition; furthermore, according to the burial customs of that period, close relatives were buried next to each other. All of these facts combined suggest that the person resting in the fifth grave may have been Prince Álmos of the Árpád Dynasty, who was buried next to Predslava of Kiev (†1116) in the fourth grave. (Up until this point, we do not know of any brick-lined Árpád-era graves in the Royal Basilica). The five graves and the skeletons they contained were drawn at the site by the engineer Varsányi (Figure 13), who was brought to Székesfehérvár by Érdy.

On December 17, 1848, Érdy brought only four skeletons to Budapest: the bone fragments in the fifth grave were found to be in such poor condition that they were not kept. Back in Budapest, a medical committee of unknown members examined the four

skeletons in December 1848. They wrote the following about the find in the fourth grave: “female, aged between 20 and 30 years; the left side of the pelvis indicates that the fetal remains were between 7 and 8 months old. The position she was found in shows that she died while pregnant” (Hungarian National Museum Historical Records Archive). As the woman’s skeleton related to the fetus was lost, the current skeletons do not contain a skeleton that could be linked to the fetus, given the currently available archaeological data (Biczó 2016). As noted, however, the genetic study conducted on the fetus in 2019 does suggest the identity of the mother and father.

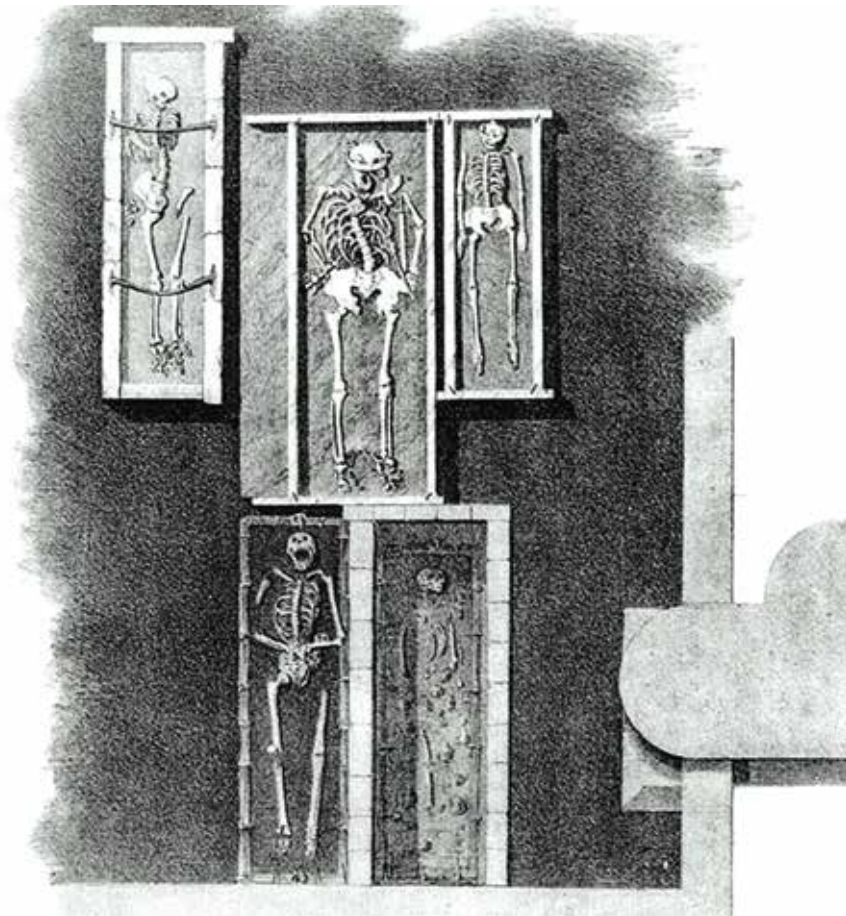


Figure 13. The five graves opened by János Érdy as drawn by János Varsányi. The separate picture is an enlarged drawing of skeleton II/52_3's grave: it is clearly discernible that the grave is made of stone and thus should have been classified as a Category I (royal grave) and not a Category II (earth grave). According to archaeological data, skeleton II/52_3 clearly dates from before Béla III. According to 12th century burial customs, only royal persons were interred in stone graves inside the Basilica, and immediate family members were buried next to each other (Engel 1987).

During a presentation at the János Érdy memorial meeting, Pirooska Biczó addressed the matter of the ground layers of the excavations of 1848 in detail (Biczó 1999). New excavations were started in the area of the grave: “while working, wall plaster crumbled below in the ground layer stood out to him immediately” he writes and then goes on to list the layers that followed each other during the excavation. The half-sentence “while working, wall plaster crumbled below in the ground layer stood out to him immediately” suggests that besides the area uncovered by earthworks, Érdy had begun exploring new layers not yet disturbed. Based on the drawing of the recovered artifacts, which depicts the third pillar’s southern stone base, which survives to this day, the grave sites which have since been destroyed can be accurately reconstructed. Two drawings of the layers explored survive to this day: a colour pen drawing by Varsányi, stored in the Hungarian National Museum Historical Records Archive, and the drawing in Érdy’s (1853) publication inspired by the former. The draft of the sectional view was probably drawn at the site, but it has been lost. Érdy had travelled to Fehérvár, along with the drawing’s author, Varsányi, “who as an engineer and a clever sketch artist, had accompanied me to many archaeological exploits of mine already”. The technical style of the colour pen drawing and the publication’s drawing is identical; however, the colour drawing contains more letters to denote the differences between the layers, but does not elaborate on them, and we do not hear more about them in Érdy’s written legacy either. The drawing that was published is not as rich in data as it could have been, but it does contain all the essentials. The drawing’s significance lies in the fact that in the area of the southern aisle, the succession of layers are described in a way that

later excavations did not (Henszlmann) or could not anymore. Accordingly, every later work on the building and its excavations must use Érdy's drawing and his writings as a basis.

In recognition of his work, at the very least János Érdy deserves to have his portrait shown (Figure 14).



Figure 11 *Portrait of János Érdy (Sunday Newspaper, 1871, 18th year, Issue 21, May 21).*

The remains Érdy found were brought to the Matthias Church in two metal chests in 1862. At first, in 1883, the two chests were brought to the Anthropological Institute of the University of Budapest, and according to the description, in addition to the royal couple, the remains of the fetus and the remains of an additional, incomplete skeleton were found. After this, the bones were placed in a sarcophagus in the crypt of the Matthias Church. Along with those

from the excavations of Érdy in 1848, skeletons from the excavations by Henszlmann between 1862 and 1874 were also put into additional copper caskets in the crypt. This means that the skeletons were from all over the area of the Church. Érdy's and Henszlmann's excavations could not have become mixed up during the processing, since the remains from 1848 were brought to the Matthias Church before Henszlmann's excavations (on July 10, 1862 and September 15 – November 12, 1862). The proceedings that occurred afterwards were summarized by Biczó (2016) as follows: Henszlmann only took and returned to the city the skeletons which he deemed to be important (royal). In 1893, Aurél Török wanted to have the skeletons examined, as he theorized that the female skeleton found in the fourth grave and the fetus may have been Béla III's grandchild. By that time, however, some of the remains had been lost and mixed up. Some of the skeletons from the crypt were preserved despite the losses. The medical examination of 1862 in Székesfehérvár was conducted on three skeletons buried next to each other in the southern aisle. One of the graves contained the skeleton said to be 50 years old by the doctors at Fehérvár (later labelled II/109). In spite of Henszlmann's opinion about the royal nature of the grave, its depth suggests that it was from the late Middle Ages. The round bronze buckle found next to him could be from the Árpád era, but the drawing suggests that the grave goods described by Henszlmann as a little hook and a little ring may have had a French connection (Henszlmann 1864, 197-198). In this scenario, the grave could not be from before the 14th century. The grave goods could neither confirm nor disprove that a royal person had been buried there. The two men found south of the woman's grave were 8-12 inches (21-31 cm) deeper than the previous

woman's grave. According to Henszlmann's dating, one of the men (later labelled II/53) was 26 years old, the other (skeleton labelled II/54) may have been below the age of 30. Whether they were from the Árpád period could not be confirmed nor ruled out. With the lack of any grave goods, nothing could be determined about their rank in society. Our genetic investigations ruled out a relation to the Árpád Dynasty and supplied clues suggesting their identity (see Chapter 10).

In 1874, Henszlmann unearthed four stone-lined graves: both the way they were built and their level suggested they were from the Árpád period. Of the four graves, two seem to have their tops and bottoms aligned with a previous surface level: this may have been the surface level of the church at the time of its great rebuilding before the 12th century, i.e. in the first half of the 12th century (graves E and G= grave I/3). The tops of the other two were 30 centimetres higher: these could have been aligned to the 20-25 cm higher, post-rebuilding floor from the 12th century (graves F and H = grave I/4). Thus, out of the four graves, grave I/3 G is probably from the first half of the 12th century, while grave I/4 H is from the latter half of the 12th century or early 13th century. The findings from grave H were a bronze ring with the inscription "*Agnus Dei*". The use of these rings started in the 13th century. The ring has since been lost, which makes it harder to date it properly. Nothing could be said about the roles played in society by the people buried in the four graves. Bronze rings are common in many layers of society. The person buried in the lost E grave stands out as having been of higher rank, since the reliquary bronze cross stands out as special compared to the usual chest cross. This skeleton was identified in 1874 as female; based

on that, it was probably a secular burial ground and they may have been members of the royal family. We cannot confirm the gender identification, however, since the skeleton has been lost. The northern isle was usually the burial ground of the clergy during the Middle Ages. In the 14th and 15th centuries, two provostship chapels stood on the north side (the chapel built by provosts Miklós Bodó Györgyi and Domonkos Kálmáncsehi). The two persons buried in grave F contradict the theory of a royal burial, but these skeletons have also been lost. The second person's bones formerly buried here were put to the back of the grave. In the Middle Ages, this was a common practice, but it is hardly imaginable in the case of the royal family. If an important person's remains were in the way, they were put in a small chest and reburied elsewhere. Regrettably, besides the remains of Béla III and his wife, not only are we missing the skeletons of four out of our other five 12th century kings, we cannot even confirm their final resting places.

In 1839, on the church's western side, 4+1 graves were excavated. Of these, one was from the Árpád period beyond doubt: based on the gold jewellery, the person may have been a royal or related to the royal family. Henszlmann saw the graves' location and shows them to be on the main aisle's northern half on his map. It is unknown, however, whether he was able to correctly perceive their location: they may well have been at the northern aisle. In that case, the possibility of a royal burial in the northern aisle should be considered. Figure 15 shows the schematics for the Royal Basilica of Székesfehérvár, where the graves excavated by János Érdy, Imre Henszlmann, Alán Kralovánszky and Piroska Biczó, and the grave of Princess Katalin are marked.

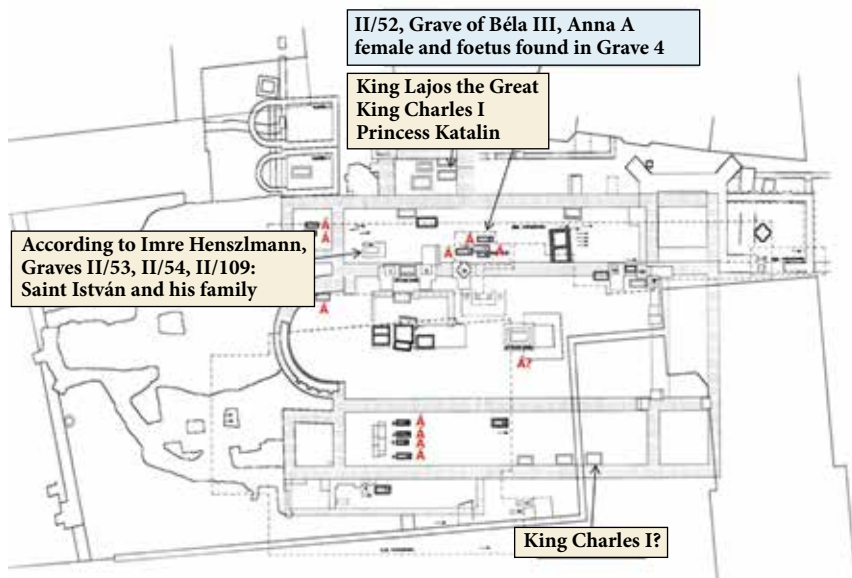


Figure 15. Diagram of the Royal Basilica of Székesfehérvár with the graves displayed on the maps excavated by Érdy (1848) and Henszlmann (1862-1874). Drawing by Zoltán Szabó (2002). The marking of grave sites took place according to the directions of Piroska Biczó based on the excavations of Alán Kralovánszky between 1965 and 1993, and Piroska Biczó between 1994 and 2002 (Á = graves from the Árpád era).

The remains unearthed by Érdy and sent to the National Museum in metal caskets arrived at the Matthias Church in 1862. For the duration of the church's reconstruction, they were taken from there to the Anthropological Institute of the University of Budapest in 1883, where medical anthropologist Aurél Török conducted studies on them and even wrote his thesis on this subject. At this time, he found the male skeleton from the third grave to be between age 20

and 22 (Török 1883). It was also he who first called attention to the similarities between Béla's skull and the herm of Saint László (Török 1894a; 1894b; 1900).

In 1898, the skeletons were returned from the University to the reconstructed Matthias Church. The royal couple was placed in the side chapel in ornate copper caskets, while the skeletons found by Érdy and Henszlmann were placed in the crypt's sarcophagus in a total of four copper caskets decorated with crosses. The first copper casket contained the headless skeleton (later labelled II/52_3) found in the first grave by Érdy, and the skeleton of the 7- to 8-month-old unborn fetus. The second copper container was used to hold the remains of the skeleton (later labelled I/3_G5) exhumed by Imre Henszlmann in 1874 in the crypt marked "G". The skeleton (labelled I/4 H6) found by Henszlmann in the course of his 1874 excavations in the crypt marked "H" was placed in the third copper container. The fourth container's contents included: Skeletons of the 14 persons found during Henszlmann's excavations between 1862 and 1874 and sorted out one by one, identified as either belonging to the earth graves of the Basilica (11 persons, grave type II), or to the area east of the Basilica (grave type VI).

The third examination of the royal bones took place in 1968. Anthropologist Tibor Tóth was asked to perform the anthropological study, while Gyula Regöly-Mérei (1968) was tasked with surveying the remains from a medical standpoint. The extent of the examination included the analysis of age, height, and, through Zoltán Zsebők's radiological records, pathology, while forensic doctor László Harsányi analyzed blood types and reassessed the persons' ages. Their results were in complete alignment: Béla III's age was

determined to be between 48 and 52, standing at 180.67 centimetres tall (mid estimate). They observed a sunken, circular area on the lambdoid suture of Béla III, which we later identified as a figurative trepanation. They believed the deformation may have been caused by skin cancer. Anne of Antioch's anthropological average age was 35 years; her bones showed signs of deficiency and osteoporosis, which they linked to her seven pregnancies. According to x-rays, the possibility of rachitis came up, but this could not be supported by palaeopathological surveys.

The bone samples held in the Matthias Church were examined for the fourth time by the Royal Grave Committee (Éry 1999), after the sarcophagus was opened on November 13, 1984, under the authority of Dr János Fábrián, pronotarian canon. The anthropological remains were transported to the National Museum for further analysis. In their work *“Jegyzőkönyv a székesfehérvári királyi bazilikából származó és a budavári Nagyboldogasszony Főplébánia altemplomában másodlagosan elhelyezett embertani leletek kiemeléséről és visszatemetéséről”* [Protocols of the extraction and reburial of the anthropological remains from the royal basilica of Székesfehérvár later sent to the Main Parish of the Blessed Virgin Mary of Buda Castle] dated April 16, 1986, the Royal Grave Committee notes that the opening of the four copper containers occurred in the presence of the president of the Royal Grave Committee, chief director Dr Ferenc Fülep and his secretary, head of department Dr Alán Kralovánszky (Figure 3). They determined that the first copper container decorated with an isosceles cross contained the skeleton found in 1848 directly adjacent to King Béla III and Queen Anne of Antioch's grave, which was brought to the Matthias Church in 1862 with its jaw and skull missing by that time, as well as

the fetus found in the female skeleton's pelvis, unearthed alongside the former. These two skeletons received the serial numbers 3 and 4, respectively.

The second copper container contained the complete skeleton labelled "G" (number 5) recovered from the northern aisle, the third copper container contained the complete skeleton labelled "H" (number 6) next to the one in grave "G", while the fourth copper container contained a large quantity of skull fragments, and, the skeletal remains of the 14 persons arranged separately. In the current investigations, these were numbered II/53_7, II/109_8, II/54_9, II/55_10, II/56_11, whereas the fractured skulls were numbered VI/1_13, VI/2_15, VI/3_17. The opening of the containers and the various investigations were documented in film, photographs and writing, which are kept in the National Museum. In this case, the anthropological studies were conducted by János Nemeskéri, Antónia Marcsik, Ferenc Szalai and Kinga Éry, while stomatologist Gábor Kocsis carried out the dental investigations. Radiological diagnostics were conducted by radiologist György Luzsa. Other examinations were conducted by the following persons: trace elements by physicists József Bacsó and Imre Uzonyi, RH antigens by serologist Zsófia Santora, and blood types by forensic doctors Árpád Szabó and Zsuzsanna Váczy. Geologist József Konda and chemical engineer Éva Orcsik studied the imprints left on the marble bottom of the graves by the bodies of Béla III and Queen Anne of Antioch. Also on the committee was medical biophysicist Dr József Tigyí. Based on all of the above, the investigation concluded unequivocally and in alignment with Érdy's original determination that the royal couple was indeed Béla III and his wife, Queen Anne of Antioch (Éry 2008; Luzsa et al. 1988; 1989).

In the opinion of historian Dr György Szabados, the above statement is worth noting as the royal couple's identification that was widely accepted in professional circles since Pauer and Érdy has recently been challenged by archaeologist Endre Tóth.

However, the identification as Béla III and Queen Anne of Antioch seems to be supported by the following:

1. The archaeological arguments themselves; thus, the theory of the cross being a "procession cross", and the identification as King Kálmán based on it are not convincing. It is questionable why would it have been necessary to refer to the title of bishop during Kálmán's burial, when that title had already lost its function more than 20 years earlier at that time. The cross was identified as a cross of pilgrimage in older scientific literature, which has a straightforward explanation. Béla III had vowed to launch a crusade, but he could not embark on it, due to his impending death. In his will, he left the duty of pilgrimage to the Holy Land to his second son, King András II (1205-1235), who fulfilled his father's final wishes 21 years later. Therefore, the cross placed in the King's grave may have been a symbol of the unfulfilled promise of a pilgrimage. In fact, the shaft cross was an insignia of Christian rulers (Uzsoki 1984). Moreover, the grave goods being somewhat old-fashioned compared to their time does not necessitate a chronological revision: after all, why should they have put only items manufactured in 1196 next to the King's body in 1196?
2. Palaeopathological studies showed that the condyles of the pubic bones had departed from each other greatly, suggesting that several births had taken place. Regöly-Mérei had found

that Anne of Antioch's earthly remains show signs of a type of osteoporosis that is caused by giving birth many times (Regöly-Mérei 1968). The fact is that Queen Anne had seven children, while Kálmán's first wife Felicia only had three.

3. At the National Institute of Oncology's Centre of Oncological Imaging and Invasive Diagnostics, Dr Mária Gödény generated computed tomographic (CT) images. The *Chronicon Pictum* states that Kálmán had been suffering from severe headaches, and his doctor put a bandage on his ear, through which a "large part of the King's brain leaked out". The chronicler was actually describing the symptoms of pustulous ear infection. This disease always comes with bone erosion, but the person whose male skull was unearthed in 1848 had a perfectly intact skull (Figure 16), so he could not have suffered from the disease that afflicted Kálmán the Learned.

The scientific literature cited above conclusively disproves Tóth's theory and confirms the contemporary scientists. Accordingly, we quote Érdy's conclusion, which still applies today: "Marble sarcophagus I and II therefore, belong to Hungarian King Béla III and his wife, Queen Anne, since the reasons cited could be applied to them alone and to no other Hungarian King and his wife that lived in the 12th century".

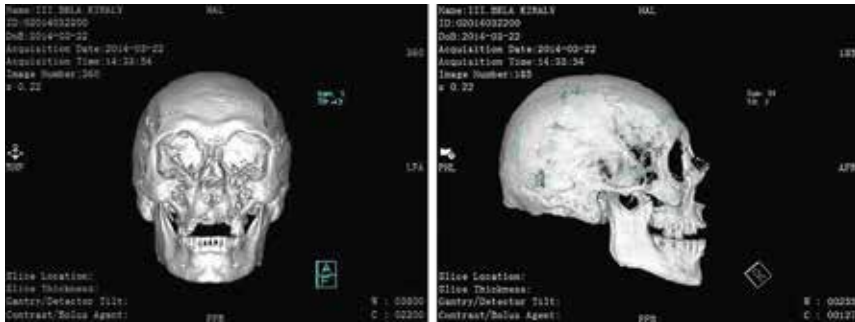


Figure 16. A: *The reconstructed 3D skull of Béla III based on hundreds of layers is a good illustration of the fact that both the cranium cerebrale, cranial base and the facial bones are intact. There are no signs of bone disease in either the sinus or ear region.*
B: *The lateral image also shows that the ear's opening is fully intact.*

Éry's working group of anthropologists performed extremely detailed anthropological investigations on the royal couple and the skeletons of persons I/3 G5, and I/4 H6 (Éry 2008). In the course of this, they calculated the anthropological age at the time of death (displayed in Figure 3) and the body heights as follows: Béla III: 186.61 cm (or 185.83 cm), Anne of Antioch: 161.69 cm (or 161.46 cm), skeleton I/3 G: 162.07 cm, skeleton I/4 H: 178.18 cm. It was considered, merely theoretically, that the skeleton labelled G may have been one of the Árpád Dynasty Kings buried at Székesfehérvár. The names of Kálmán the Learned (†1116), Béla II the Blind (†1141), Géza II (†1162), László II (†1163) and István IV (†1165) were considered, but all of them were ruled out due to a small difference between the known age of these kings and the anthropologically determined age. In Kálmán's case, the ear infection lacks osteological evidence, while Béla II was ruled out from the theoretical possibilities

due to a lack of osteological evidence of having been blinded at a young age (Éry 2008, 89–91).

According to archaeological data, the grave marked “H” dates back to the first half of the 13th century, but no adult king was buried at Székesfehérvár at that time. This skeleton showed clear signs of DISH (Diffuse Idiopathic Skeletal Hyperostosis, Forestier disease), but the condition was not specified. The final diagnosis was given by Józsa (2010) and Józsa and Forgács (2009): both having studied this condition, they note that this disease was extremely common among monks, as it was essentially an occupational hazard, while it occurred very rarely, if at all, among the general population. If we consider that the northern aisle of the royal basilica of Székesfehérvár, where grave “H” was found is – generally speaking – the place where clergymen would be buried in the Middle Ages, it is not hard to come to the conclusion that a high-ranking member of the clergy may have been buried there.

For an easier overview, in Table 3, we display the anthropological age of the skeletons genetically studied as determined by the various working groups at various points in time. Out of these, the anthropological ages of the skeletons labelled II/53_7, II/109_8, II/54_9, II/55_10 are presented only in the monograph by Éry (2008) in Table 15/b. The table shows that the anthropological ages given by the various examiners can differ from each other. One difference stands out especially in the case of skeleton II/52_3. At the time of exhumation, János Érdy determined the anthropological age of the skeleton from grave 3, later labelled II/52_3, to be above 30 (the skull had not been lost yet). Despite this, the age estimated by Aurél Török was between 20 and 22 years (the skull had been lost already). This

Persons	Estimated anthropological age of the skeletons at the time of death (in years)						
	A	B	C	D	E	F	G
Béla III († 48 years old)				50		48-52	45-49
Anne A. († 31 years old)	40			30-40		35	37-41
Grave 3, male, II/52_3	Above 30				20-22		Not identical
Grave 4, female		20-23					lost
Grave 4, fetus		7-8 months					
I/3 G5, male							36-40
I/4 H6, male							37-41
II/53, male			26				21-27
II/54, male			Below 30				32-38
II/55, male							36-42
II/109, female							35-41

Table 3. The table displays the anthropological age of the skeletons originating from the Royal Basilica of Székesfehérvár and kept in the Matthias Church which underwent genetic analyses. Also displayed are the actual ages of Béla III and Anne of Antioch.

A: Age determined by Érdy at the time of exhumation (December 5, 1848).

B: December 1848, dating of a medical committee consisting of unknown members from Budapest.

C: Age given by Henszlmann and the medical committee from Székesfehérvár in 1862.

D: Anthropological dating carried out by a medical committee of a scientific university in Budapest before the royal couple's final interment in the Matthias Church.

E: Aurél Török's (1883) estimated age data.

F: Age calculated by Regöly-Mérei (1968) and László Harsányi.

G: Anthropological dating of Éry's working group (2008).

age data was accepted by Éry's working group and used as proof of their hypothesis that the skeleton had been swapped.

The monograph edited by Éry (which is considered a fundamental work to this day) entitled "A székesfehérvári királyi bazilika embertani leletei 1848–2002" [*The anthropological finds of the Royal Basilica of Székesfehérvár 1848–2002*] contains the anthropology working group's investigations, as well as those of the other disciplines, such as dentistry, radiology, the study of trace elements, studies of the blood type RH0 and the antigen Rh(D), the study of the DNA mycobacterium leprae and mycobacterium tuberculosis, and non-metric analysis of the skull bones. Samples were taken from the humerus bone of skeleton II/52_3 (see Figure 25) and sent to Germany for genetic analysis. This attempt, however, did not prove successful.

Éry's book (2008) contains an unbelievably large amount of anthropological measurement data in a table on page 219. Naturally, we will not attempt to expound the valuable insights of the book, but we do wish to discuss two details.

1) Figurative trepanation of Béla III's skull

Éry and her working group found that on Béla III's cranial vault, at the intersection between the sutura coronalis and sutura sagittalis, on the area of the ossified large fontanelle, there was a 9-mm diameter, 2-mm deep bone defect, which had no trace in the cranium's inner surface, which was deemed to be a surgical intervention, a so-called figurative trepanation (a photo is included in Éry's work). The intervention happened during the King's life, moreover, during his adult life, since the edges were somewhat washed out due to the

wound healing. We also observed and recorded this change in the bone (Figure 17).

Figurative trepanation is observed on Hungarians from the 10th and 11th centuries on skull finds from the Carpathian Basin, as well as on the route of Hungarians as they embarked on The Conquest, for example on skulls found around the Volga river and graves around the Caucasus. This practice is not known among other European peoples. In absence of written records, no one can tell why the practice existed, but it certainly did not have a healing purpose; instead, it may have been part of a shamanic ritual or rite of initiation. Figurative trepanation was only observed on adult men and women, and it was done in such a clever way that the wound never became infected and healed well. The scientific literature documents some two hundred cases of figurative trepanation. With the spread of Christianity, the practice disappeared, which is why it is strange that clear signs of it were found on King Béla III's skull, long after the conversion to Christianity.

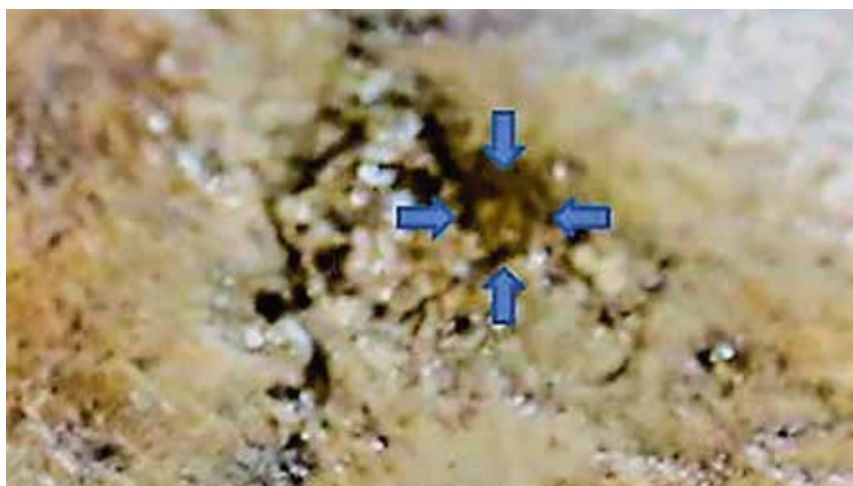


Figure 17. *Circular trace of figurative trepanation on Béla III's skull.*

2) Exclusion from Éry's investigation of the skeleton labelled II/52_3 from the third grave excavated by Érdy

In light of the later events, it is worth noting that the skeleton found by Érdy in the third grave, which was later interred in the Matthias Church and found again without its head in 1883, was also studied by Éry and her working group. The remains from the third grave were probably given the designation II/52_3 at this time, which remained the same later as well, while the fetus found in the fourth grave was labelled II/52_4, but we did not use this subsequently. The investigation that took place involved a comparison of the number and type of bones that can be seen on Varsányi's drawing of the bones found in 1848 with the skeleton we have; extra bones and differences in type were found. In addition, the skeleton's anthropological age turned out to be lower than the 30 years given when it was found, and thus the working group leaned toward the dating of Aurél Török (20-22 years). The skeleton had a yellowish colour, but they believed it should have been brownish, because it had been lying in brownish-black mud. For this reason, the working group's members believed that the skeleton that was found in 1883 could not be the same as that of the man found in the third grave and labelled II/52_3, so they did not include the skeleton in the aforementioned monograph (in Table 15^a the fetus is labelled as II/52) (Éry 2008, 19). However, Éry's working group did not consider the following:

- The bones found by Érdy in 1848 were placed in the Matthias Church much earlier and could not have been mixed up.
- After finding the skeleton and removing it from the grave, the mud was washed off, and thus the brownish black colour was

no longer visible. Dr Judit Olasz, however, found dissolved mud residue in the reaction buffer while isolating the DNA from the skeleton's bone sample.

- They did not consider that the contemporary drawing of the grave (Figure 13) may have been inaccurate, resulting in the number of bones in the drawing differing from those of the actual skeleton under investigation.
- In Chapter 7, we compare the A-STR marker pattern of the various bones of skeleton II/52_3 with each other and King Béla III's corresponding marker pattern. The analysis demonstrated that all of the bones belonged to skeleton II/52, and we also found that all of the A-STR markers contain a (maternal) allele that is identical to King Béla III's corresponding marker pattern. This information is genetic proof that the skeleton of the man found in 1883 was not swapped.

The conclusion from all of this is that such firm opinions, like the one held by Éry's working group, i.e. that the skeleton had been swapped, should be based on much more extensive evidence.

After the investigations, Éry's working group wrapped up the skeletons individually, marked their outer layer with metal threads, and put them back in the copper containers made in 1898, which were in turn reinterred on April 18 in the sarcophagus in the crypt of the Matthias Church.

Dr László Józsa (2014) wrote a review of the monograph edited by Éry entitled "*Vajúdtak a hegyek és egeret szültek*" [*The mountains are in labor, a ridiculous mouse will be born.*] which has not been published to this day. His work contains several critical statements, but according to the author the main issues were:

- No attempt was made to explain or harmonize the results obtained by the anthropologists and their colleagues from other fields, in order to arrive at unified conclusions relating to the public health situation of that day and age.
- The analyses of the inflammatory processes contain the most errors. The authors only mention three kinds of inflammation (syphilis, leprosy, tuberculosis), when in reality, there are many more inflammatory processes, most of which palaeopathologists can easily spot even with macroscopic observation. They did not notice, however, otitis media or mastoiditis in a single case, even though according to both foreign and domestic experience during that era, half of the population showed signs of the disease. In some cases, these processes can only be recognized by the deformity of the hearing canal, with a small enlargement.
- The tumour-like bone deformities were diagnosed on a visual basis alone, and their size was given inaccurately. Professional pathologists whose knowledge and equipment could have provided a much broader scope of processing and more accurate diagnosis were not involved.

With all of this in mind, Dr Józsa asks on the reader's behalf: Who was this gigantic work made for? Was it for historians and archaeologists? They are unable to use the wealth of anthropological data. Or was it for doctors and medical students studying ancient times? Hardly, since they cannot use the severely inaccurate diagnoses of pathological lesions. We have no explanations, conclusions or summary of the anthropological, or at least the biological data. In the end, the question remains: who is this indubitably gigantic work supposed to benefit?

The fifth (and at the time of this book's writing latest) opening of the sarcophagus of Béla III and Anne of Antioch in the Matthias Church and subsequent investigations took place on March 17, 2014. When it was sealed, the log placed alongside the rest of the documents in the glass cylinder contained the following quote: "Since the detailed anthropological analysis of the royal couple's earthly remains between November 1984 and April 1986, advances in genetics and other scientific fields allow for further study of the only Árpád Dynasty burial site preserved in an undisturbed state, and for the individual identification and genetic mapping of the anthropological remains from the royal graves in the Basilica of Székesfehérvár, which survived the turbulent tides of history. On this basis, the bones can be placed with appropriate piety in a national place of remembrance" (see Figure 3).

The project was initiated, organized and led by Prof Dr Miklós Kásler, director-in-chief of the National Institute of Oncology at the time. At the opening of the sarcophagi, the following persons were present and assisted: Dr Zoltán Szentirmay (National Institute of Oncology), Dr Béla Melegh (Scientific University of Pécs, Department of Medical Genetics), Dr Elek Benkő and Dr Balázs Mende (MTA Liberal Arts Research Centre, Department of Archaeology), Dr Piroska Biczó (National Museum of Hungary), Piroska Rácz (King Saint István Museum, Székesfehérvár) and head nurse Dr Éva Zoltánné Csorba (National Institute of Oncology). The minutes were signed on April 1, 2017 by Dr László Süllel prebendary, parson (Matthias Church) and Prof Dr Miklós Kásler director-in-chief (National Institute of Oncology).

In conclusion, we can state that in the course of the current investigations which we initiated, we planned to genetically identify

as many skeletons stored in the Matthias Church as possible with the use of Béla III and Queen Anne of Antioch's genetic marker patterns. Our goals included the identification of the Árpád lineage as well. We did not wish to study the relics either from scientific or pious reasons. Instead, we wanted to bring the historical, archaeological and anthropological data we had collected, along with radiocarbon dating and morphological analysis of the bone structure in accordance with the genetic data, so that jointly they could help us with the planned identification of the unknown skeletons.

SUMMARY: The graves found within the walls of the Royal Basilica of Székesfehérvár and previously believed to have been from the Árpád age were hard to locate after the fact, and identifying the skeletons found within also proved to be difficult. For example, the skeletons extracted from the graves underwent six different anthropological studies before arriving at their final resting places, the two sarcophagi of the Matthias Church. Also problematic was the fact that the subsequent anthropological studies were not contrasted with the earlier data. Breaking from traditional practice, we compiled the various ages of each skeleton that were given at various points in time in a table, so we could thus check the accuracy of the anthropological dating. We encountered two cases where the dating showed a higher-than-average dispersion; we explained this with the severe post-mortem damage the bones had sustained. The skeletons of Béla III and Queen Anne of Antioch were recovered from the only untouched grave, and their identities were accepted by science to this day. In the recent past however, archaeologist Endre Tóth

challenged the professional consensus on the royal couple, as he deemed the grave goods to be too archaic for Béla III's time. One of the items, for example, he believed to have been a processional cross and emphasized that it is not the insignia of a king, but one used by bishops. Medical science supports the identification as Béla III and Queen Anne. After the birth of Queen Anne's seven children, the condyles of the pubic bones departed significantly from each other. Régöly-Mérei (1968) also noted that the woman's earthly remains show signs of a type of osteoporosis that is caused by giving birth many times, while Kálmán the Learned's first wife, Felicia only had three children. We know that there was a purulent inflammation in Kálmán the Learned's right ear and nearby ethmoids and that a massive amount of pus flowed out from his ear. Such diseases always cause serious damage to nearby bones, and this can be verified with imaging diagnostics. Dr Mária Gödény created detailed computed tomographic (CT) images of Béla III's skull. The skull reconstructed in 3D based on several hundred high resolution layers clearly shows that the skull, the viscerocranium and the neurocranium are all intact. Based on this knowledge, Endre Tóth's proposal was rejected. Éry's colleagues conducted detailed investigations on the skeletons interred in the Matthias Church and presented their results in the monograph entitled "*A székesfehérvári királyi bazilika embertani leletei 1848–2002*". Of the myriad of valuable results of their working group, we would only like to highlight two: Béla III's figurative skull trepanation, the purpose of which is unknown, and the supposed swapping of the skeleton found by Érdy in grave 3 in 1848. Figurative trepanations were only observed in

adults. With the spread of Christianity, the custom disappeared, which makes it all the more peculiar, and inexplicable to this day, that King Béla III's skull without a doubt showed figurative trepanation long after the conversion to Christianity took place. Éry and her working group believed that the skeleton from the third grave labelled II/52_3 held to this day in the crypt of the Matthias Church was of unknown origin and not authentic, and had been swapped with another, so they did not include it in their monograph. When determining the origin to be unknown, they relied too much on anthropological dating, but that is not reliable in case of heavily damaged bone structure, and as we see, their conclusion proved to be incorrect.

CHAPTER FOUR

LÁSZLÓ JÓZSA, ZOLTÁN SZENTIRMAY

PALAEOPATHOLOGICAL INVESTIGATIONS

Previous findings:

Regöly-Mérei Gy (1968): *Béla király és Antiochiai Anna csontvázának paleopatológiai vizsgálata [Palaeopathological investigation of King Béla III and Anne of Antioch's skeletons]*, Orvosi Hetilap, 109, 423–427.

Luzsa Gy, Gáspárdy G, Nemeskéri J, Éry K (1988): *Paleoradiológiai tanulmány a székesfehérvári királyi bazilika 15 csontvázmaradványáról [Palaeoradiological study of the 15 skeletal remains from the Royal Basilica of Székesfehérvár]*, Magyar Radiológia, 62, 39-50.

Luzsa Gy: *Radiológiai vizsgálat III. Béla király és Antiochiai Anna királyné csontváz leletein [Radiological study of King Béla III and Queen Anne of Antioch's skeletal remains]*.

Éry K (ed.) (2008): *A székesfehérvári királyi bazilika embertani leletei 1848–2002 [The anthropological finds of the Royal Basilica of Székesfehérvár 1848–2002]*, Balassi Kiadó, Budapest (pp. 148-159).

Prof Dr László Józsa, pathologist-palaeopathologist, conducted a detailed pathological analysis of the four skeletal finds.

King Béla III's skeleton:

In vertebrae IX (11×6 mm diameter), XII (17×9 mm size), and L I. (14×9 mm extent), in the upper layer, a roughly central Schmolli hernia.

Spina bifida sacralis (open spine) on segments IV-V.

Spondylosis of the thoracic and lumbar vertebrae. The small joints are intact. The manubrium sterni and the corpus sterni are separate.

Arthrosis in both sternoclavicular joints, which is more pronounced on the right side.

Bone crest formation at the medial head of the musculus gastrocnemius on both tibiae, 11 centimetres long on the right side and 10.5 cm long on the left side. A bone crest formed on the surface of both tibia fibularis.

The two patellae differ in size. The right kneecap length is 44x40x22 mm, the left one is 49x45x24 mm. Bone crest on the edge of the left patella, millet-sized arthrosis on the knee joint surface.

Bone deposition on the frontal edge of the upper left hock joint. The condyles of the astragalus are intact.

The two femoral necks are uneven, the right femoral neck is 28 mm long, its angle is 123 degrees. The left cervix is 25 mm long, its angle is 121 degrees.

Enthesopathia formation by the Achilles tendon's adhesion on both calcanei, more pronounced on the left side. Both tuberositas tibiae are forward leaning, their surface uneven (healed

Osgood-Schlatter disease?). The tibiak layer find is not mentioned by Luzsa, and the anteroposterior direction of the image published does not indicate whether it is really healed Osgood-Schlatter disease that caused the ruberositas tibiae's forward lean? The lower jaw had been fixed, so I could not take any tartar from the incisors. Otherwise, there seemed to be only a minimal amount of tartar on the teeth.

Anne of Antioch's skeleton:

Both patellae contain enthesopathic bone deposition at the base of the ligamentum patellae.

The right femur's collodiaphyseal angle is 130 degrees, the left one could not be measured. The right femoral neck is 19 millimetres long, in a steep position (coxavalga).

The shin bones differ in size. The right tibia and fibula are 32 centimetres long, while the left tibia and fibula are both 36 centimetres long, and the latter is curved.

Ribs: 10 on the right, 11 on the left, both scapula, clavícula hummerus, radius, ulna are free of macroscopic abnormal deviations. There was no tartar on the teeth.

Skeleton I/3 G5:

Arthrosis in the left temporomandibular joint. The upper left 5th, the upper right 1st and 4th teeth, as well as the lower left 3rd and lower right 6th and 8th teeth are post-mortally missing. No sign of tartar build-up on the teeth.

The laryngeal cartilage is ossified, but in regular shape, intact.

Arthrosis in both sternoclavicular joints.

A 1-2 millimetre bone deposit (spondylosis) on the edge of the thoracic and lumbar vertebrae's body. The small joints are intact. Spina bifida sacralis on the surface of segment V.

Bilateral coxarthrosis, medium severity. The femoral neck is in a steep position, the left angle is 140 degrees, the right angle is 145 degrees. Enthesopathic bone deposition at the adhesion of the Achilles tendon on both calcaneus.

Skeleton I/4 H6:

Sutura metopica on the frontals.

The upper left 7th and 4th, as well as the upper right 1st, the lower left 8th and the lower right 6th and 8th teeth are post-mortally missing. The teeth are intact, no reasonable amount of scrapings can be collected due to the tartar build-up being insignificant. The clivus (base of the skull) is flat, only 40 degrees (the normal value is around 60 degrees). Severe arthrosis on both condylus occipitalis, as well as on the jointifying atlas vertebra's joint surface, the epistropheus processus dentatus is deformed (arthrosis). Sacroileitis osteochondritis ossis pubis. Spina bifida sacralis in segment V. The right femoral neck degree is 120, the left is 125. Pronounced spondylosis on vertebrae C I-V. The ligamentum longitudinale anterius ossified on the area of vertebrae X-LT, the width of the ossification (spread) increases toward the distal. The vertebral bodies are attached to each other, but the small joints in between the vertebrae are free, the discs in between the vertebrae are not calcified (Forestier's disease, Figure 18).



Figure 18. Forestier's disease in skeleton I/4 H6 (*Diffuse Idiopathic Skeletal Hyperstosis, DISH*)

Józsa (2010), as well as Józsa and Forgács (2009) published an excellent summary on the pathomorphology and history of Forestier's disease. In the following, we provide direct quotes from these studies, which we edited at our own discretion.

“Forestier and Rotes-Querol described the disease later named after them as ‘ankylosing hyperostosis’ in 1950. They separated its clinical and x-ray-morphological image from other diseases of the spine that involved fusion of the bones. In the early stage, the following were observed: focal ossification of the ligamentum longitudinale anterius, degeneration of the annulus fibrosus, L-, T-, or Y-shaped terolateral extension of the annulus’ peripheral

fibre. Forestier et al. (1983) found that ossification of the frontal long ligament is formed from several nodules; the heterotopic bone islands begin to form along the middle part of the vertebral body, they spread in distal and proximal directions, but they have no connection to the vertebral bodies' cortex during the early stages. Not only our own observations (Józsa 2010), but every microscopic analysis suggests that calcification could be ruled out, which, in pathological terms means amorphous calcification, and it forms around foreign bodies or necrotic nodules, in rare cases, without a known cause (e.g. lime gout).

Forestier disease is one of the most ancient skeletal degenerations. It first appeared during the age of the dinosaurs, and in the following 150 million years, many species (both extinct and contemporary) were shown to exhibit it. It is still not uncommon among mammals living in natural conditions, or older pets... the most surprising finding is that it can develop in recent fishes at sea and in freshwater. In monkeys from the old world (gorilla, macaque, baboon, rhesus monkey, etc.) its incidence rate is between 4 and 21 percent. It does not appear any more often in captive primates than in wild ones.”

Among the hominoids (human like), the Proconsul skeleton (an ancestor of modern gorillas) exhibited the disease 10-12 million years ago. However, on the remains of Australopithecus (precursor to humans, 3 – 1 million years prior today), Homo habilis (archaic human, 2.5 – 1 million years ago), and Homo erectus (upright ancient man, lived 1.5 million – 300,000 years ago) no signs of Forestier's disease were found (although it should be noted that we only know about 200-300 incomplete skeletons of these three hominid (human) species).

It has often been observed on the spine of ancient Neanderthals (which lived 300,000 – 20,000 years before our time). Waldron (1985) published a report in the British Medical Journal on investigating the remains of monks from the Augustinian priory at Merton (operated between 1140-1540). In the publication's title, he referred to Forestier's disease as being so common among monks that he would consider it a "new occupational disease". Janssen et al. (1999) compared the materials of a cloister graveyard and a civil cemetery. All of the skeletons in the graveyard that died between the age of 43 and 75 showed signs of DISH (Diffuse Idiopathic Skeletal Hyperostosis, Forestier's disease), but the graveyard which contained skeletons of peasants and merchants not a single find with Forestier's disease was made.

The knowledge we have gained since the publication of the quotes above point to the role of *genetic factors* (such as the cumulative incidence within the Medici family), as well as *environmental dietary factors* (Fornaciari–Giuffra 2013). Our knowledge of Forestier's disease, however, is still limited. Some pathogenetic knowledge we took from analogous entities, such as the ligamentum longitudinale posterior (OPLL) (Mader et al. 2017). According to the basic concept the growth factors, such as insulin, insulin-like growth factor 1, transforming growth factor- β 1, platelet-derived growth factor-BB, prostaglandin E1/E2 and the overproduction of endothelin-1 are the main causes of the disease, which could cause mesenchymal cells to transform into fibroblasts and osteoblasts. On the other hand, we could perhaps take into account the inhibition of bone-promoting peptides such as the matrix Gla protein, bone morphogenetic protein-2 or Dickkopf-1. Most recently, while conducting investigations

pertaining to OPLL, Nakajima et al. (2016) found that the spondin 2 (RSPO2) gene expression's pronounced decrease could play a role. The RSPO2 gene (a member of the RSPO gene family) regulates the gene expression of β -catenin. Patients with colon cancer were found to have RSPO2 (and RSPO3) fusion transcripts, which occur with the gene mutation of APC to the exclusion of each other, however, they probably activate the Wnt signal and could promote colon cancer (OMIM 8/16/2016).

Nakajima et al. (2016) studied a hereditary DNA sequence variation marked rs374810 identified during studies, which can be found in the RSPO2 gene's supposedly real promoter region, 116 basepairs (bp) before the RSPO2 gene's transcription starting point. The variation of the rs374810 SNP -116T>C allele connects differently to nuclear proteins, and in an experimental environment, the "C" allele has a significantly lower promoter activity than the "T" allele in the HSCO2/8 chondrocyta cell line. This observation proves that the genotype of rs374810 SNP CT and CC can be considered a risk allele variation, which leads to significantly lower RSPO2 gene expression than the TT genotype in vivo. All of this leads us to the conclusion that the RSPO2 gene, when in the presence of the risk allele of the rs374810 SNP predisposes to OPLL, albeit to a small degree. With regards to the fact that the pathomechanisms of OPLL and Forestier's disease are probably identical (one being ossification of the ligamentum longitudinale next to the vertebrae on the back side, while the other is the same on the front side), we could presume that the RSPO2's significantly decreased transcription activity plays a role in the emergence of Forestier's disease along with dietary factors.

SUMMARY: The palaeopathological survey conducted by Dr Józsa uncovered several new aspects. Firstly, he described anomalies in bone development, for example, sacralis spina bifida (open spine) that could often be observed, which in our case was present on the skeleton of Béla III and skeletons I/3 G5 and I/4 H6. On skeleton I/4 H6, Forestier's disease was diagnosed for the first time, which is related to hereditary predisposition via a sequence variation and to dietary factors that cause the disease. Describing Forestier's disease pointed toward the conclusion that skeleton I/4 H6 could be a very high-ranking member of the clergy, confirmed by the burial circumstances (stone-lined grave, first half of the 13th century). As for relation to the Árpád Dynasty, however, the genetic analyses presented below ruled this out. Several types of degenerative bone diseases were precisely described on the skeletons studied by Dr Józsa; by our contemporary standards, these diseases seem unusual for the age of the skeletons at the time of death, and thus we can compare them with the occurrence of similar diseases in modern times.

CHAPTER FIVE

ELEK BENKŐ, BALÁZS MENDE

RADIOCARBON DATING

Radiocarbon dating was performed at the initiative of Dr Elek Benkő by the Radiocarbon Institute of Glasgow, where such dating procedures had previously been conducted for Professor Benkő on several occasions (Table 4).

Persons	Bone sample	Calibrated year (68.2%)	Calibrated year (95.4%)
Béla III	Bone dust, femur	993–1012	977–1025
Anne of Antioch	Phalange II	901–1012	895–1019
II/52_3	rib	1040–1153	1035–1155
II/53_7		Not investigated	Not investigated
II/54_9	rib	1400–1435	1320–1443
II/55_10	rib	1429–1457	1415–1487
II/56_11	rib	976–1020	889–1028
II/109_8	rib	1429–1453	1415–1477
I/3 G5	rib	133–218	84–245
I/4 H6	vertebra	1191–1257	1162–1265

Table 4. Radiocarbon dating

The results for three samples are clearly flawed. Béla III and Anne of Antioch's skeletons were covered with some sort of resin when they were placed in the Matthias Church, which made the dating older (and also caused difficulties with DNA isolation). Skeleton I/3 G5 is clearly from the Árpád era according to archaeological dating, and thus its age could not be in alignment with the interval in the table.

CHAPTER SIX

LÁSZLÓ MÓDIS, TÜNDE TERDIK
(WITH CONTRIBUTION BY ZOLTÁN MÉSZÁR)

MORPHOLOGICAL ANALYSIS OF THE BONE STRUCTURES

The investigation was conducted by Prof Dr László Módis, anatomist, who has many years of experience in conducting ultrastructural investigations of bone- and cartilage structures at the University Of Debrecen's Medical Centre, Institute of Anatomical and Developmental Studies. The purpose of the investigation was to learn about the structure of bones outlined below that are at least six hundred years old, to what degree their structure remained intact, and how that relates to the quality of the DNA isolated. The microscopic images were created at the Institute of Anatomical and Developmental Studies.

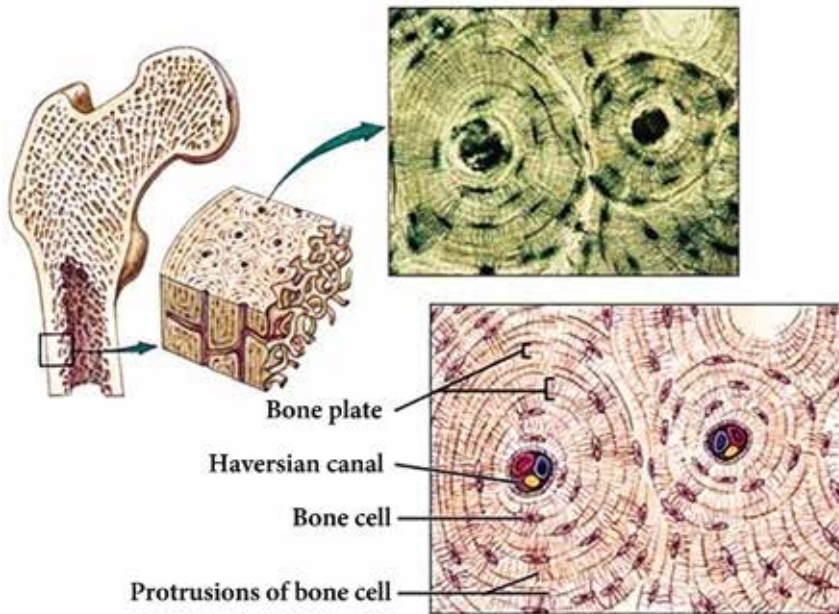


Figure 19. Bone structure of the femur. The bones consist of osteons, which themselves consist of bone plates organized into concentric circles around the Haversian canals, among them are a large number of bone cells, the lamellae of which produce type I collagen fibres, which are horizontal and perpendicular. Inside the Haversian canal run arteries, veins and nerve fibres (Váradi et al. 2015, images downloaded from the internet).

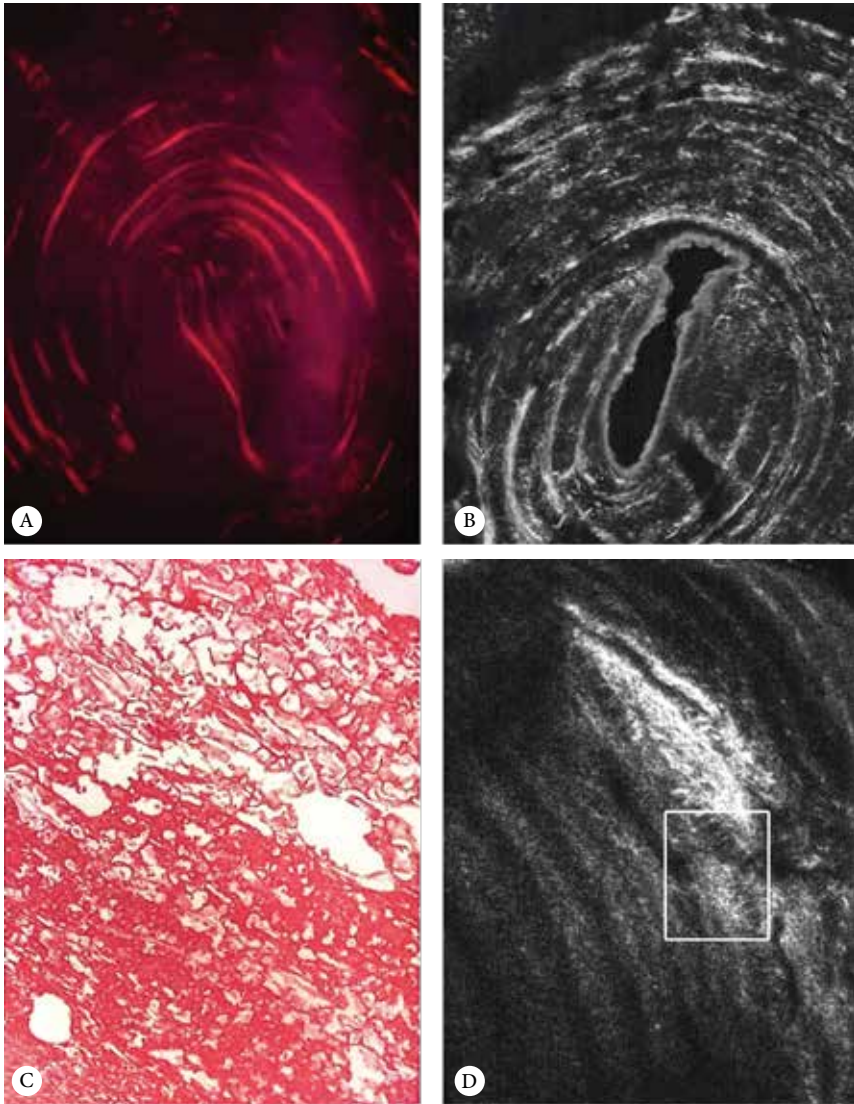


Figure 20. A: Bone structure of the cortex of Béla III's femur as shown on segments created after decalcination. Picrosirius staining, 20x zoom. The staining molecules attaching to the collagen fibres greatly enhance their own birefringence, and the phenomenon can be seen very clearly in the dark field of view created by the crossing polar

filters in the polarizing microscope. The outline of osteons appears through the birefringence of the collagen fibres running circularly around the Haversian canals.

B: Here we can see the two-photon absorption fluorescence microscopic image of the same bone tissue. The excitation laser's energy reaches the necessary light intensity required for the two-photon excitement attached to the collagen fibre only in the $\sim 0.3 \mu\text{m}$ diameter, 0.1 femtoliter volume focal point, which excites the fluorescent indicator connected to the collagen fibre, and through glowing light, reveals the same structure, which is shown by the birefringence in the polarization microscope. The perpendicular collagen fibre arrangement cannot be detected.

C: The cortex of skeleton II/52_3's femur. After picosirius staining following decalcination, heavily damaged bone structure can be seen. The osteon structure is broken up, the Haversian canals cannot be seen. 40x zoom.

D: The two-photon microscopic image is of the cancellated area by the intramedullary part of the femur's corticalis region. Instead of the broken up parts of the osteon structure, only collagen fibre clusters can be seen stuck together. The framed area shows the femur's denser corticalial area, but this area also lacks preserved bone structure, only a very small number of collagen fibres can be seen stuck together.

In 1967, the skeletons of the royal couple were studied by Gyula Regöly-Mérei, pathologist-palaeopathologist, from Budapest. At that time, a cortical histological section was made based on Anne of Antioch's left thigh, an 8×6.3 enlarged image of which was published (Regöly-Mérei 1968, 445, Figure 5). This reveals a bone structure that sustained severe post-mortem damage, which is similar to Figure 20 C, but Regöly-Mérei mentions Haversian canals that were preserved. This histological survey, however, supported our observation that the DNA in Anne of Antioch's skeleton could be considerably degraded due to post-mortem damage to the bone structure (see Chapter 7, Point 5). In addition to the condition of the bone structure and the DNA fragmentation in relation to this, the locus of some A-STR markers, as well as the chromosome region corresponding to the locus influences the detectability of the surveyed markers via PCR amplification. Thus, we must be very careful in interpreting the results and check them via DNA sequencing whenever possible.

SUMMARY: In the case of the thigh bone of skeleton II/52_3, microscopic morphological surveys revealed that there is no intact bone structure (Figure 20 C and D); thus, the DNA sample from it is not suitable for A-STR marker analyses, even in rare cases where short marker alleles may have occurred. The longer allele which sometimes appears is probably a faulty PCR result. In Béla III's case, the femur's bone structure is better preserved, but here again the issue arises of faulty PCR products created during the amplification of DNA samples of some alleles.

CHAPTER SEVEN

ERZSÉBET CSERNÁK, SUSANNE HUMMEL,
JUDIT OLASZ, VERENA SEIDENBERG,
ZOLTÁN SZENTIRMAY
(WITH CONTRIBUTION BY BÉLA MELEGH)

GENETIC INVESTIGATIONS

1. Taking samples from the bones held in the Matthias Church for genetic studies

In the church, dressed in sterile surgical attire, wearing a surgical mask and rubber gloves, Prof Dr Miklós Kásler handles the bones wrapped in white textile after the opening of the glass containers (Figure 4/C). He did not unwrap the textile, and the royal couple's skeletons were placed back in the glass containers after having been checked by touch. After that, the glass containers were placed into special delivery packages made of paper for this exact purpose and transported them to one of the NIO's sterile operating rooms, where samples were taken. The skeletons from the crypt were left in their original caskets and transported to the NIO sealed with tape.

The cut-out samples were individually placed into sterile tubes, similarly to the residual bone dust (Figure 21).



Figure 21. Dr Miklós Kásler cutting out a bone sample using a vibratory bone saw; even the small amount of resulting bone dust was vacuumed up, in order to avoid contamination of other bones.

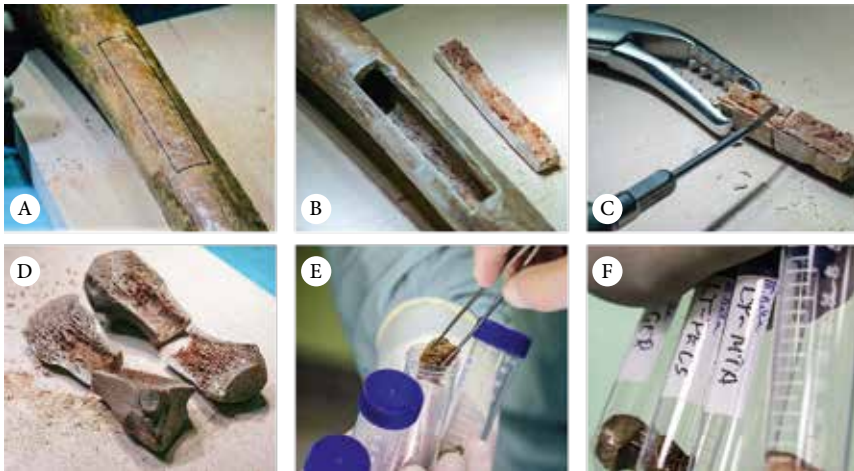


Figure 22. A: Drawing of the bone sample on Béla III's femur for cutting out.

B: The cut-out bone sample.

C: Division of the cortical bone slab into four samples.

D: Metatarsal cut in four parts. E: Individual bone samples are placed in separate sterile tubes. F: Bone sample series from a skeleton.

Four samples were taken from each bone (Figure 22). Two series of bone samples were given to Dr Béla Melegh, which he in turn provided to Dr Hummel's laboratory in Göttingen, while Professor Johannes Krause in Berlin also received part of it. One series was given to Dr Elek Benkő and Dr Zoltán Szentirmay; the latter provided the samples to Dr Judit Olsz at the NIO Pathogenetics Department (Laboratory-1). The data on the samples taken is summarized in Table 5.

Skeleton	Femur	Humerus	Tibia	Tarsus-1	Tarsus-2	Vertebra	Rib	Sternum	Bone dust
Béla III	X			X ₁	X	X			X
Anne A.	X					X	X		X
Fetus				X					
II/52_3	X			X ₁			X		X femur
II/53_7						X	X	X	
II/54_9	X					X	X		X
II/55_10	X					X	X		X
II/56_11		X ₂				X	X		X
II/109_8			X			X	X		
I/3 G5	X				X		X		X
I/4 H6	X				X	X			X

Table 5. Bone samples from the royal graves in the Matthias Church. X1: Chosen from two separately held tarsi for the sake of better analysis. There are samples of skull fragments from II/57_12, II/58_14, II/59_18, VI/1_13, VI/2_15 and VI/3_17, which we are currently not analyzing. By the term “bone dust” we mean the “bone sawdust” which was created when using the vibratory bone saw; since it never gets into the air, it is not vacuumed out of the air (Figure 21). We collected it on an individual basis during sample-taking from each skeleton (see Figure 22). The bone dust can be used when conducting genetic tests, such as DNA isolation.

The royal couple, as well as skeletons II/52_3, the fetus, I/3 G5 and I/4 H6 are displayed before taking the samples (Figures 23-27). Genetic analyses were conducted for each skeleton.



Figure 23. Béla III's skeleton and skull.



Figure 24. Anne of Antioch's skeleton and skull.



Figure 25. Skeleton II/52_3, who was identified through genetic analysis as a Hungarian King from the Árpád Dynasty. The area of earlier sample takings can be seen on the tarsus and, if enhanced, on the insert (see Chapter 11, Section 3).



***Figure 26.** The remaining female fetus of the woman lying in the fourth grave, who died during childbirth and was later identified as a royal princess (see Chapter 11, Section 3).*



Figure 27. I/3 G5 and I/4 H6: The skeletons found by Henszlmann in the Royal Basilica of Székesfehérvár's northern aisle, in stone-lined graves. The architecture of the graves, as well as the level data, confirms that they are indeed from the Árpád era. According to the genetic studies, neither is a relative of the Árpáds; skeleton I/3 G5 is still unknown, while I/4 H6 is probably a member of the clergy (see Chapter 11, Section 3).

2. Scientific background on the study of STR markers

Repeating (microsatellite) short sequences that can be found in the genome in great numbers are called STR (Short Tandem Repeat). The repeats are made of several (1-6) DNA bases, and the STRs typically consist of 5-50 repeats. The variants of a given STR marker, containing a different number of repeats are called *alleles*. STR markers have two main types, autosomal markers and Y-chromosome markers.

Autosomal STR (A-STR) markers. These markers are located on the somatic chromosomes (called autosomes); thus an individual inherits one allele from the father and one from the mother. The alleles from the distinct parental sources may differ from each other in length (i.e. in the number of repeats). Sometimes the successor inherits parental alleles with very little difference from the marker alleles of either the father's or the mother's side, and therefore, they allow us to trace family relations. In Figure 28, we display the A-STR marker's chromosomal loci and one of the potential hereditary patterns of the D13S317 marker. The alleles are marked by a number on the figure, these are the numbers of the repeats. An A-STR DNA database has existed since 1995 in the United Kingdom, while the US Federal Bureau of Investigation (FBI) created a national DNA database on October 13, 1998. This database contained over 1.5 million A-STR DNA sequences by the end of 2003 and is known as Combined DNA Index System, abbreviated to CODIS. This system was copied by 22 DNA laboratories and out of the possible microsatellite sequences they chose the 13 A-STR sequences which can be used for personal identification in everyday use. These are the following: D13S317, D21S11, D18S51, TH01, D5S818, FGA, D16S539, CFS1PO, D7S820,

VWA, TPOX, D3S1358 and D2S441. When investigating the ancient bones, this set of markers proved to be insufficient, and therefore another eight A-STR markers were added, in such a manner that instead of TPOX we used the marker D10S1248. The newer markers were also chosen based on international experience.

Possible chromosomal localizations of A-STR markers:

2, 3, 4, 5, 7, 8, 11, 12, 13, 16, 18, 19, 21

Example

Name of marker	Localization	Sequence	Number of repetitions
D13S317	13q31.1	TCTA	8–15

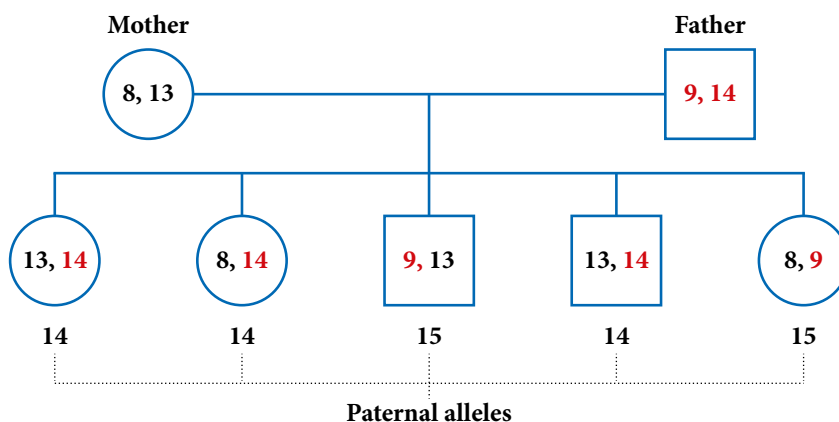


Figure 28. One of the possible hereditary patterns of A-STR marker D13S317. The alleles marked with black are maternal, the red ones are paternal in origin. The paternal D13S317 marker consists of 9 or 14 repeating units (TATC), the maternal marker consists of the same 8 or 13 repeating markers. Only one paternal and one maternal allele is inherited, and they are distributed randomly.

Y-chromosome STR (Y-STR) markers. Only a single allele from the father is transmitted to the descendant, and therefore we can call it the paternal inheritance. These STR-markers are located on non-coding parts of the Y-chromosome. Accordingly, in this case, only paternal markers are inherited, which is why they can be used to conduct retrospective hereditary (haplogroup) analyses (Figure 29).

Y-STR inheritance in male heirs only

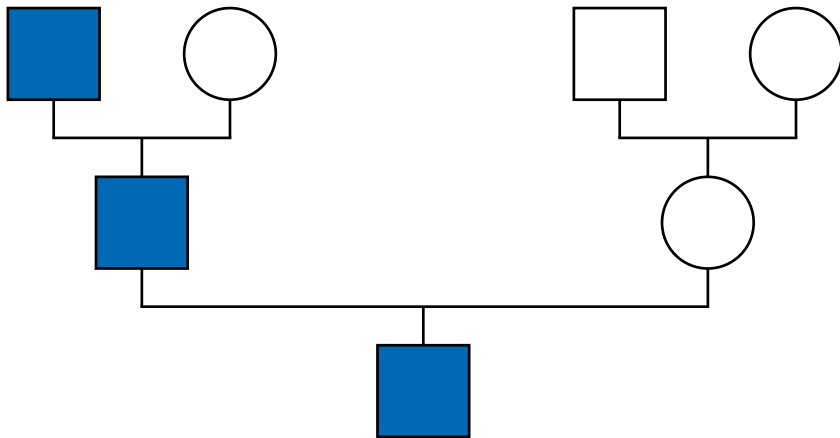


Figure 29. *There is a DNA sequence which does not recombine during meiosis, and consequently it is passed on from generation to generation unchanged. In this case, only cumulative mutations cause changes, which is why this region has a genetic history that is easier to determine.*

The detection of STR markers is done by taking the isolated DNA from the sample and, using the PCR method with the corresponding factory reagent kit, multiplying the chromosome region in which the marker is located. In the course of amplification, the PCR product receives a fluorescent marker, which is necessary to make the final result visible. The manufacturer of the kit used to detect STRs provides a marker set (ladder) that is equal in length to each marker's allele length, to which the PCR products are then compared and marked on a scale (electropherogram), from which the actual allele length of the marker can be read. We should note here, however, that when observing fragmented DNA samples extra peaks occurring at the wrong places or missing peaks can occur.

3. Circumstances related to A-STR markers that influence the analysis results. Summary of the literature.

The effect of mutation rates on studying hereditary relations

The high mutation rates of A-STR markers are especially important when it comes to the analysis of paternal/hereditary relations. When examining hereditary, and by extension father-son relations, we suppose that the alleles remain the same when they transfer over to the next generation. This is not necessarily true, however, because several factors independent of hereditary ones can influence the length of alleles (the number of repetitions), and this can lead to false conclusions.

Data from the literature show that if the rate of mutation is below 0.1%, then for 1,000 father-son alleles transmitted there will be one mutation that is not corrected. Weber–Wong (1993) and Sajantila et al. (1999) studied 29,640 father-son allele transfers and found 18 A-STR mutations. Several studies have investigated mutations of the 13 core STR markers. Usually, 1-5 mutations happen out of 1,000 allele transfers. A higher marker mutation rate causes a higher rate of allele lengthening or shortening; in other words, a change in the number of repeating units. The lowest mutation rate can be found in the following A-STR markers: CSF1PO, TH01, TPOX, D5S818 and D8S1197. The highest mutation rates are in D21S11, FGA, D7S818, D16S539 and D18S51 (Butler 2005, Table 6.3, Appendix I).

Possible artifacts in the study of A-STR markers

During amplification of A-STR markers, several artifacts can be generated, which can interfere with the evaluation of the allele genotypes from a given DNA template. First, we have to recognize – and for this reason, we discuss in detail – the so-called triple-peak pattern, also known as the “stutter” phenomenon, as well as peaks beyond the normal allele lengths, which can cause the allele lengths to deviate from the actual length on the electropherogram. Other factors that influence STR classification include non-template addition, microvariant and “off-ladder” alleles, allele skipping (dropout) and “null (silent)” alleles (Butler 2012).

*Peaks beyond normal allele lengths and the triple-peak pattern
(three-banded pattern)*

In the examination of allele lengths, a new allele may randomly appear next to one of the real A-STR allele pairs. This causes a problem for evaluation. The peaks can be the same, longer, or shorter than the corresponding consensus allele peaks. This phenomenon can be recognized if the examination is repeated with a different A-STR detection kit and a different result is obtained. The three-banded pattern that can be observed during individual marker localizations is not the artifact of the detection process, but rather that of the individual samples which can be reproduced. This can be caused by the presence of an extra chromosome or primer point mutations, or a bad quality DNA template (Crouse et al. 1999). Up until August 4, 2016, a total of 389 three-banded patterns had been published. For example, 9 such peaks were registered at D2S1338, 11 such peaks at D3S1358, 20 such peaks at D7S820, and 12 such peaks at D19S433, and this occurred with the same markers in our cases as well. The list of allele microvariations is frequently refreshed and can be found at the STRbase website: http://www.cstl.nist.gov/biotech/strbase/var_tab.htm.

“Stutter” artifacts

The electropherogram containing the STR data may show peaks, usually smaller ones, which are usually one repeat shorter or longer than a real PCR product. In the case of a microsatellite unit composed of several bases, the stutter artifact at one repeating unit can be longer or shorter than a real PCR peak. According to the model of the

mechanism of stutter artifacts, the fragmented DNA strand hybridizes in a flawed manner (mispairing) with a DNA template. This creates a non-base-paired loop, and causes PCR amplification slippage (Figure 30). As a result of this, the template is multiplied incorrectly during the reaction. This phenomenon depends on the following circumstances:

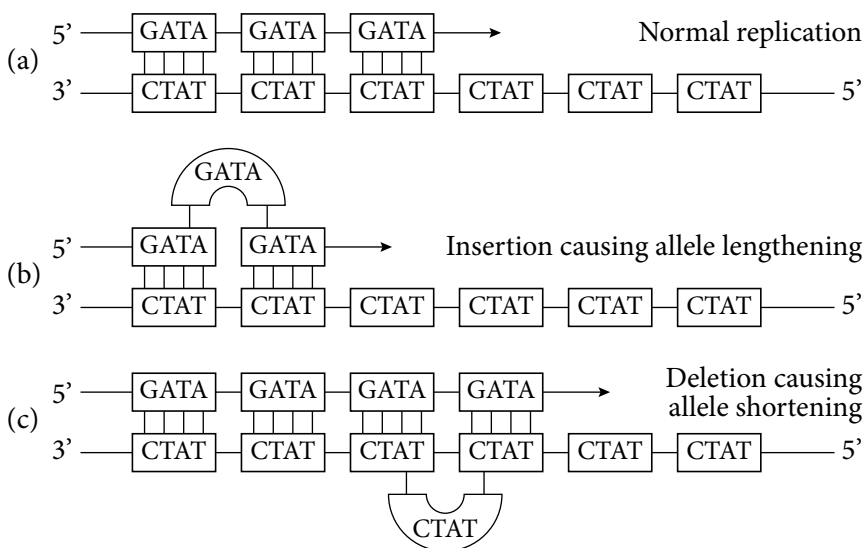


Figure 30. Stutter artifact appears due to the DNA strand not attaching correctly. (a) During a normal replication, the two DNA strands hybridize without error, and the repeating units will be the same length. DNA strands that are created later can reattach easily and normal DNA replication can resume. (b) If the repeating unit ‘loops out’ during a newly synthesized extension in an upcoming PCR cycle, this causes insertion and allele lengthening. (c) If the looping of the repeating unit occurs on the template strand, the synthesized new strand slides forward and it will be a unit shorter than the full-length STR allele (Butler 2012).

(a) for the most part, the extent of the DNA template's degradation, (b) the PCR circumstances and the Taq polymerase used, (c) it is more common in the case of longer alleles, within the given marker, and (d) further unique characteristics of the marker.

Non-template addition

Taq polymerase often adds an extra nucleotide to the end of a PCR product, mostly an adenine (called "adenylation"). In the case of partial adenylation some of the PCR products do not have the extra adenine (-A peaks), other products do (+A peaks). All of this results in the peak becoming broader (in the case of poor resolution), or in the case of good resolution, a split peak can be seen. In the case of several samples, variation in the adenylation status affected the marker's length and genotype. For example, the 12 allele of the non-adenylated D2S441 marker is the same length as the completely adenyated D2S441 11.3 allele, as both contain the same number of repeating microsatellite units, and base number variation only exists within one repeating unit. The same applies, for example, to alleles TH01 10 and TH01 9.3. Therefore, it is important to amplify purely +A or -A samples instead of investigating +/- mixed samples (Butler 2012). Several methods exist for the pure +A or -A conversion of samples, but we did not perform these.

Microvariant and "off-ladder" alleles

Human populations may contain DNA markers which differ from common STR allele variants by one or more basepairs. Differences

can be insertions, deletions and nucleotide variations. Alleles that contain an incomplete repeat unit are defined as microvariant alleles, or “off ladder” alleles (Butler 2012). Microvariant alleles are not rare: they are most often found in polymorph STR markers such as FGA, D118S51 or D21S11.

Alleles of equal length, but differing sequences

Some STR marker alleles contain variable repeating blocs, but the number of basepairs is the same as the consensus allele length. This could be an artifact and is formed during PCR amplification. This phenomenon could only be detected through sequencing (because the PCR based STR genotyping only takes the allele length as a basis), but the sequencing routine is not used in hereditary investigations.

Allele dropout and “null” (silent) alleles

During the amplification of fractured DNA strands containing STR repetitions the phenomenon of allele dropout can occur. We know that DNA sequence polymorphism can be within the repeating sequences, around the -5' or -3' ends of the STR, or within the primer binding sites. If the basepair swap occurs at the primer binding site, hybridization of the primer does not occur, and thus the marker on the template will not be detected. This phenomenon is called a null allele. Fortunately, this happens very rarely during routine paternity tests, as the STR's environment is stable and does not change. The danger posed by null alleles within a given laboratory does not become a problem if the same primer is used. Investigating the same

sample in a different laboratory with different primers or comparing the samples with samples stored in genotype-databases can lead to false negative results, or inconsistency between the two samples compared. The presence of a “null allele”, however, can never be discounted when dealing with degraded DNA samples.

4. Göttingen, Final Report-2. Final report on the investigations conducted on the skeletons from the Matthias Church. DNA isolation

Table 6 contains the list of bone samples used in the analyses.

Samples	Femur		Tarsus-1		Tarsus-2		Vertebra		Ribs		Sternum	
	G	B	G	B	G	B	G	B	G	B	G	B
Laboratories (G/B)												
Béla III		X	X	X	X	X		X				
II/52_3	X		X*	X						X		
II/53_7								X	X	X**	X	
II/54_9	X								X	X		
II/55/10	X								X	X		
I/3 G5	X		X						X	X		
I/4 H6			X	X	X	X						
Anne of Antioch	X						X		X	X		
Fetus							X					
II/109_8							X		X			

Table 6. Bone samples used for DNA isolation. G: Göttingen, B: Budapest-1 *: isolated from tarsus 1 and 2. **: isolated from two rib samples (see Table 5).

It was possible to isolate DNA from every bone sample investigated, but optimization of the DNA isolation protocol was necessary for this. This was especially important in the case of Béla III, since these bones were treated with some sort of resin, and this treatment may have caused the severe fragmentation of the DNA isolated from the bone samples (and may have also affected the radiocarbon dating). Optimizing the DNA isolation was conducted on Béla III's tarsus, and four special DNA isolating kits were tried in six combinations. The fourth, fifth and sixth variations proved to be appropriate, and these were used to isolate DNA from Béla III's tarsus, as well as from various bones of other skeletons (Figure 31, Fehren-Schmitz et al. 2015).

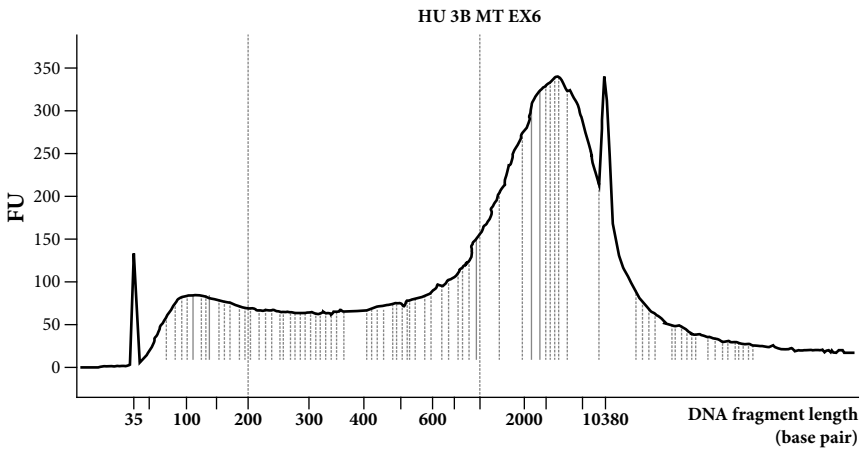


Figure 31. Sixth DNA extraction iteration from Béla III's metatarsal sample with the use of QiaVAc MinElute Organic kit, Aglient fluorescent gel electrophoresis figure. The fragment lengths are displayed in the denominator in basepair (bp) units. (Fehren-Schmitz et al. 2015).

As we can see, the isolated DNA was suitable for the amplification of A-STR markers (determining the genetic fingerprint). This amplification was initially performed using the Heptaplex kit (Seidenberg et al. 2012). This kit is capable of detecting 6 A-STR markers and the amelogenin marker (for gender determination). For preliminary orientation, Figure 32 shows the compiled results of six A-STR markers; this gives us an overview of the detectability of the chosen markers and of the artifacts that were generated. The result of the modified DNA isolation also allows for the conclusion that the DNA sample thus obtained can also be used for next generation sequencing (NGS).

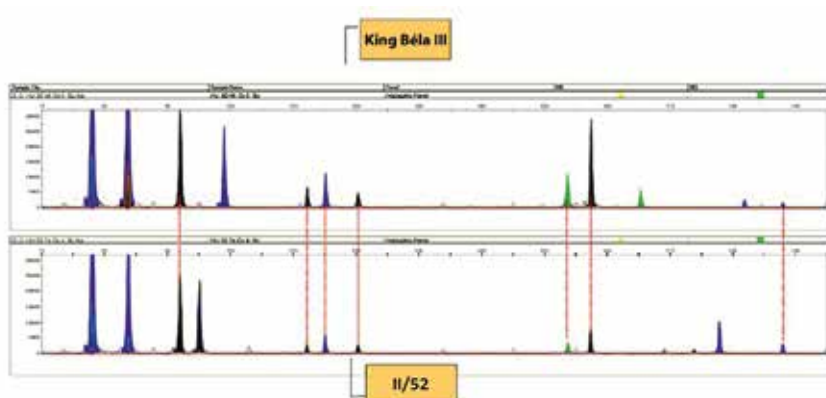


Figure 32. Six A-STR localizations and the amelogenin profile detected on the bone samples of Béla III and skeleton II/52_3 using the Heptaplex kit. The identical markers are marked by a red line (Göttingen laboratory). Additionally, the figure contains alleles which are not identical in the two bone samples (Seidenberg et al. 2012).

At the Göttingen laboratory, after implementing 20 A-STR marker panels, the DNA isolated from the femur of skeleton II/52_3 was found to be of very poor quality, to which the possibility of DNA contamination contributed, because this DNA sample was previously investigated by Péter Nagy in the USA, and thus the result was no longer considered when studying family relations. The DNA isolated from the 2nd tarsus provided the best result. Even so, several missing alleles (dropouts, null alleles) were observed during amplification of the alleles for each marker. This meant that in many cases, the allele could be detected once during a trial of 4-8 amplifications. All of this indicated severe degradation of the DNA sample, in which very few target sequences were present. According to the Göttingen laboratory's experience, such heavily degraded DNA samples containing very few target sequences tend to generate more and more stutter artifacts and other PCR errors.

5. DNA template quality for all skeletons and the detectability of individual A-STR markers depending on allele length

The detectability of A-STR markers depends on two things: (1) *Length of the alleles*. We found that A-STR markers with longer alleles are much harder to detect. Such markers include D1S1656, D2S1338, D12S391, D19S433 and SE33. (2) *The preservation of bone structure*. It was possible to isolate fragmented DNA from the analysed bones, the length of which was 150-250 bp. Depending on the soundness of the bone structure, we split the skeletons into two groups. The DNA isolated from the bone samples of the first

group (Béla III, II/54, II/55, I/3 G5, I/4 H6 and II/109) contained several longer fragments, which made the detection of A-STR markers more effective. The DNA isolated from the bone samples of the second group, which included II/52, II/53, Anne of Antioch and the fetus, was quite fragmented and contained significantly fewer template sequences than the previous group's DNA samples, making it much more difficult to detect A-STR markers. The results are shown in Figure 33.

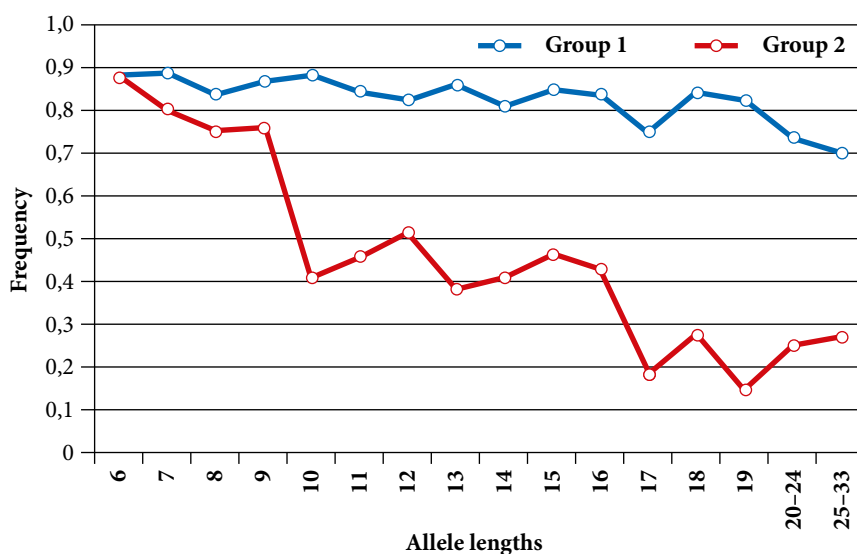


Figure 33. The probabilities of 20 A-STR markers' detectability displayed in relation to allele length. The bone samples belonging to group 1 (Béla III, II/54, II/55, I/3 G5, I/4 H6, II/109) are the ones from which the isolated DNA contained several longer fragments, thus facilitating the detection of A-STR markers (blue line). The DNA isolated from the members of group 2 (skeletons II/52, II/53,

Anne of Antioch and the fetus) were quite degraded compared to the other group's DNA samples, and thus it was much harder to detect A-STR markers (red line). Frequency of detectability in the cases of individual STR markers: number of accepted identical (fingerprint) allele lengths/ number of total attempts. The calculation was made using the Final Report-2 data from the Göttingen laboratory.

6. Comparison of A-STR markers of the bones of skeleton II/52_3. PCR study

The purpose of the comparison was the numerical presentation of the Göttingen laboratory's observations pertaining to the DNA isolated from skeleton II/52_3, but we also wished to gather data on the correspondence between the A-STR markers from the femur and tarsus-1, in order to interpret the results. In Table 7, we compare 20 A-STR markers of the femur from skeleton II/52_3 to the marker data of tarsus-1 generated by two different laboratories. At this point, we would like to note that all of the bones of skeleton II/52_3 belong to the same individual, and thus in the period following their discovery, sorting and reassembly of the mixed-up bones using anthropological methods was done without error. The laboratories in Budapest and Göttingen both received one of each bone sample; the boxes highlighted in yellow show the allele data from the A-STR investigation that differ from each other, suggesting a PCR error. Due to the poor bone structure of skeleton II/52_3, some marker alleles could not be detected even after several attempts (see Chapter 6, Figure 20 C and D).

II/52 A-STR	Femur (Göttingen)		Tarsus-1 (Göttingen)		Tarsus-1 (Budapest-1)		Rib (Budapest-1)	
	A1 (Freq)	A2 (Freq)	A1 (Freq)	A2 (Freq)	A1 (Freq)	A2 (Freq)	A1 (Freq)	A2 (Freq)
D1S1656	(0/4)	(0/4)	12 (1/4)	17,3 (1/4)	12 (6/8)	17,3 (4/8)	n.a.	n.a.
D2S441	9 (1/3)	11 (3/3)	9 (1/4)	10 (1/4)	10 (1/1)	10 (1/1)	10 (1/1)	(0/1)
D2S1338	(0/4)	(0/4)	(0/7)	(0/7)	20 (3/3)	25 (1/3)	20 (1/1)	(0/1)
D3S1358	15 (1/4)	18 (3/4)	14 (5/8)	(0/8)	14 (3/3)	(0/3)	n.a.	n.a.
D5S818	(0/2)	12 (1/2)	10 (4/8)	12 (6/8)	n.a.	n.a.	n.a.	n.a.
D7S820	n.a.	n.a.	8 (1/5)	9 (3/5)	8 (1/2)	9 (1/2)	(0/2)	9 (2/2)
D8S1179	(0/4)	(0/4)	12 (2/8)	14 (1/8)	12 (1/1)	14 (1/1)	n.a.	n.a.
D9S1120	n.a.	n.a.	15 (3/3)	16 (2/3)	15 (1/1)	16 (1/1)	n.a.	n.a.
D10S1248	13 (3/4)	13 (3/4)	13 (2/4)	13 (2/4)	12 (1/8)	13 (8/8)	13 (1/1)	13 (1/1)
D12S391	(0/4)	18 (2/4)	17 (1/4)	18 (1/4)	17 (3/4)	18 (2/4)	n.a.	n.a.
D13S317	8 (1/2)	(0/2)	8 (7/10)	13 (7/10)	8 (4/4)	13 (4/4)	8 (4/4)	13 (2/4)
D16S539	(0/4)	12 (2/4)	10 (2/12)	11 (8/12)	10 (6/7)	11 (11/11)	10 (2/2)	11 (1/2)
D18S051	15 (1/6)	(0/6)	13 (8/12)	17 (4/12)	13 (5/6)	17 (4/6)	13 (2/3)	17 (2/3)
D19S433	(0/4)	(0/4)	13 (3/4)	14 (1/4)	13 (4/4)	14 (1/4)	n.a.	n.a.
D21S11	(0/8)	(0/8)	30 (7/12)	32.2 (8/12)	30 (4/6)	32.2 (3/6)	30 (1/1)	(0/1)
D22S1045	12 (1/4)	16 (1/4)	15 (1/4)	17 (1/4)	15 (7/7)	16 (1/7)	15 (1/1)	(0/1)
CSF1PO	n.a.	n.a.	n.a.	n.a.	9 (5/5)	11 (5/5)	9 (2/2)	11 (1/2)
FGA	21 (1/6)	22 (1/6)	21 (5/12)	25 (5/12)	21 (5/6)	25 (2/6)	n.a.	n.a.
TH01	9 (4/4)	9,3 (1/4)	9 (13/15)	9,3 (11/15)	9 (6/8)	9,3 (8/8)	9 (1/1)	9,3 (1/1)
VWA	16 (1/4)	21 (1/4)	(0/8)	(0/8)	16 (2/2)	17 (2/2)	n.a.	n.a.

Table 7. Allele lengths of the A-STR markers of DNA isolated from the femur, tarsi and ribs of skeleton II/52_3 are displayed. In the fractions in parenthesis, the numerator stands for the number of fingerprint allele detections, while the denominator indicates the total number of attempts. The matching alleles detected from different bones are displayed in bold numbers. The tarsus-1 samples of the laboratories in Göttingen and Budapest-1 are from the same bone, but despite this, the allele length data of these bones differed in the case of three markers (boxes highlighted in yellow); this points to a potential PCR artifact.

Regarding the A-STR markers from skeleton II/52's femur, we were only able to take into account 12 marker data points out of 20 in the comparison. When comparing the femur and tarsus-1 samples at Göttingen, the paternal and maternal alleles of TH01 marker were both identical, while in the case of another three markers, only one allele of the femur was detectable, but that allele was identical to one of the alleles from tarsus-1. At markers D10S1248 and D22S1045, in the laboratories in Göttingen and Budapest, one tarsus-1 allele length belonging to the same markers in each facility are different from each other, but are identical to the corresponding femur marker length data investigated at the laboratory in Budapest. The alleles of the femur's D3S1358 marker are composed of 15 and 18 repeating units, while one of the D18S51 marker's detectable allele lengths is made up of 15 repeating units. These were not identical to either allele length from tarsus-1, but the consensus allele lengths of these very same markers are identical to all the tarsus-1 marker data. Based on our studies, we have every reason to believe that the PCR amplification of alleles with such a large repeat number would not give a valid result. If skeleton II/52_3's consensus A-STR marker data are taken into account, then it is apparent that all of the twelve evaluable marker data of the femur are identical to either the tarsus-1 and/or the rib; thus, all of the bones investigated belong to the same person. If we compare the twelve detected marker data of skeleton II/52 with the corresponding marker data for Béla III, we find that for 10 markers all the alleles are the same length in both skeletons. The D2S441 marker data are indeed different in the two skeletons. We could only detect one allele of marker D18S51, so this difference must not be accepted as valid.

The significance of the above is that these data also disprove the opinion of Éry and her working group about the originality of the

II/52 skeleton. This observation confirms that there was no skeleton swap, and thus further A-STR and Y-STR studies were conducted on the original skeletons. In Varsányi's drawing (Figure 13) skeleton II/52_3 is shown as though one of its leg bones was broken, but this cannot be seen on the skeleton II/52_3 that is interred in the Matthias Church. Therefore, the drawing is inaccurate and unable to serve as proof that the skeletons were swapped.

The question arises: Why is tarsus-1 more suitable than the femur for the investigation of family relations by A-STR markers? The bone structure of the femur cortex is wider and more compact than that of the tarsus, and thus the DNA isolating process from it is modified. Decalcination takes more time, which in turn could lead to further DNA fragmentation, and possibly the DNA also fragmented further during the subsequent isolation steps, and thus fewer DNA strands suitable as a PCR template remained.

7. Determining the A-STR markers and gender of the skeletons in the Göttingen and Budapest-1 laboratories

In May 2014, ten Árpád-age bone samples arrived from the Matthias Church in Budapest at the Historical Anthropology and Human Ecology laboratory of the Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology at the University of Göttingen for researchers Verena Seidenberg and Susanne Hummel, along with the request to isolate DNA from the samples of a quality suitable for next generation sequencing. They optimized the method of DNA isolation. The investigation results for the A-STR marker are presented in Figures 8.1 and 8.2, as well as Figures 9.1 and 9.2.

Skeletons	Béla III		II/52		II/53		II/54		II/55	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
A-STR										
D1S1656	13 (6/10)	17.3(6/10)	n.v.a.	n.v.a.	15 (2/8)	n.v.a.	15 (4/8)	17/16(2/8)	15.3(5/6)	16 (6/6)
D2S441	11(7/10)	11.3(7/10)	n.v.a.	n.v.a.	12 (7/8)	14 (8/8)	10 (7/8)	11 (4/8)	11 (6/6)	11 (6/6)
D2S1338	17 (4/14)	n.v.a.	n.v.a.	n.v.a.	n.v.a.	n.v.a.	21(3/12)	n.v.a.	17 (6/8)	24 (7/8)
D3S1358	15 (9/14)	17 (10/14)	14(4/12)	14(5/12)	14 (5/12)	n.v.a.	16(5/12)	17(5/12)	14 (7/8)	16 (7/8)
D5S818	10 (6/25)	12 (7/25)	10(4/10)	12(7/10)	12 (7/12)	n.v.a.	9 (7/10)	11(6/10)	11(11/14)	12(8/14)
D7S820	10 (4/4)	11 (4/4)	9 (2/4)	n.v.a.	10 (3/4)	12 (3/4)	11 (2/8)	n.v.a.	9 (2/2)	10 (2/2)
D8S1179	13 (6/14)	14 (7/14)	12(2/12)	14(2/12)	14 (3/12)	n.v.a.	11 (4/12)	14 (3/12)	13 (7/8)	13 (7/8)
D9S1120	15 (4/4)	16 (4/4)	15 (3/4)	16 (2/4)	16 (3/4)	n.v.a.	15 (2/4)	16 (2/4)	15 (2/2)	16 (2/2)
D10S1248	13 (6/10)	13 (6/10)	13 (5/8)	13 (5/8)	13 (8/8)	13 (8/8)	13 (4/8)	15 (4/8)	13 (5/6)	14 (5/6)
D12S391	18 (6/10)	19 (5/10)	n.v.a.	n.v.a.	18 (1/8)	25**(1/8)	23(3/8)	19**(1/8)	17 (5/6)	22 (5/6)
D13S317	9(6/25)	13(6/25)	8 (7/10)	13(6/10)	12(9/11)	n.v.a.	11(8/10)	13(8/10)	11(18/20)	13(18/20)
D16S539	11(12/15)	12(12/15)	10(2/12)	11(6/12)	12 (8/12)	13 (7/13)	11(8/12)	12(6/42)	12 (7/8)	13 (7/8)
D18S051	13(12/35)	16(12/35)	13(8/18)	17(4/18)	14(10/18)	15(7/18) 19(3/18)	14(10/18)	17(11/18)	12(17/22)	16(17/22)
D19S433	15 (9/14)	16.2(8/14)	13(4/12)	n.v.a.	13 (4/42)	14 (3/12) 17.2*(4/12)	13 (5/12)	14(6/12) 17.2*(3/12)	13 (7/8)	14 (7/8) 17.2**(1/8)
D21S11	31(8/39)	32.2(8/39)	30(7/22)	32.2(5/22)	26(7/22)	28(2/22)	29(12/22)	30(2/22)	30(18/24)	31(17/24)
D22S1045	15 (6/9)	16 (6/9)	n.v.a.	n.v.a.	14 (4/8)	n.v.a.	14 (4/8)	15 (3/8)	12 (6/6)	15 (6/6)
FGA	21(12/35)	21(12/35)	21(6/18)	25(5/18)	20(5/18)	22(6/18) 24(5/18)	21(11/18)	23(8/18)	22(16/22)	23(17/22)
SE33	20 (5/10)	27.2(2/10)	n.v.a.	n.v.a.	n.v.a.	n.v.a.	18(3/8)	28.2(2/8)	26.2(5/6)	34.2(5/6)
TH01	7 (13/35)	9 (12/35)	9(16/18)	9.3(11/18)	8 (9/18)	10(14/18)	6(18/18)	9.3(14/18)	6(20/22)	9.3(20/22)
vWA	17 (9/14)	17 (9/14)	n.v.a.	n.v.a.	19 (2/12)	n.v.a.	14 (4/12)	16 (4/12)	16 (7/8)	16 (7/8)

Table 8.1. Autosomal STR-marker values / Göttingen laboratory. The data are from the summary “Final Report-2”. In the fraction in parenthesis, the numerator shows the number of fingerprint allele detections, the denominator indicates the number of total attempts. *: probably an artifact (Seidenberg and Hummel, Göttingen) n.a.: not analyzed; n.v.a. / **: invalid data.

Skeletons	Béla III		I/3 G5		I/4 H		Anne		II/109		Fetus	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	n.a.	n.a.
A-STR												
D1S1656	13 (6/10)	17.3(6/10)	13 (5/6)	17.3 (4/6)	11 (8/9)	17.3(9/9)	n.v.a.	n.v.a.	12(6/8)	16(5/8)	n.a.	n.a.
D2S441	11(7/10)	11.3(7/10)	11 (6/6)	11 (6/6)	10 (9/9)	11 (9/9)	11 (2/9)	n.v.a.	11(8/8)	14(6/8)	n.a.	n.a.
D2S1338	17 (4/14)	n.v.a.	24 (6/8)	25 (4/8)	18 (10/13)	18(10/13)	19(2/11)	n.v.a.	18(4/12)	19(4/12)	n.a.	n.a.
D3S1358	15 (9/14)	17 (10/14)	14 (8/8)	17 (8/8)	15(11/11)	19(11/11)	16 (2/11)	n.v.a.	15(11/12)	18(8/12)	n.a.	n.a.
D5S818	10 (6/25)	12 (7/25)	11(10/10)	12(10/10)	10(14/22)	12(18/22)	14(4/24)	n.v.a.	11(8/10)	11(8/10)	12(3/8)	13(3/8)
D7S820	10 (4/4)	11 (4/4)	8 (2/2)	12 (2/2)	11 (2/2)	12 (2/2)	n.v.a.	n.v.a.	8(4/4)	10(4/24)	n.a.	n.a.
D8S1179	13 (6/14)	14 (7/14)	13 (8/8)	13 (8/8)	12 (9/11)	12(9/11)	13(2/11)	n.v.a.	12(6/12)	15(7/12)	n.a.	n.a.
D9S1120	15 (4/4)	16 (4/4)	16 (2/2)	16 (2/2)	16 (2/2)	16 (2/2)	n.v.a.	n.v.a.	15(4/4)	16(3/4)	n.a.	n.a.
D10S1248	13 (6/10)	13 (6/10)	14 (6/6)	15 (6/6)	13 (9/9)	13 (9/9)	14 (2/9)	n.v.a.	14(8/8)	15(8/8)	n.a.	n.a.
D12S391	18 (6/10)	19 (5/10)	15 (6/6)	21 (6/6)	18 (9/9)	18 (9/9)	n.v.a.	n.v.a.	17(5/8)	23(6/8)	n.a.	n.a.
D13S317	9 (6/25)	13 (6/25)	8(10/10)	13(9/10)	8(14/22)	13(15/22)	11(6/29)	11(6/29)	9(9/10)	11(8/10)	12(3/8)	n.v.a.
D16S539	11(12/15)	12(12/15)	11 (8/8)	11 (8/8)	12(11/11)	14(11/11)	11(6/11)	12(4/11)	10(10/12)	13(10/129)	n.a.	n.a.
D18S051	13(12/35)	16(12/35)	19(16/16)	23(11/16)	14(26/31)	14(26/31)	18(2/36)	n.v.a.	14(13/18)	15(11/18)	19(2/8)	n.v.a.
D19S433	15 (9/14)	16.2(8/14)	13 (8/8)	14 (6/8)	14(9/11)	16((10/11)	n.v.a.	n.v.a.	13(8/12)	13(8/12)	n.a.	n.a.
D21S11	31 (8/39)	32.2(8/39)	25(18/19)	31.2(13/18)	30(21/33)	32.2(21/33)	33(3/40)	32.2** (1/40)	31 (6/22)	33.2 (8/22)	30.2 (2/8)	n.v.a.
D22S1045	15 (6/9)	16 (6/9)	15 (6/6)	16 (6/6)	15 (8/9)	16 (8/9)	11 (2/9)	n.v.a.	15(7/8)	16(6/8)	n.a.	n.a.
FGA	21(12/35)	21(12/35)	19(14/16)	20(16/16)	19(25/31)	25(25/31)	n.v.a.	n.v.a.	22(11/18)	22(11/18)	20(3/8)	25(3/8)
SE33	20 (5/10)	27.2(2/10)	18 (6/6)	19.2(3/6)	22.2(9/9)	28.2(6/9)	n.v.a.	n.v.a.	19.2(6/8)	29.2(4/8)	n.a.	n.a.
TH01	7 (13/35)	9 (12/35)	7(16/16)	9(16/16)	6(26/31)	7(26/31)	7 (5/36)	9.3(12/36)	8(15/18)	9(14/18)	6(6/8)	9.3(6/8)
vWA	17 (9/14)	17 (9/14)	14 (5/8)	19 (7/8)	17 (9/11)	18(10/11)	n.v.a.	n.v.a.	14(8/12)	19(8/12)	n.a.	n.a.

Table 8.2. Autosomal STR-marker values / Göttingen laboratory. The data are from the summary “Final Report-2”. In the fraction in parenthesis, the numerator shows the number of fingerprint allele detections, the denominator indicates the number of total attempts. n.a.: not analyzed; n.v.a. / **: invalid data.

Evaluation of A-STR analysis of ten skeletons from the Mathias Church (Göttingen)

1. In the course of these analyses, the optimized DNA isolation method was applied (for details see Chapter “*Investigation of bone samples and methods*”).
2. The final form of the various A-STR markers was usually accepted after 8×-40× detection attempts, with the criterion of obtaining the same allele lengths three times, which were acceptable as the correct (fingerprint) alleles. However, it was not always possible to fulfil the latter criterion.
3. Several attempts were needed during the analysis of Béla III’s skeleton, because the DNA isolated from the bone samples chosen (tarsus-1 and tarsus-2) was strongly fragmented and contained only a few longer DNA strands suitable as a target sequence. This DNA degradation cannot be attributed to the bone structure being damaged after death, because the structure was quite well preserved (Figure 20 A and B) and it was possible to isolate much better quality DNA from bones as well preserved as this. The DNA fragmentation is obviously due to the treatment of the skeletons with resin, which was done before their interment in the Matthias Church, perhaps in order to preserve them.
4. We previously established that skeletons II/52, II/53, Anne of Antioch and the fetus’ skeleton sustained severe damage after death, and due to this, it was difficult to isolate DNA strands of the right length that are suitable for A-STR marker detection. In these cases several repetitions were needed to detect the markers,

and thus the results were not always acceptable, or only one allele of a given A-STR marker was detectable.

5. In the case of markers where the allele's length consists of 18 or more repeating units with four bases, a large number of flawed PCR products are generated during PCR amplification, and these are not always easy to detect. In the case of marker SE33, frequent PCR errors were obvious, which is why it was advisable to exclude this marker from the analysis of family relations. All of this underscored the fact that when investigating the family relations of certain skeletons the data cannot be evaluated in a routine manner.
6. It was also clearly found that in some cases it is not the length of the allele, but rather the molecular structure of the chromosome region that influences the detectability of the A-STR marker. This phenomenon was particularly apparent in the case of the following three A-STR markers. (1) D2S1338: in several cases the PCR detection produced unacceptable results, even after numerous attempts. (2) D7S82: detection of this marker with various kits was problematic, because with some DNA samples, at least one PCR primer did not readily attach to the appropriate chromosome region due to a sequence variation in the template, and thus the PCR product was not created (see Chapter 8, Figure 38). (3) D19S433: this marker caused the biggest problem. It is located in a chromosome region with a very complicated structure. Furthermore, when detecting this marker, Verena Seidenberg and Susanne Hummel (Göttingen) found many flawed PCR products. During PCR amplification of the DNA samples from three skeletons, a PCR artifact as long as 17.2 bp appeared (Final Report-2).

7. Markers that have alleles consisting of no more than 13 repeating units can be detected unambiguously with fewer attempts, and the possibility of flawed PCR products that can interfere with the result is lower. Accordingly, these results are most acceptable when it comes to investigating family relations.

Skeletons	Béla III		II/52		II/53		II/54		II/55	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
A-STR										
DI1656	13 (3/3)	17.3 (3/3)	12 (5/7)	17.3(2/7)	n.a.	n.a.	15 (2/2)	17 (2/2)	16 (2/2)	18**(1/2)
D25441	11 (5/5)	11.3 (5/5)	10(11/11)	10(11/11)	12 (3/4)	14 (4/4)	10 (2/2)	11 (2/2)	11 3/3	11 (3/3)
D2S1338	17 (7/7)	17 (7/7)	20 (4/4)	25 (2/4)	20 (5/5)	21 (4/5)	21 (4/4)	23 (4/4)	17 (3/3)	24(3/3)
D3S1358	15(2/29)	17 (2/2)	14 (3/3)	14 (3/3)	9**(1/2)	14 (2/2)	16 (2/2)	17 (2/2)	14 (2/2)	16 (2/2)
D7S820	10(5/6)	11(6/6)	8 (6/6)	9 (2/6)	10 (3/5)	12 (5/5)	11 (2/2)	12 (2/2)	9 (2/2)	10 (2/2)
D8S1179	13 (1/1)	n.v.a.	n.a.	n.a.	14**(1/1)	n.a.	11 (2/2)	14 (2/2)	n.a.	n.a.
DI10S1248	13 (4/4)	13 (4/4)	13 (8/8)	13 (8/8)	11**(1/4)	13 (4/4)	13 (2/2)	15 (2/2)	13 (3/3)	14 (3/3)
DI2S391	18 (3/3)	19 (3/3)	17 (2/3)	18**(1/3)	n.a.	n.a.	19 (2/2)	23 (2/2)	17 (2/2)	22 (2/2)
DI3S317	9 (5/6)	13 (6/6)	8 (8/8)	13 (6/8)	12 (4/4)	12 (4/4)	11 (2/2)	13 (2/2)	11 (2/2)	13(2/2)
DI6S539	11 (7/7)	12 (7/7)	10 (8/9)	11 (8/9)	13 (7/8)	13 (7/8)	11 (4/4)	12 (4/4)	12 (5/5)	13 (4/5)
D18S51	13 (8/8)	16 (6/8)	13 (5/10)	17 (6/10)	14 (7/7)	15 (5/7)	14 (4/4)	17 (4/4)	12 (5/5)	16 (4/5)
DI9S433	15 (2/2)	16.2(2/2)	13 (3/3)	13 (3/3)	n.a.	n.a.	13 (2/2)	14 (2/2)	13 (2/2)	14**(1/2)
D21S11	31(4/6)	32.3(4/6)	30 (5/7)	32.2(3/7)	26 (5/6)	28 (5/6)	29 (3/3)	29 (3/3)	30 (3/4)	31 (4/4)
D22S1045	15 (3/4)	16 (4/4)	15 (8/8)	17 (4/8)	14(2/2)	15**(1/2)	14 (2/2)	15 (2/2)	12**(1/2)	15 (2/2)
CSFIPO	11 (5/5)	12 (5/5)	9 (7/7)	11 (6/7)	10 (5/5)	10 (5/5)	12 (2/2)	12 (2/2)	11 (2/2)	13 (2/2)
FGA	21 (7/7)	21 (7/7)	21 (6/6)	25 (5/6)	20 (5/6)	22 (6/6)	21 (3/3)	23 (3/3)	22 (5/5)	23 (3/5)
TH01	7 (4/4)	9 (4/4)	9 (8/9)	9.3 (9/9)	8 (2/4)	10 (4/4)	6 (2/2)	9.3(2/2)	6 (3/3)	9.3(3/3)
vWA	17 (3/3)	17 (3/3)	16 (2/2)	17 (2/2)	18 (2/2)	19**(1/2)	14 (3/3)	16 (3/3)	14**(1/3)	16 (3/3)

Table 9.1. Autosomal STR markers / Budapest-1 laboratory. The data are from the summary report of September 28, 2015. In the fraction in parenthesis, the numerator shows the number of fingerprint allele detections, the denominator indicates the number of total attempts. n.a.: not analyzed; n.v.a. / **: invalid data.

Skeletons	Béla III		I/3 G5		I/4 H		Anne		II/109	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
A-STR										
D1S1656	13 (3/3)	17.3 (3/3)	13 (2/3)	17.3 (3/3)	11 (2/2)	17.3(2/2)	12** (1/1)	n.a.	n.a.	n.a.
D2S441	11 (5/5)	11.3 (5/5)	11 (4/4)	11 (4/4)	10 (3/3)	11 (3/3)	10** (1/1)	14** (1/1)	n.a.	n.a.
D2S1338	17 (7/7)	17 (7/7)	24 (4/4)	25(2/4)	18(4/4)	18(4/4)	20 (2/2)	27 (2/2)	18(3/3)	19(2/3)
D3S1358	15 (2/29)	17 (2/2)	8** (1/1)	17** (1/1)	15** (1/1)	19** (1/1)	n.a.	n.a.	n.a.	n.a.
D7S820	10 (5/6)	11 (6/6)	8 (3/3)	12 (3/3)	11 (3/3)	12(3/4)	8(2/4)	10(3/4)	8 (3/3)	10 (3/3)
D8S1179	13** (1/1)	n.v.a.	13 (2/2)	n.a.	12** (1/1)	n.a.	n.a.	n.a.	n.a.	n.a.
D10S1248	13 (4/4)	13 (4/4)	14 (4/4)	15 (2/4)	13 (3/3)	13(3/3)	15** (1/1)	n.a.	n.a.	n.a.
D12S391	18 (3/3)	19 (3/3)	15 (3/3)	21** (1/3)	18 (2/2)	n.a.	18** (1/1)	n.a.	n.a.	n.a.
D13S317	9 (5/6)	13 (6/6)	8 (4/4)	13 (3/4)	8 (4/4)	13(3/4)	10** (2/4)	11(4/4)	9 (2/3)	11(3/3)
D16S539	11 (7/7)	12 (7/7)	11 (6/6)	11 (6/6)	12 (3/4)	14 (4/4)	10 (3/3)	11** (1/3)	10 (3/3)	13(3/3)
D18S51	13 (8/8)	16 (6/8)	19 (6/6)	23 (5/6)	14 (4/4)	14 (4/4)	16 (4/4)	18 (4/4)	14 (3/3)	15 (2/3)
D19S433	15 (2/2)	16.2(2/2)	13** (1/1)	14** (1/1)	14** (1/1)	16** (1/1)	n.a.	n.a.	n.a.	n.a.
D21S11	31(4/6)	32.3(4/6)	25(2/4)	31.2(4/4)	30 (4/4)	32.2(3/4)	29 (2/3)	30 (2/3)	31(2/3)	33.2(3/3)
D22S1045	15 (3/4)	16 (4/4)	15 (2/3)	16 (2/3)	15 (2/2)	16 (2/2)	11** (1/1)	n.a.	n.a.	n.a.
CSFIPO	11 (5/5)	12 (5/5)	11 (4/4)	12 (3/4)	11 (3/3)	12 (3/3)	12 (3/3)	12 (3/3)	10 (3/3)	13 (3/3)
FGA	21 (7/7)	21 (7/7)	19 (4/4)	20 (4/4)	19 (4/4)	25 (3/4)	21 (2/3)	23 (3/3)	22 (3/3)	n.a.
TH01	7 (4/4)	9 (4/4)	7 (3/3)	9 (3/3)	6 (4/4)	7(4/4)	7** (1/1)	9** (1/1)	n.a.	n.a.
VWA	17 (3/3)	17 (3/3)	14** (1/1)	19.2** (1/1)	17 (2/2)	18 (2/2)	n.a.	n.a.	n.a.	n.a.

Table 9.2. Autosomal STR marker values / Budapest-1 laboratory. In the fraction in parenthesis, the numerator shows the number of fingerprint allele detections, the denominator indicates the number of total attempts. The data are from the summary report of September 28, 2015. n.a.: not analyzed; n.v.a. / **: invalid data.

Evaluating the detection of A-STR markers on bone samples from nine skeletons using PCR amplification (Budapest-1)

1. We investigated a total of 18 A-STR markers at our laboratory in Budapest, as the SE33 marker was excluded from Tables 9.1 and 9.2, due to the large number of PCR errors.
2. We conducted the PCR analysis of Anne of Antioch's bone samples with much fewer repetitions (2x-8x) compared to the laboratory in Göttingen, but we were using different PCR kits.
3. The fingerprint alleles of markers D3S1368, D8S1179 and D19S433 and the female skeletons (Anne A., II/109) had to be accepted after 2 trials at most, and thus the criteria were less strict than at the Göttingen laboratory. The result of a single PCR amplification is also displayed.
4. Samples marked with ** are not considered acceptable data.
5. The PCR results of markers with alleles longer than 17 repetitions (D2S1338, D21S11, vWA) show a high degree of variation.

Combined results of the Göttingen laboratory and Budapest-1

With a few exceptions, the A-STR marker results from Göttingen and Budapest are the same; the same results were obtained from the PCR analysis of Béla III and the partially different bone samples of skeleton II/52_3. In the case of every other skeleton, one allele difference occurred, but this was related to the condition of the bone structure. Thus, the differences between the A-STR data from Göttingen and Budapest were the following: only a single different marker in the case of skeleton G5, four markers in the case of II/53,

and six markers for the skeleton of Anne of Antioch. The differences occurred primarily with bone samples with less intact structures, or in the case of markers with long alleles. Three markers were only investigated at the Göttingen laboratory, one marker only at Budapest, and in the case of several markers only one of the laboratories obtained acceptable results (Tables 8.1, 8.2 and 9.1, 9.2).

According to 12th-century burial customs, only royal persons and their family members were buried inside the Royal Basilica of Székesfehérvár, with immediate family members placed next to each other whenever possible (Figure 13). This archaeological observation raised the possibility that skeleton II/52_3, which was buried earlier, could be an Árpád Dynasty King and be closely related to King Béla III. By collating the A-STR data determined via PCR from the laboratories in Göttingen and Budapest, we were able to compile a marker set with consensus alleles. The comparison made with consensus alleles indicated that there were not any shared alleles between the two skeletons in the case of 5 markers out of 20 (Table 10). This contradicts the hypothesis of a father-son relationship. Instead, the grandfather, Béla II (the Blind), or the father's two brothers, László II and István IV, could be considered (Olasz et al. 2018). The possibility of burial next to the uncles has very little support among historians for the following reasons: László II and István IV were pretenders, and thus it is unlikely that Béla III and his wife Anne of Antioch would have been buried next to them. It was also found that out of the five different chromosome markers, in the case of at least three, the molecular structure of the region where a given marker was located interfered with the detectability of the A-STR marker, and we also had to account for

the technical errors of PCR amplification. Thus, we decided that we should investigate these chromosome regions in the next generation sequencing analysis as well.

Skeletons	Béla III		II/52	
	A1	A2	A1	A2
A-STR				
D1S1656	13	17.3	13	17.3
D2S441*	11	11.3	10	10
D2S13388*	17	17	18	18
D3S1358*	15	17	14	14
D5S818	10	12	10	12
D7S820*	10	11	8	9
D8S1179	13	14	12	14
D9S1120	15	16	15	16
D10S1248	13	13	13	13
D12S391	18	19	18	18
D13S317	9	13	8	13
D16S539	11	12	10	11
D18S51	13	16	13	17
D19S433*	15	16.2	13	13
D21S11	31	32.2	30	32.2
D22S1045	15	16	15	17
CSF1PO	11	12	9	11
FGA	21	21	21	25
SE33	20	27.7	n.a.	n.a.
TH01	7	9	9	9.3
vWA	17	17	16	17

Table 10. The joint (consensus) A-STR marker data for Béla III and skeleton II/52 from Göttingen and Budapest aimed at revealing family relations. The markers with distinct alleles are marked with a star in the list. The identical A-STR alleles of the two skeletons are highlighted in bold. Marker SE33 is probably unusable due to the large number of PCR artifacts and is thus disregarded.

We show the gender determination results based on the amelogenin gene in Table 11. The gender of the fetus had remained unknown up to this point.

Skelette Labore	Béla III.	II/52_3	II/53	II/54	II/55	I/3 G5	I/4 H6	A. Anna	Fötus	II/109
Göttingen	X/Y	X/Y	X/Y	X/Y	X/Y	X/Y	X/Y	X/X	X/Y	X/X
Budapest	X/Y	X/Y	X/Y	X/Y	X/Y	X/Y	X/Y	X/X	X/X	X/X

Table 11. *Determining the gender of the investigated skeletons via PCR examination of the amelogenin gene. The Göttingen laboratory incorrectly determined the gender of the fetus as male based on a value measured in a heavily fractured DNA sample. According to several tests we conducted, the fetus is actually female.*

SUMMARY: Opening of the glass containers in the Matthias Church where the skeletons were kept occurred under sterile conditions. The royal couple's skeletons wrapped in textile were not removed. Instead, they were immediately placed back in the open glass containers after they had been checked by touch and were subsequently transported to a sterile operating room at NIO in special shipping containers, where the samples were taken. Preliminary experiments indicated that all of the bone samples were suitable for DNA isolation, but in order to carry out the procedure, the DNA isolation protocol had to be optimized. All of the DNA isolated from bone samples from the Matthias Church could be fit into one of two groups. Less fragmented DNA samples were isolated from the skeletons of Béla III, II/54, II/55, I/3 G5, I/4 H6 and II/109 (group 1), and the probability of

the detection of the alleles is 80-90%, depending on their length. The DNA samples of group 2, consisting of II/52, II/53, Anne of Antioch and the fetus, were so degraded that only a few template DNA strands suitable for amplification could be found. Because of this, the detectability of the A-STR allele decreased dramatically based on the number of repeating units to a frequency of 10%. 20-33 repeat long alleles could no longer be viewed as realistically acceptable.

We studied the A-STR markers of skeleton II/52_3's femur extensively, and during the course of this, we compared them to A-STR markers from other parts of the skeleton and to the marker pattern of Béla III's skeleton as well. This series of studies is important, because it proves that the bones, which had become mixed up in the period following exhumation, were then correctly sorted using anthropological methods and reassembled into a whole skeleton, while at the same time, these data disprove the opinion of Éry and her working group that we no longer have the original skeleton in the Matthias Church.

Regarding the autosomal STR-marker investigation, it should be highlighted that the unique characteristics and structure of the chromosome region where a given marker is located affects detectability.

With a few exceptions, the A-STR marker results from Göttingen and Budapest were identical, and the differences occurred mostly with bone samples with less-than-intact structures or at markers with long alleles. If we merge the A-STR data of skeleton II/52 and Béla III's skeleton obtained through PCR analysis in the Göttingen and Budapest laboratories, then we can build a marker set with

consensus alleles. The comparison conducted with consensus alleles showed that using PCR analysis, the two skeletons differ in 5 markers out of 20 due to allele length difference. This contradicts a father-son relationship, and instead the grandfather, Béla II (the Blind), could be considered as a possibility. The possibility of burial next to uncles has little support from historians, since László II and István IV were pretenders, and thus it is unlikely that III Béla and his wife, Anne of Antioch, would be buried next to them.

CHAPTER EIGHT

ERZSÉBET CSERNÁK, JÁNOS MOLNÁR,
ZOLTÁN SZENTIRMAJ

PCR AND NGS INVESTIGATIONS

1. PCR and NGS analysis of the chromosome regions of five selected A-STR markers

As we shall see later, the allele data for these markers are different in the case of Béla III and skeleton II/52_3, and this necessitated the DNA sequence analysis of the chromosome regions that belong to these markers. More specifically, we only wished to sequence the five A-STR marker regions, the alleles of which were different in the DNA samples isolated from the bones of Béla III and skeleton II/52 using PCR analysis. These markers were the following: D2S441, D2S1338, D3S1258, D7S820 and D19S433. We analyzed the template DNA strands generated in the PCR-amplified chromosome region after sequence capture, followed by magnetic separation and subsequent amplicon library creation.

Tarsus-1 belonged to skeleton II/52_3, while tarsus-2 belonged to the skeleton of Béla III. We took two bone samples for both skeleton

II/52_3 and Béla III, and to distinguish between them, we designated them Budapest-1 (B1) and Budapest-2 (B2). We isolated DNA for sequencing from these separately. The DNA isolated from sample B1 was marked A1, while DNA isolated from sample B2 was marked A2. The results from the study of DNA samples A1 and A2 were designated M1 and M2 in the case of skeleton II/52, while they were designated M3 and M4 in the case of Béla III (Figure 34).

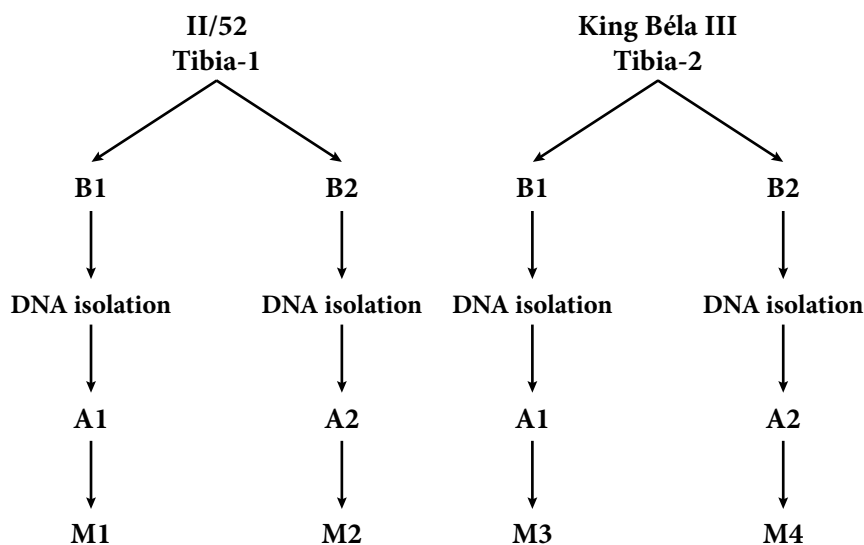


Figure 34. Workflow of the next generation sequencing conducted on the following A-STR markers' chromosome regions of the skeletons II/52_3 and Béla III: D2S441, D2S1338, D3S1358, D7S820 and D19S433.

We used the “Short Tandem Repeat DNA Internet Database” (STRBase) operated by the US National Institute of Standards and Technology to compare STR-marker sequencing data (Butler – Reeder 1997; Ruitberg et al. 2001). The following data can be found in the database: name and alternate name, the precise chromosome location, GenBank availability, the structure of repeats, the PCR primer sequences necessary for the investigation of the regions, the allele sequences attributed to allele lengths, and the region of the repetitions expressed in basepairs (bp). The manufacturer of the kit needed for STR detection supplies a marker set (ladder) corresponding to each marker’s allele length, i.e. to the number of repeating units, and we compare the PCR products to these. It is important to note that in some cases the chromosome region in question has such a complicated structure that the consensus allele length defined by the ladder is not necessarily identical to the number of repeating units in a given sample. This is the situation in the case of marker D19S433. (The STRbase database notes: “nomenclature for supplied allelic ladders does not agree with repeat structure shown”.)

The individual marker’s chromosome location, the genome region denoting its location, and the PCR primer we used can be found in the Chapter entitled *Investigation of bone samples and methods*. The results are presented in Table 12.

III/52_3											
Marker	PCR				NGS				Fp. allele		
	A1	Ratio	A2	Ratio	M1	Read no.	M2	Read no.	Read no.		
D2S441-B1	n.v.a.	-	n.v.a.	-	10	A1=8376	10	A1=7682	10/10		
D2S441-B2	10	11/11	10	11/11	10	A2=7682	10	A2=7682	17/20		
D2S1338-B1	n.v.a.	-	n.v.a.	1/12	18	A1=481	17	A1=1680	14/14		
D2S1338-B2	20	4/4	25	2/4	20	A2=1171	20	A2=750	8/9		
D3S1358-B1	14	4/12	14	5/12	14	A1=19747	13	A1=5400	12/13		
D3S1358-B2	14	3/3	14	3/3	14	A2=19747	14	A2=37062			
D7S820-B1	9	2/4	n.v.a.	0/4	8	A1=446	8	A1=790			
D7S820-B2	8	6/6	9	2/6	n.a.	-	9	A2=195			
D19S433-B1	13	4/12	n.v.a.	0/12	12	A1=405	12	A1=800			
D19S433-B2	13	3/3	13	3/3	13	A2=3480	13	A2=7845			
Béla III											
Marker	A1	Ratio	A2	Ratio	M3	Read no.	M4	Read no.	Fp. allele		
D2S441-B1	11	7/10	11.3	7/10	11	A1 = 666	11	A1 = 1404	11/11.3		
D2S441-B2	11	5/5	11.3	5/5	11.3	A2 = 288	11.3	A2 = 953	17/19		
D2S1338-B1	17	4/14	n.v.a.	-	17	A1 = 371	17	A1 = 128	14/15		
D2S1338-B2	17	7/7	17	7/7	19	A2 = 211	n.a.	-	8/-		
D3S1358-B1	15	9/14	17	10/14	14	A1 = 4479	14	A1 = 724	13/13		
D3S1358-B2	15	2/29	17	2/2	15	A2=9871	15	A2 = 5940			
D7S820-B1	10	4/4	11	4/4	n.a.	-	8	A1 = 2			
D7S820-B2	10	5/6	11	6/6	n.a.	-	11	A2 = 1			
D19S433-B1	15	9/14	16.2	8/14	12	n.v.a.	13	A1 = 2083			
D19S433-B2	15	2/2	16.2	2/2	13	A1 = 21	13	A2 = 2083			

Table 12. Comparison of the A-STR marker data generated using the PCR method and DNA sequencing in DNA samples isolated from the skeletons of Hungarian Árpád Dynasty kings II/52_3 and Béla III. The bone samples selected can be found in Tables 6 and 7. The data from the Göttingen and Budapest-1 laboratories are displayed separately for each lab, along with the frequency of detectability. B1 and B2 after the marker names refer to the Budapest-1 and Budapest-2 laboratory samples. We display every single piece of sequencing data as a sample, with the allele's coverage (the number of reads that detect them). Abbreviations: A1: allele 1, A2: allele 2: Ratio: the numerator shows the fingerprint allele detection's number, the denominator indicates the number of total attempts. Fp. allele: fingerprint allele; Read no.: number of sequenced DNA strands (coverage).

In the course of sequencing, we obtain either a forward sequence (D2S441, D3S1358) or a reverse sequence (D2S1338, D7S820, D19S443). This is visible in the sequencing diagrams attached, but the following table shows the repeating unit with its forward sequence in all cases (Table 13).

Marker	Skelett	Allel (bp)	Sequenz der Wiederholungseinheit [in Klammern] und am Rand befindliche (obere/untere) Sequenzteile	
D2S441	II/52 M1	A1 = 10	5'-TCTATGAAAAC[TCTA] ₁₀ TATCATAACACC-3'	
		A2 = 10	5'-TCTATGAAAAC[TCTA] ₁₀ TATCATAACACC-3'	
	II/52 M2	A1 = 10	5'-TCTATGAAAAC[TCTA] ₁₀ TATCATAACACC-3'	
		A2 = 10	5'-TCTATGAAAAC[TCTA] ₁₀ TATCATAACACC-3'	
	Béla III. M3	A1 = 11	5'-TCTATGAAAAC[TCTA] ₁₁ TATCATAACACC-3'	
		A2 = 11.3	5'-TCTATGAAAATC[TCTA] ₁₂ -T TATCATAACACC-3' (T del.)	
	Béla III. M4	A1 = 11	5'-TCTATGAAAAC[TCTA] ₁₁ TATCATAACACC-3'	
		A2 = 11.3	5'-TCTATGAAAATC[TCTA] ₁₂ -T TATCATAACACC-3' (T del.)	
D2S1338	II/52 M1	A1 = 18	5'-CTTGGCCT[TGCC] ₇ [TTCC] ₁₁ GTCCTTCCTTCCTCCTGCA-3'	
		A2 = 20	5'-CTTGGCCT[TGCC] ₇ [TTCC] ₁₃ GTCCTTCCTTCCTCCTGCA-3'	
	II/52 M2	A1 = 17	5'-CTTGGCCT[TGCC] ₇ [TTCC] ₁₀ GTCCTTCCTTCCTCCTGCA-3'	
		A2 = 20	5'-CTTGGCCT[TGCC] ₇ [TTCC] ₁₃ GTCCTTCCTTCCTCCTGCA-3'	
	Béla III. M3	A1 = 17	5'-CTTGGCCT[TGCC] ₆ [TTCC] ₁₁ CTCCTGCAATCC-3'	
		A2 = 19	5'-CTTGGCCT[TGCC] ₇ [TTCC] ₁₂ CTCCTGCAATCC-3'	
	Béla III. M4	A1 = 17	5'-CTTGGCCT[TGCC] ₆ [TTCC] ₁₁ CTCCTG.-3'	
		A2 n.a.	n. a.	
	D3S1358	II/52 M1	A1 = 14	5'-GGCTTGCATGTA[TCTA] ₁ [TCTG] ₂ [TCTA] ₁₁ TGAGACAGGGTC-3'
			A2 = 14	5'-GGCTTGCATGTA[TCTA] ₁ [TCTG] ₂ [TCTA] ₁₁ TGAGACAGGGTC-3'
II/52 M2		A1 = 14	5'-GGCTTGCATGTA[TCTA] ₁ [TCTG] ₂ [TCTA] ₁₁ TGAGACAGGGTC-3'	
		A2 = 13	5'-GGCTTGCATGTA[TCTA] ₁ [TCTG] ₂ [TCTA] ₁₀ TGAGACAGGGTC-3'	
Béla III. M3		A1 = 14	5'-GGCTTGCATGTA[TCTA] ₁ [TCTG] ₂ [TCTA] ₁₁ TGAGACAGGGTC-3'	
		A2 = 15	5'-GGCTTGCATGTA[TCTA] ₁ [TCTG] ₂ [TCTA] ₁₂ TGAGACAGGGTC-3'	
Béla III. M4		A1 = 14	5'-GGCTTGCATGTA[TCTA] ₁ [TCTG] ₂ [TCTA] ₁₁ TGAGACAGGGTC-3'	
		A2 = 15	5'-GGCTTGCATGTA[TCTA] ₁ [TCTG] ₂ [TCTA] ₁₂ TGAGACAGGGTC-3'	
D7S820		II/52 M1	A1 = 8	5'-GTCATAGTTTGAATGAAC TAACG[GATA] ₈ GACAGATTGATAGTTT-3'
			A2 = 8	5'-GTCATAGTTTGAATGAAC TAACG[GATA] ₈ GACAGATTGATAGTTT-3'
	II/52 M2	A1 = 8	5'-GTCATAGTTTGAATGAAC TAACG[GATA] ₈ GACAGATTGATAGTTT-3'	
		A2 = 9	5'-GTCATAGTTTGAATGAAC TAACG[GATA] ₉ GACAGATTGATAGTTT-3'	
	Béla III. M3	A1 = n.a.	n. a.	
		A2 = n.a.	n. a.	
	Béla III. M4	A1 = 8	5'-GTCATAGTTTGAATGAAC TAACG[GATA] ₈ GACAGATTGATAGTTT-3'	
		A2 = n.v.a.		
D19S433	II/52 M1	A1 = 12	5'-AAGGAAAG[AAGG] ₁ [TAGG] ₁ [AAGG] ₁₀ AGAGAGGAAGAAAGAGAGAA-3'	
		A2 = 13	5'-AAGGAAAG[AAGG] ₁ [TAGG] ₁ [AAGG] ₁₁ AGAGAGGAAGAAAGAGAGAA-3'	
	II/52 M2	A1 = 12	5'-AAGGAAAG[AAGG] ₁ [TAGG] ₁ [AAGG] ₁₀ AGAGAGGAAGAAAGAGAGAA-3'	
		A2 = 13	5'-AAGGAAAG[AAGG] ₁ [TAGG] ₁ [AAGG] ₁₁ AGAGAGGAAGAAAGAGAGAA-3'	
	Béla III. M3	A1 = n.v.a.	n. v. a.	
		A2 = n.v.a.	n. v. a.	
	Béla III. M4	A1 = 13	5'-AAGGAAAG[AAGG] ₁ [TAGG] ₁ [AAGG] ₁₁ AGAGAGGAAGAAAGAGAGAA-3'	
		A2 = 13	5'-AAGGAAAG[AAGG] ₁ [TAGG] ₁ [AAGG] ₁₁ AGAGAGGAAGAAAGAGAGAA-3'	

Table 13. *Repeating units consisting of 4 bases in square brackets, and the number of repetitions detected per allele. A mutated tetramer unit became inserted between the repeating units in markers D2S1334, D2S1334 and D19S443, which counts towards the allele length. The upper (5') and lower (3') genominal DNA sequence parts in contact with the repetitions are also shown.*

2. Evaluating the sequencing data

D2S441: The PCR and NGS results for skeleton II/52 and Béla III were exactly the same. Thus, in this case the NGS result confirmed the PCR result that the A1 and A2 alleles of Béla III and skeleton II/52 are different from each other. Due to the short allele lengths, no PCR artifact can be seen (Table 13, Figure 35).

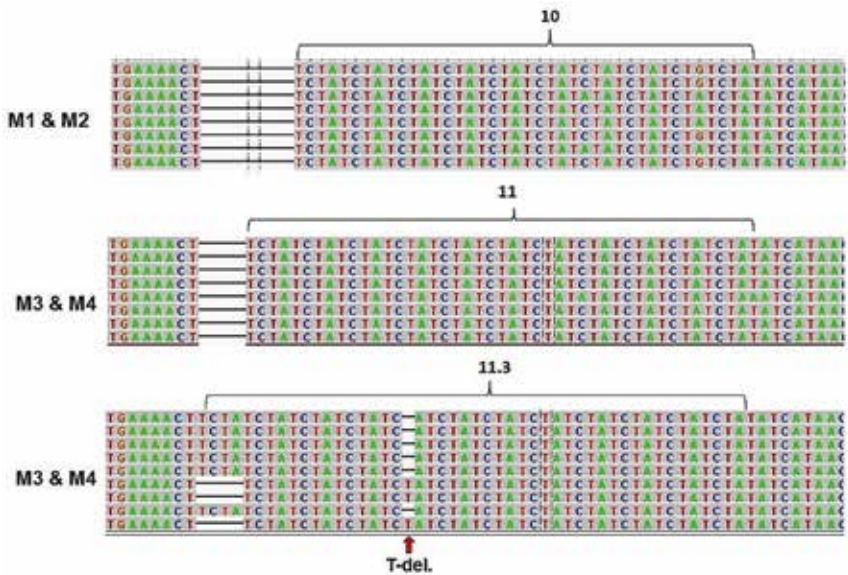


Figure 35. Marker D2S441, forward sequence, TCTA repeating units are in brackets. The fourth repeating unit contains a T deletion, which resulted in a truncated TCA sequence and an allele length of 11.3.

D2S1338: The PCR marker data for skeleton II/52 and Béla III differ. During the next generation sequencing, it turned out that in skeleton II/52's M1 sample taken from the same DNA isolate sample as the M2 sample, there is a longer A1 that occurs in a smaller proportion (probably a “stutter” artifact) than the A1 allele found in M2 which is one unit shorter, but occurs more frequently.

Aside from the above, the A1 allele data of skeleton II/52 and Béla III are identical (Table 13 and Figure 36).

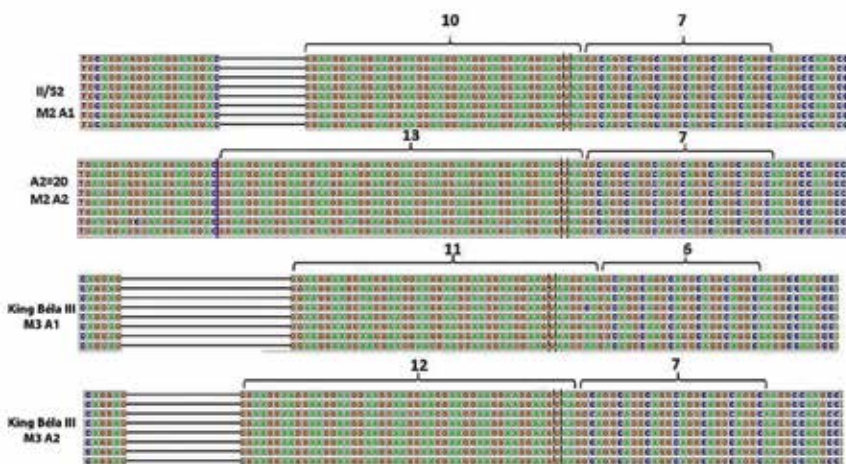


Figure 36. Marker D2S1338, reverse sequence. Repeating units GGAA (motif) are in brackets. The A1 M2 sample of skeleton II/52 is identical to Béla III's A1 M3 sample (allele length 17 repeat.).

D3S1358: The M1, M2 and M3 analysis results for skeleton II/52 and Béla III are completely identical. The allele length is 14 repeat. In sample M4, some of the alleles are 14 repeat and 15 repeat long. In samples M3 and M4, there is G>A mutation, the result of which is that reads with equal lengths but different DNA sequences can be seen (Table 13, Figure 37).

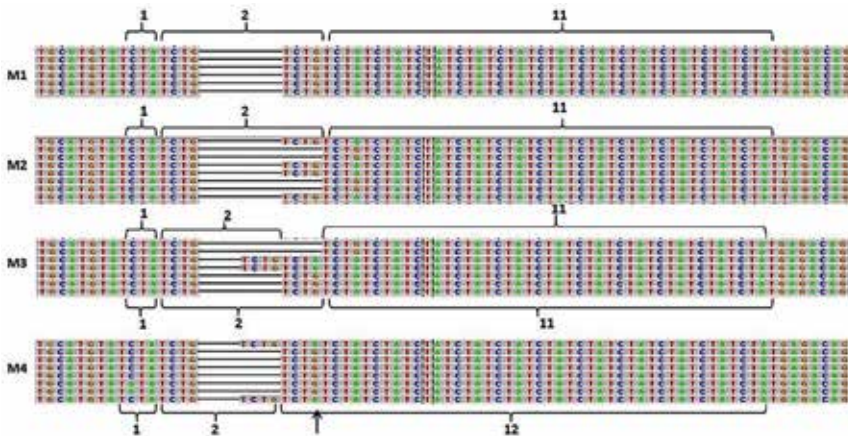


Figure 37. D3S1358 marker, forward sequence, TCTA motifs are in brackets. Samples M2, M3 and M4 contain stutter artifacts; this is the result of loop development during amplification and it caused the reads to lengthen or shorten (Figure 30). In the area marked by the arrow, a G>A sequence can be seen on the reverse strand. A severely truncated read can also be seen due to a PCR artifact (not marked).

D7S820: In the sample (M1, M2) from skeleton II/52, this marker was much easier to analyze both via PCR and NGS compared to Béla III's DNA sample (M3, M4). The structure of Béla III's skeleton is better preserved, but despite this, the DNA extracted from it – as mentioned before several times – was more fragmented than the sample of the less well preserved skeleton II/52. In the case of skeleton II/52, the A2 allele could not be detected in the Göttingen laboratory, not even with sequencing, but the A1 alleles detected via PCR and NGS were identical. The marker alleles detected in the bone samples from Béla III were different from the above PCR data. We did not obtain an evaluable M3 result via sequencing, but the

A1 allele we obtained from M4 and the A1 allele of skeleton II/52 were completely identical. The above difficulties in detection may have been due to other reasons besides DNA fragmentation, since in the area marked by an arrow on the figure, sequence variation can be seen, which is equivalent to A>G transition on the reverse strand, and T>C transition on the forward strand, which influenced hybridization of the PCR primer. We know that DNA sequence polymorphism can occur within or in nearby repeating sequences. If the base swap is in accordance with the primer binding site, as in this case, then hybridization of the primer cannot occur or only occurs at a lower hybridization temperature, and thus the marker on the template will not be detectable. This was probably the situation that rendered detection of the D7S818 marker via PCR and NGS technologies more difficult and was responsible for the formation of the PCR artifact (Table 11, Figure 38). Despite the above technical difficulty, the bone samples of skeleton II/52 and Béla III are identical in terms of the 8-bp allele length.

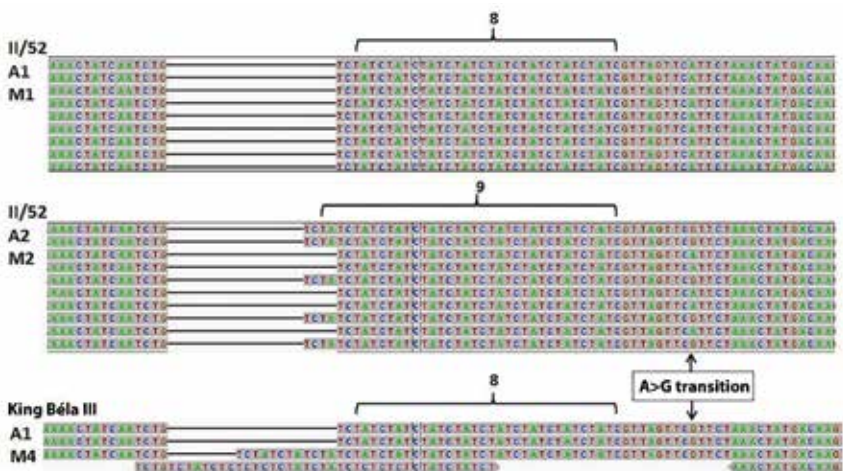


Figure 38. D7S818 marker, reverse sequence, CTAT motifs are in brackets. In the area marked by the arrow, on the -3' end of the reverse strand, an A>G sequence variation can be found very close to the repeating sequences, which significantly inhibited hybridization of the reverse primer and caused the result to be of limited value.

D19S433: Analysis of this marker was performed by János Molnár, biologist-bioinformaticist, who ended up facing many difficulties. He found that duplicated or shorter/longer flawed reads and sequence variations occurred quite often. For this reason, he handled the reads individually, while ignoring base differences (sequencing errors) and only taking fully matching ones into account. The M1 and M2 samples of skeleton II/52 were much easier to analyze. 12/13 genotypes were identified. The M1 and M2 sequences taken into account are the following:

13 motif:

AAGGAAAG–AAGGTAGG AAGG AAGG AAGG AAGG AAGG AAGG
AAGG AAGG AAGG AAGG AAGG–AGAGAG

12 motif:

AAGGAAAG–AAGGTAGG AAGG AAGG AAGG AAGG AAGG AAGG AAGG
AAGG AAGG AAGG–AGAGAG

The sequencing data for Béla III were much harder to evaluate, because the M3 sample contained 13 and 15 allele genotypes, while sample M4 contained 11 and 13. Short and long repetitions only occurred in a small percentage and could be classified as PCR artifacts. The accepted final genotype is 13/13.

Sample M3, motif 15 (coverage 21):

AAGGAAAG–AAGG TAGG AAGG AAGG AAGG AAGG AAGG
AAGG AAGG AAGG AAGG AAGG AAGG AAGG AAGG–
AGAGAGGAAGAAAGAGAGAAGATTTTTATTTCGGGTAATGGGTGC

Sample M4, motif 13 (coverage 2083):

AAGGAAAG–AAGG TAGG AAGG AAGG AAGG AAGG
AAGG AAGG AAGG AAGG AAGG AAGG AAGG –
AGAGAGGAAGAAAGAGAGAAGATTTTTATTTCGGGTAATGGGTGC

Based on the above, we can conclude that the number of A2 repeating sequences of the skeletons II/52 and Béla III are the same, 13 motif.

We also analyzed this marker and found numerous PCR artifacts (Figure 39). In the case of both skeletons, there were stutter artifacts; we did not find evaluable alleles in Béla III's M3 samples, while the M4 sample was evaluable. Along with truncated reads, among the repetitions, a couple of base swaps also occurred, out of these we only show one, the T>A base swap (with the arrow). In the PCR analysis conditions there are no DNA repair systems, and thus the PCR artifacts remain, causing evaluation errors in some cases.

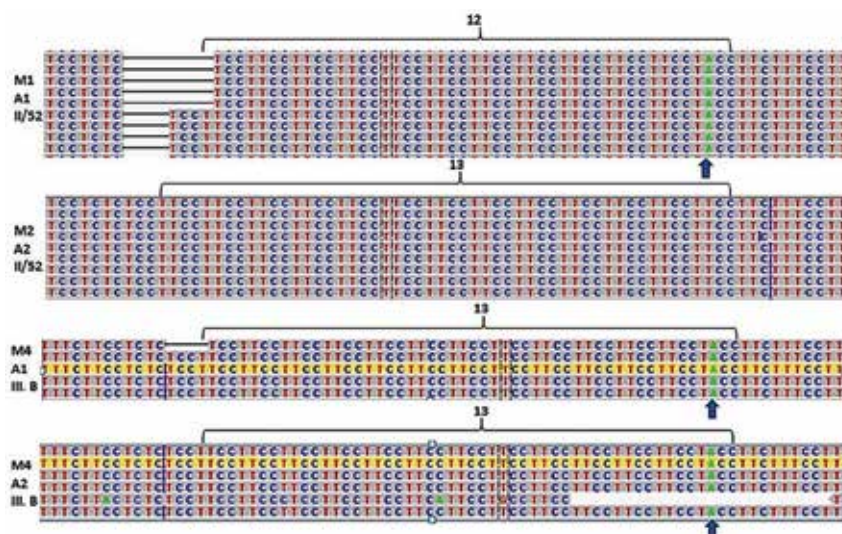


Figure 39. D19S433 marker, reverse sequence, TTCC motifs are in brackets. (The forward motif is AAGG.) T>A sequence variation occurs at the area marked by the arrow on the reverse strand, which is equivalent to an A>T transition on the forward strand. It is located on the -3' end of the DNA strand and may have been caused by a microsatellite error of the Taq polymerase and multiplied further. A severely truncated read can also be seen due to a PCR artifact (not marked).

3. Possible explanation for the differences between PCR and sequencing data

The question arises as to what could have caused the differences shown in Table 12 between the traditional PCR-based A-STR detection and the marker detection via sequencing. When investigating bone samples containing ancient, degraded DNA, several difficulties must be considered.

1. During amplification of repeating sequences the DNA polymerase can slip which causes allele length differences. This phenomenon is called a “stutter” flaw/error. In our case, this phenomenon can be detected very well via NGS, when we see that two parallel DNA libraries launched from the same template of A-STR markers with longer alleles show different sequencing data. This error probably occurred in every marker investigated with the exception of D2S441.
2. The phenomena of allele dropout and “null” (silent) alleles is suggested by the analysis of the D7S820 marker from Béla III’s sample. We know that DNA sequence polymorphism can occur within or in nearby repeating sequences. At the D7S818 marker, on the -3’ end of the sequence motif series a A>G base swap can be seen near the repeating sequences. If the base swap is according to its primer connection area on the DNA template – and this is the case here – then hybridization of the PCR primer either cannot occur or can only occur at lower hybridization temperatures, and thus the marker on the template will either not be detectable at all or be very hard to detect.

3. A new allele could randomly appear next to one of the real A-STR allele pairs during the allele length analyses, which could cause a problem with the evaluation. This is what we call “peak outside normal allele length”. The peaks can be longer, shorter, or the same length as the corresponding consensus alleles. This phenomenon could theoretically occur with markers D2S1338 and D19S433. In the Göttingen laboratory, an allele length (25/-) only appeared with the D2S1338 marker of skeleton II/52 once out of 13 attempts, which is a significant difference compared to both the results of the Budapest-1 laboratory achieved with a different detection kit, and the results of sequencing as well. For this reason, it could not be accepted as a fingerprint.
4. The Göttingen laboratory mentions some technical problems which made A-STR marker investigations more difficult and affected the results: (a) The remaining samples of the DNA isolated from Béla III’s tarsus returned from Dr P.N. from the USA may have become contaminated. (b) The marker results obtained from the DNA isolated from the femur of skeleton II/52_3 were different in three cases from the results obtained from the DNA of the two tarsi. In this case too, the possibility of severe contamination was considered, as well as the possibility of PCR artifact errors described above. The possibility of the femur belonging to another individual was ruled out by the comparative analysis described in Section 6 of Chapter 7, as well as by the Budapest-1 laboratory’s mitochondrial DNA analysis (see Table 7). (c) There is a suspicion of an artifact in the case of the 17.2 allele of marker D19S433 of skeletons II/53 and II/54.

SUMMARY: The D2S441 markers of skeleton II/52 and Béla III are shown to differ from each other, not just with PCR but with sequencing as well. The DNA sequence analysis also showed that another 4 traditional PCR differences of marker 1 alleles were shown to be identical after all. All of this means that out of the 20 A-STR markers of the Árpád Dynasty King labelled II/52 and King Béla III 19 are identical, which points toward close relatedness. In the human population a subset of STR alleles differs from the reference allele variants only in one or more basepairs. The alleles which contain sequence variations (SNP) and differ from unchanged alleles only to a small degree are called microvariant alleles. Microvariant alleles are no different in length from consensus alleles. The first allele (A1) of the D3S1358 marker of both skeleton II/52 and Béla III shows the following microvariant sequence: TCTA [TCTG]₂ [TCTA]₁₂, which is different from the consensus variation of the marker (TCTA [TCTG]₃ [TCTA]₁₁), but the allele lengths are the same. Furthermore, in marker D7S820, before the repeating sequences on the 5'-end, we found C>T sequence variation (SNP) in both skeletons' samples. The same sequence variations found in both person II/52 and King Béla III occur in such a way only if they are directly related.

The differences between the PCR and sequencing data may have several explanations, such as a broken (fragmented) DNA strand hybridizing with another DNA strand in a flawed way, reproducible artifacts of the samples, the DNA sample returned to us being contaminated, or the above mentioned PCR amplification error.

CHAPTER NINE

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VERENA SEIDENBERG, ZOLTÁN SZENTIRMAY,
GÁBOR TUSNÁDY

STATISTICAL AND GENETIC INVESTIGATIONS FOR THE PURPOSE OF PERSONAL IDENTIFICATION

1. Statistical analysis of family relations based on the A-STR markers of Béla III and skeleton II/52_3

The analysis was conducted by Dr Gábor Tusnáy, using his own log-likelihood method (see the Chapter *Investigation of bone samples and methods*).

The starting points and parameters are the following:

a) The skeleton marked II/52_3 is male and was buried in the 12th century. In the analysis of family relations, only those kings were considered, who were buried in the 12th century in the Royal Basilica of Székesfehérvár, and whose skeletons could have been transported from there to the Matthias Church. These are the following: Béla II

(the Blind) (†1141), Géza II (†1162), László II (†1163), István IV (†1165) and Béla III (†1196).

b) Autosomal STR (A-STR) marker analyses were conducted in the laboratories of Dr Susanne Hummel (Göttingen, Germany), and Dr Judit Olasz (Budapest, laboratory-1), while the DNA sequencing was carried out at the Tumour Pathology Centre of the National Institute of Oncology (Budapest, laboratory-2).

c) With regard to the fact that in our investigations we worked with fragmented DNA samples isolated from ancient bones, the CODIS-13 A-STR markers were increased to a total of 20 markers in the Göttingen laboratory, and to a total of 18 in the Budapest laboratory.

d) We indicated the incidence rate and mutation rate of the alleles for every marker.

e) The statistical analysis was conducted using the latest validated measurement data.

f) Using traditional PCR methods, skeleton II/52 and Béla III's genotypes were found to have 4 differences out of 20 A-STR markers in the Göttingen laboratory, and 5 differences out of 20 in the Budapest-1 laboratory. The 5 differences were investigated with next generation DNA sequencing analysis as well in the Budapest-2 laboratory, and only one of them could be confirmed. In the course of statistical processing, however, we considered the allele data of all three laboratories individually and not as consensus data.

g) The statistical analysis considered varying degrees of probabilities of measurement errors. At higher measurement error probabilities, the statistical analysis showed skeleton II/52_3 to be Béla III's father, while at smaller ones, it showed him to be his grandfather. The result of the statistical analysis is displayed in Tables 14/A/B/C/D.

Table 14/A

Relational neglog-likelihood					
Error probability 0.12500					
FATHER	GRAND-FATHER	UNCLE	NON RELATED	DIFF.	A-STR
7.8165	8.2296	8.5249	8.9458	0.4131	D1S1656
8.2421	6.7098	6.3790	6.1309	-1.5323	D2S441
9.0463	8.8645	8.7846	8.7107	-0.1818	D2S1338
7.1077	6.1455	5.8762	5.6643	-0.9622	D3S1358
4.7313	4.9018	4.9993	5.1075	0.1705	D5S818
10.7437	10.1016	9.8889	9.7137	-0.6421	D7S820
4.8872	5.0714	5.1780	5.2974	0.1842	D8S1179
3.8043	3.9025	3.9555	4.0114	0.0982	D9S1120
2.3701	2.8852	3.2960	4.0056	0.5151	D10S1248
6.5607	6.8570	7.0462	7.2798	0.2962	D12S39
8.4157	8.8384	9.1436	9.5851	0.4227	D13S313
6.1300	6.4897	6.7336	7.0569	0.3597	D16S539
9.3674	9.9280	10.3994	11.3213	0.5606	D18S51
7.5888	7.1716	7.0141	6.8780	-0.4173	D19S433
8.5171	8.8779	9.1228	9.4479	0.3608	D21S11
4.7118	4.9335	5.0661	5.2189	0.2218	D22S1045
4.5013	4.7181	4.8472	4.9954	0.2168	CSF1PO
5.2315	5.6549	5.9609	6.4040	0.4234	FGA
4.1589	4.1589	4.1589	4.1589	0.0000	SE33
6.8308	7.1226	7.3082	7.5363	0.2918	TH01
3.4368	3.6483	3.7736	3.9168	0.2115	VWA
134.2000	135.2107	137.4572	141.3866	1.0106	TOTAL

Table 14/B

Relational neglog-likelihood					
Error probability 0.06250					
FATHER	GRAND-FATHER	UNCLE	NON RELATED	DIFF.	A-STR
7.5391	7.9880	8.3211	8.8241	0.4489	D1S1656
9.3893	6.3393	5.9498	5.6702	-3.0500	D2S441
10.2740	9.8306	9.6658	9.5244	-0.4435	D2S1338
8.2966	6.1072	5.7398	5.4717	-2.1894	D3S1358
5.3011	5.5358	5.6776	5.8429	0.2346	D5S818
11.7512	10.4430	10.1320	9.8951	-1.3082	D7S820
4.9987	5.2727	5.4443	5.6514	0.2740	D8S1179
4.0246	4.1575	4.2313	4.3109	0.1330	D9S1120
2.0962	2.6352	3.0770	3.8880	0.5390	D10S1248
6.6653	7.0412	7.3001	7.6504	0.3759	D12S39
8.1888	8.6557	9.0089	9.5597	0.4669	D13S313
5.7884	6.1723	6.4388	6.8033	0.3839	D16S539
9.4988	10.0754	10.5698	11.5898	0.5766	D18S51
8.8130	7.5453	7.2384	7.0038	-1.2677	D19S433
9.0230	9.4206	9.7005	10.0906	0.3976	D21S11
4.7665	5.0383	5.2081	5.4127	0.2718	D22S1045
4.9447	5.2514	5.4492	5.6961	0.3067	CSF1PO
4.9741	5.4299	5.7706	6.2913	0.4558	FGA
5.5452	5.5452	5.5452	5.5452	0.0000	SE33
6.4715	6.7922	7.0018	7.2673	0.3207	TH01
3.4433	3.7053	3.8675	4.0613	0.2620	VWA
141.7934	138.9822	141.3375	146.0502	-2.8112	TOTAL

Table 14/C

Relational neglog-likelihood					
Error probability 0.03125					
FATHER	GRAND-FATHER	UNCLE	NON RELATED	DIFF.	A-STR
7.3389	7.7955	8.1371	8.6601	0.4567	D1S1656
10.6591	6.1118	5.7098	5.4239	-4.5474	D2S441
11.5741	10.7522	10.5051	10.3072	-0.8219	D2S1338
9.5689	6.0037	5.6077	5.3248	-3.5653	D3S1358
5.6832	5.9522	6.1198	6.3212	0.2690	D5S818
12.9438	10.9328	10.5729	10.3089	-2.0110	D7S820
5.0455	5.3699	5.5827	5.8532	0.3244	D8S1179
4.1475	4.2993	4.3848	4.4783	0.1518	D9S1120
1.9651	2.5091	2.9576	3.7923	0.5440	D10S1248
6.7061	7.1190	7.4143	7.8351	0.4130	D12S39
8.0006	8.4771	8.8412	9.4197	0.4765	D13S313
5.5752	5.9644	6.2360	6.6101	0.3891	D16S539
9.8917	10.4732	10.9745	12.0271	0.5814	D18S51
10.0933	7.6026	7.2252	6.9518	-2.4907	D19S433
9.5974	10.0031	10.2911	10.6971	0.4057	D21S11
4.8525	5.1485	5.3374	5.5706	0.2959	D22S1045
5.2785	5.6332	5.8725	6.1878	0.3547	CSF1PO
4.8132	5.2759	5.6244	6.1637	0.4627	FGA
6.9315	6.9315	6.9315	6.9315	0.0000	SE33
6.2515	6.5787	6.7938	7.0682	0.3272	TH01
3.4509	3.7367	3.9175	4.1385	0.2858	VWA
150.3685	142.6702	145.0369	150.0710	-7.6984	TOTAL

Table 14/D

Relational neglog-likelihood					
Error probability 0.01563					
FATHER	GRAND-FATHER	UNCLE	NON RELATED	DIFF.	A-STR
7.2254	7.6839	8.0275	8.5552	0.4584	D1S1656
11.9891	5.9912	5.5865	5.2993	-5.9979	D2S441
12.9170	11.6080	11.2968	11.0599	-1.3090	D2S1338
10.8952	5.9301	5.5270	5.2405	-4.9651	D3S1358
5.9149	6.2014	6.3828	6.6046	0.2865	D5S818
14.2336	11.5181	11.1350	10.8586	-2.7154	D7S820
5.0679	5.4184	5.6539	5.9625	0.3504	D8S1179
4.2131	4.3746	4.4663	4.5673	0.1615	D9S1120
1.9011	2.4463	2.8963	3.7366	0.5451	D10S1248
6.7280	7.1590	7.4729	7.9330	0.4310	D12S39
7.8900	8.3687	8.7354	9.3206	0.4787	D13S313
5.4589	5.8492	6.1220	6.4984	0.3904	D16S539
10.4303	11.0137	11.5180	12.5847	0.5834	D18S51
11.4215	7.5739	7.1756	6.8915	-3.8476	D19S433
10.2264	10.6341	10.9239	11.3337	0.4076	D21S11
4.9232	5.2311	5.4298	5.6781	0.3078	D22S1045
5.5122	5.8911	6.1529	6.5086	0.3789	CSF1PO
4.7256	5.1900	5.5402	6.0840	0.4644	FGA
8.3178	8.3178	8.3178	8.3178	0.0000	SE33
6.1333	6.4620	6.6784	6.9550	0.3287	TH01
3.4567	3.7541	3.9443	4.1793	0.2974	VWA
159.5813	146.6166	148.9835	154.1690	-12.9647	TOTAL

Tables 14/A/B/C/D. *Relatedness between Béla III and skeleton II/52_3 at error probabilities of 0.1250, 0.06250, 0.03125 and 0.01563 (12.5%, 6.25%, 3.125% 1.563%). In the table, the data are displayed in a negative logarithmic likelihood format, so the probability of a connection increases as the related likelihood value decreases. The numerical value in the difference (DIFF) column denotes the likelihood difference between grandfather and father; positive values represent a probable father, while negatives represent a probable grandfather. The higher the value of the negative likelihood difference, the higher the probability of the result.*

We gradually decreased the allele detection (measurement) statistical error probability pertaining to skeleton II/52 which was ultimately taken into account in the statistical analyses until we reached 1.563% from 12.5%. The starting value is the error probability of 0.1250, the corresponding total likelihood difference is 1.016. This value suggests that skeleton II/52_3 and Béla III were father and son. As we gradually decreased the probability, we observed the changes in likelihood values. At a measurement error of 0.06250, the total likelihood difference is already at an error probability -2.8112, which suggests that skeleton II/52 and Béla III were grandfather and grandson. The probability of a grandfather-grandson relationship between skeleton II/52 and Béla III gradually increases between measurement error probabilities 0.03125 and 0.01563, and the total likelihood values are -7.6984 and -12.9647, respectively. Accordingly, the statistical analysis strongly suggests that skeleton II/52 is Béla II.

2. Paternal A-STR genotype determined via consensus A-STR and NGS data

Based on Tables 8/1-2 and 9/1-2 and the sequencing data from 5 markers (Table 12), it was possible to determine the A-STR marker genotype of the Árpád Dynasty kings. The common A-STR genotype is shown in Table 15. Amongst other things, this is important because using this, we can draw realistic conclusions about the family relations between individual skeletons with the help of further supplementary data (Y-STR, archaeological, historical, anthropological considerations, radiocarbon dating).

Skeleton Marker	Béla III PCR		Béla III NGS		II/52 PCR		II/52 NGS		II/53		II/54		II/55		I/3 G5		I/4 H6		Árpád Dyn. paternal genot.
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	
Alleles	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	
D1S1656	13	17.3			13	17.3			15	n.a.	15	17	15.3	16	13	17.3	11	17.3	17.3
D2S441*	11	11.3	11	11.3	10	10	10	10	12	14	10	11	11	11	11	11	10	11	11
D2S1338*	17	17	17	19	18	18	17	20	20	21	20	21	17	24	24	25	18	18	17
D3S1358*	15	17	14	15	14	14	14	14	14	n.v.a.	16	17	14	16	14	17	15	19	14
D5S818	10	12			10	12			12	n.a.	9	11	11	12	11	12	10	12	12
D7S820*	10	11	8	11	8	9	8	9	10	12	11	12	9	10	8	12	11	12	8
D8S1179	13	14			12	14			14	n.a.	11	14	13	13	13	13	12	12	14
D9S1120	15	16			15	16			16	16	15	16	15	16	16	16	16	16	16
D10S1248	13	13			13	13			13	13	13	15	13	14	14	15	13	13	13
D12S391	18	19			18	18			18	25	19	23	17	22	15	21	18	18	18
D13S317	9	13			8	13			12	12	11	13	11	13	8	13	8	13	13
D16S639	11	12			10	11			12	13	11	12	12	12	13	11	12	14	11
D18S51	13	16			13	17			14	15	14	17	12	16	19	23	14	14	13
D19S433*	15	16.2	13	13	13	13	12	13	13	14	13	14	13	14	13	14	14	16	13
D21S11	31	32.2			30	32.2			26	28	29	29	30	31	25	31.2	30	32.2	32.2
D22S1045	15	16			15	17			14	15	14	15	12	15	15	16	15	16	15
CSF1PO	11	12			9	11			10	10	12	12	11	13	11	12	14	14	11
FGA	21	21			21	25			20	22	21	23	22	23	19	20	19	25	21
SE33	20	27.7			n.a.	n.a.			n.a.	n.a.	18	28.2	26.2	34.2	18	19.2	22.2	28.2	n. v. a.
TH01	7	9			9	9.3			8	10	6	9.3	6	9.3	7	9	6	7	9
vWA	17	17			16	17			18	19	14	16	16	16	14	19	17	18	17

Table 15. *Determining the genotypes of Árpád Dynasty Kings using PCR A-STR and next generation sequencing (NGS) analyses from the bone samples of Béla III and skeleton II/52_3. A-STR analyses were performed at the same time in the Göttingen laboratory and one of the Budapest laboratories (number 1), while the NGS data came from the other Budapest laboratory (number 2). The table shows the allele lengths of individual consensus markers. The markers for which the allele lengths determined by PCR for Béla III and skeleton II/52_3 were different from each other are marked with *. Identical allele lengths, whichever methods were used to obtain them, are highlighted in bold. For marker SE33, the data is scattered to such an extent that we believe a severe PCR artifact is present, and thus we do not take this marker into account in determining the Árpád Dynasty genotype. n.a.: no data, n.v.a.: no valid data.*

3. Genetic investigation of Y-STR and mtDNA markers

Y-STR marker data:

Y-STR	Béla III	II/52	II/53	II/54	II/55	I/3 G5	I/4 H6
DYS19	16	16	16	16	14	15	14
DYS385	11-13	11-13	11-15	11-14	11-14	12-17	11-16
DYS389 I	13	13	13	13	13	14	13
DYS389 II	33	33	30	30	29	30	29
DYS390	25	25	25	25	25	23	24
DYS391	11	11	10	10	10	10	10
DYS392	11	11	11	11	14	13	13
DYS393	13	13	13	13	13	12	13
DYS437	14	14	14	14	16	14	15
DYS438	11	11	11	11	12	10	12
DYS439	10	10	10	10	13	11	11
DYS448	20	n.a.	20	20	19	21	29
DYS456	16	16	15	17	15	15	16
DYS458	15	15	15	15	18	19.2	17
DYS635	23	23	22	23	23	21	23
GATA H4	13	13	11	12	11	11	11
Marker match	16/16		10/16	11/16	4/16	1/16	4/16

Table 16. 16 consensus Y-STR marker haplotypes based on data from the Göttingen and Budapest-1 laboratories. The Y-STR markers of Béla III and skeleton II/52 are completely identical. In the case of the other skeletons, we indicated identical alleles in bold numbers and highlighted in red the ones which also match with Béla III's markers.

Y-STR haplotypes and genealogical relationships

Determination of the genetic (relational) distance between two individuals is based on Y-STR marker sets and is possible by determining the genetic (allele) distance and with the help of the mathematical formula “Calculate Time to Most Recent Common Ancestor” (TMRCA):

http://www.nevgen.org/NevGen_TMRCA_Calculator.html
<https://genealogy.stackexchange.com/questions/9186/genetic-distance-to-generations-calculation-for-y-str-dna-tests>.

Every anthropologically identified male skeleton has an X/Y genotype, while anthropologically identified female skeletons have X/X genotype. The fetus was proven to be female. The Y-STR markers only show probable hereditary relationship based on allele matches (haplotypes). A total of 16 Y-STR markers were completely identical in the case of King Béla III and skeleton II/52_3 (Table 16); this marker number suggests a lineage relation. Every other skeleton showed at least four Y-STR marker differences: in other words, differing haplotypes from both skeleton II/52 and Béla III, as well as from each other, rendering a relation to Béla III improbable (Table 17, Figure 40).

Probability of family relations

Number of Y-STR markers: 16	Y-STR match						
	16/16	15/16	14/16	13/16	12/16	11/16	10/16
	Close relative	Relative	Probably relative	Probably not relative	Not relative		

Figure 40. Probability of hereditary relations based on Y-marker matching, determined using the TMRCA mathematical formula.

In the case of Y-chromosome STR markers, in a direct father-son relation, there may be numerical changes in repeating sequences or point mutations, which can happen in the following cases: (a) longer repeating sequences, (b) repetitions of some base orders, (c) some chromosome regions, (d) a given marker's higher mutational tendency. Kayser et al. (2000) found 11 Y-chromosome microsatellite tetranucleotide allele length changes in cases of father-son relationships.

The Y-STR data allow us to determine hereditary relations based on paternal haplogroups (the sum of markers) (Walsh 2011); we can obtain these using the YHRD international database (<https://yhrd.org>) and the NEVGEN prediction software (<http://www.nevgen.org>, version 2016). Only skeleton II/54's Y-STR pattern was in the YHRD database containing several hundred thousand entries. The prediction value of the haplogroups is shown through three parameters: fitness score, relative fitness score and prediction probability. The smaller the fitness score and relative fitness score are, the less reliable it becomes, due to the fact that the prediction software's current version does not conform to a great enough degree to the given sample. Detailed data are presented in Table 17.

Skeletons	YHRD database search	Haplogroup prediction	Fitness score	Relative fitness score	Probability %
II/52 and Béla III	Not identical to 145,816 haplotypes	R1a*	34.49	0.73	99.98
II/53	Not identical to 145,816 haplotypes	R1a	48.29	1.02	100
II/54	32 matches found with 145,816 haplotypes	R1a	63.67	1.35	100
II/55	Not identical to 145,816 haplotypes	R1b	38.98	0.84	99.99
I/3 G5	Not identical to 145,816 haplotypes	J1a*	29.04	0.65	30.71
1/4 H6	Not identical to 145,816 haplotypes	R1b*	28.45	0.62	99.72

Table 17. Haplogroup prediction of the male skeletons interred in the Matthias Church based on a search of the YHRD international database. The Y-STR data of Béla III and skeleton II/52 are completely identical. Haplogroup prediction is less reliable in the cases of genotypes marked with a star due to lower fitness and relative fitness score values.

The location of human Y-haplogroups around the world is shown in Figure 40. The Finno-Ugric N1c and N1b haplogroups are clearly distinct from the R1 haplogroup of the Árpáds, and thus they have no direct genetic relations.

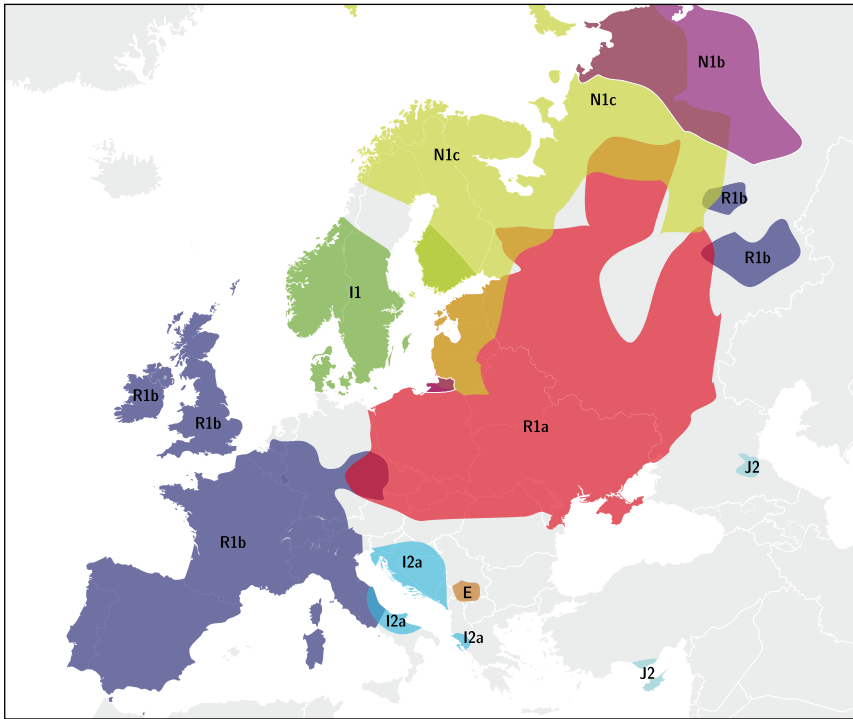


Figure 40. Global distribution of human Y-STR DNA haplogroups. The figure shows the Finno-Ugric haplogroups (N1c, N1b) and Árpád Dynasty haplogroup (R1a) as clearly distinct from each other (compiled by János Jeney, based on MMG 233 2014 Genetics & Genomics Wiki).

4. Genetic investigation of mitochondrial DNA

Analysis of the control region of the mitochondrial DNA (hypervariable region) was conducted using mtDNA isolated from skeleton II/52, Béla III, Anne of Antioch and the fetus in the Budapest-1 laboratory. In the course of this, they compared the SNPs found in the HVR1 and HVR2 variable regions of the mitochondrial

(maternal) genomes of Béla III, Anne of Antioch and skeleton II/52 and conducted a haplogroup prediction as well (Olasz et al. 2018, Table 5). Sequence variations are shown in Table 18.

Béla III	Anne of Antioch	Skeleton II/52_3
HRV1 SNV	HRV1 SNV	HRV1 SNV
16138C	16240G	16126C
16189C	16519C	16192T
16353C		16294T
		16519C
HRV2 SNV	HRV2 SNV	HRV2 SNV
263G	263G	263G
315.1C	315.1C	315.1C
		73G
		517G
Haplogroup prediction	Haplogroup prediction	Haplogroup prediction
Haplogroup: H1b (100 %)	Haplogroup: H1j & H1bz (100 %)	Haplogroup: T (81,07 %)
EMPOP: H1b	EMPOP: H	EMPOP:T2b2b1

Table 18. Comparison of the sequence variations found in the mitochondrial genes of Béla III, Anne of Antioch and skeleton II/52 in the HVR1 and HVR2 hypervariable regions (Olasz et al. 2018, Table 5). HVR: hypervariable region; SNV: single nucleotide variation

The mtDNA of skeleton II/52_3 does not resemble the mtDNA of either Béla III or Anne of Antioch, which means that skeleton II/52_3 cannot be King Béla III's son or brother. The haplogroups estimated using the prediction software EMPOP (<http://empop.online>) and HAPLOGREP (<https://haplogrep.uibk.ac.at>) are the following: Béla III → H1b, Anne of Antioch → H (H1j8 or H1bz), II/52 → T2b (T2b2b1).

The L1 mitochondrial haplogroup first appeared in Africa 120,000-150,000 years ago and then spread outside Africa 55,000-75,000 years ago, and other haplogroups formed. Haplogroup L1 occurred in Asia 40,000-70,000 years ago, and in Europe 35,000-50,000 years ago, by which time haplogroups H and T had also appeared (Figure 42). Mitochondrial haplogroup H has a 46% rate of occurrence (Richards et al. 2000) and wherever it appeared, H1b can also be found, but most commonly (5%) in Eastern and Central Europe (Loogväli et al. 2004). Haplogroup T2b is also common, especially in Northwestern Europe, with a roughly 4.16% frequency (Pala et al. 2012). Figure 42 shows the spread and diversity of mitochondrial haplogroup around the world.

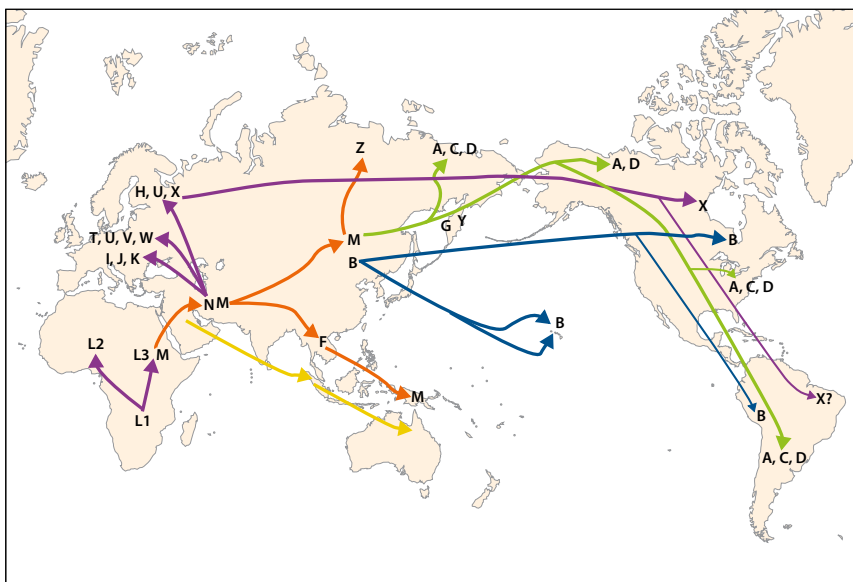


Figure 40. Migration of mitochondrial DNA haplogroups (source: Mathilda's Anthropology Blog; edited by János Jeney).

SUMMARY: Looking at all of the available German and Hungarian A-STR marker data individually instead of in a joint consensus form, and leaving in the PCR data revealed to be incorrect after sequencing, the statistical analysis suggests that skeleton II/52_3 is indeed probably King Béla III's grandfather, Béla II (the Blind). Based on the A-STR marker data investigated by a total of 15 PCRs and another 5 next generation DNA sequencing analyses, it was possible to determine a male marker genotype of the Árpád Dynasty kings (Table 15). The importance of this lies in the fact that by using this, we can draw realistic conclusions regarding the family relations between the skeletons. All of the alleles of the investigated 16 paternal Y-STR markers were completely identical in King Béla III and skeleton II/52. The other male skeletons differed in their haplotypes from Béla III, skeleton II/52 and each other in more than three markers, and this does not suggest a relation with Béla III. At this juncture, we should note that haplogroups N1c and R1a both occur among Finno-Ugric peoples. The ratio of R1a is significantly higher than that of N1c only in the Hungarian and Mordvin peoples. The ratio of N1c is highest in people who lived in relative geographical isolation, e.g. in the Finnish, the Khanty, Lappish and northern Komi, thus in the case of people who are believed to have better preserved the original Finno-Ugric gene pool. The Árpád Dynasty's R1a haplogroup is clearly distinct from these, which suggests there is no genetic relation. With the help of the sum of paternal Y-STR markers (haplogroup), the possibility of genealogical and phylogeographical determination opened up. Skeleton II/52_3 and Béla III share the same R1a haplogroup, so both of them

belong to the Árpád Dynasty. PCR analysis of the Y-chromosome from the rest of the investigated skeletons clearly showed that none of them are from the Árpád Dynasty.

Mitochondrial (maternal) DNA analyses do not rule out that skeleton II/52_3 could be Béla II (the Blind).

CHAPTER TEN

ERZSÉBET CSERNÁK, JUDIT OLASZ,
ZOLTÁN SZENTIRMAJ

(WITH CONTRIBUTION BY BALÁZS HOLCZMANN)

UNIQUE IDENTIFICATION OF THE SKELETONS

1. Origins of Béla III and the persons interred in the Matthias Church labelled II/53, II/54, II/55, I/3 G5 and I/4 H6

All five skeletons were buried in the inner area of the Royal Basilica of Székesfehérvár and all were placed in sarcophagi at the Matthias Church's crypt as it was assumed that they were all relatives of Árpád Dynasty Kings (*Reges Hungariae*). This assumption is not confirmed by the data presented in Table 19.

Skeletons	A-STR match	Y-STR match	Haplotype metapopulation	
			PCR	
Béla III and II/52_3	PCR:17/22 NGS:21/22	16/16	R1a	Middle East and Western Asia vicinity
II/53	9/20	10/16	R1a	Occurs in Africa, Southern Balkans, especially in Greece, Byzantine origin
II/54	11/20	11/16	R1a	Occurrence: Northwestern Russia, Ukraine, Belarus, Northern Poland
II/55	13/20	4/16	R1b	France, Northern Italy, Northern England, Baltic region
I/3 G5	14/20	1/16	J1a	Frequent on the Arabian peninsula, west bank of the Caspian Sea
I/4 H6	13/20	4/16	R1b	Most frequent occurrence: France and Northern Italy

Table 19. Comparison of genetic marker data of the male skeletons.

In the case of skeleton **II/53**, there were no grave goods of any kind, so its Árpád-era origin could neither be confirmed nor disproven by the archaeological investigation. The fact that the person was buried in the inner church's southern aisle does not mean that the person is necessarily of royal origin, because during this period, not only kings were buried – usually in an earth grave – in the inner church. The skeleton's anthropological age is between 21 and 27 years, the time of death by archaeological estimation is between the 14th and 15th centuries. According to A-STR data, he is not related to the Árpád Dynasty. According to the genetic investigation, skeleton **II/53** belongs to the haplogroup R1a. This haplotype can be found in Africa and the Southern Balkans, especially in Greece, which opens up the possibility, that a high-ranking person of Byzantine origin was

buried in the Royal Basilica of Székesfehérvár, whose skeleton now rests in the crypt of the Matthias Church.

The genotype determined by PCR of skeleton **II/54** belongs to haplogroup R1a. This haplogroup occurs in Northwestern Russia, Ukraine, Belarus, Northern Poland and comes from somewhat closer eastern areas than the Hungarians; it does not belong to the Árpád Dynasty. The ethnic group of person II/54 populated Northern Poland: based on this, the outlines of an early Hungarian-Polish connection can be seen. Investigations do not show a close genetic relationship with the R1a haplogroup or the most common N1b/c Finno-Ugric haplogroup.

Based on PCR analysis, skeleton **II/55** belongs to haplogroup R1b. This haplogroup occurs in France, Northern Italy, Northern England and the Baltic region, and is thus similar to the Eurasian, European and Western European metapopulations. This skeleton was also found in the inner area in the southern aisle of the Royal Basilica of Székesfehérvár; its anthropological age is between 36 and 42 years, its height is between 173 and 174 centimetres, and the archaeological estimation for its time of burial is the 15th century. This dating is also confirmed by radiocarbon dating. Based on its haplogroup, skeleton II/55 is that of a person from Western Europe who came to Hungary with an unknown purpose, possibly sent by the Pope.

In 1874, Henszlmann found four stone-lined graves, which were from the age of the Árpáds based on their architecture and level data. The graves marked G5 and H6 are among these graves.

Skeleton **I/3 G5** belongs to haplogroup J1a according to investigation by PCR method. Haplogroup J1a is common on the Arabian peninsula and the west bank of the Caspian sea. The skeleton

was recovered from the Royal Basilica of Székesfehérvár, from a stone-lined grave, its anthropological age is between 36 and 40 years, the archaeological dating places its burial in the first half of the 12th century, there is no data from radiocarbon dating. According to the A-STR and Y-STR mark, this person is a high-ranking foreign individual of unknown origin, who could not be related to the Árpád Dynasty.

Skeleton I/4 H6, found in the grave marked “H”, is from the second half of the 12th century, or possibly the early 13th century. The grave was later robbed, and thus the only grave good found was a bronze ring with an *Agnus Dei* engraving. The use of similar rings began in the 13th century and later occurred in wider strata of society. The ring was lost, which makes precise dating difficult. Archaeological methods could not reveal anything about the societal standing of those buried in the four graves found in the northern aisle, but if we consider the finds from the other two graves which have since been lost, we can form the hypothesis that the person buried was high-ranking because of the arc holder bronze cross that was found in grave E, which is a more delicate piece than the chest cross usually found in graves. Grave I/4 H6 could not be a royal grave, because at the beginning of the 13th century no Hungarian King was buried in the Royal Basilica of Székesfehérvár, and also because the A-STR and Y-STR marker analyses ruled out that possibility. The possibility of an interesting conclusion was opened up by the palaeopathological investigation by Dr Józsa, as he diagnosed Forestier’s disease (Diffuse Idiopathic Skeletal Hyperostosis) on the skeleton. This disease was described by Waldron (1985) as a “new occupational disease” of monks that lived between 1140 and 1540. Janssen et al. (1999) found signs of

Forestier's disease on the skeletons of every inhabitant from a cloister who died between the ages of 43 and 75, while none of the remains from a contemporary cemetery filled with merchants and peasants showed signs of Forestier's disease. If we consider that the northern aisle was usually the burial place of the clergy in medieval times and possibly before, our most reliable conclusion is that a high-ranking clergy person was buried in grave I/4 H6, who belongs to the R1b metapopulation according to Y-STR. The persons belonging here are mostly found in France and Northern Italy. So in theory, it is possible that the person was a high-ranking clergyman sent by the Pope. In this time period, Clement III (1187–1191), Celestine III (1191-1198) and Pope Innocent III (1198-1216) reigned, and if one of them sent an envoy to Hungary, this could have a trace in the Vatican Archives.

In summary: Skeleton II/53 is probably a high-ranking *person of Byzantine origin*. Skeleton II/54 is from the Middle East. Skeleton II/55 belongs to the Western European metapopulation. Skeleton I/3 G5 is a high-ranking person of unknown origin who came from the east. Skeleton I/4 H6 is possibly a very high-ranking clergyman sent by the Pope.

2. Identifying skeleton II/52_3

1. *Radiocarbon dating*. With a 95.4% probability, the radiocarbon dating suggests that skeleton II/52 is from between 1035 and 1155. Béla II's death falls within this interval (1141), while the deaths of Géza II, László II and István IV are 7, 8 and 10 years later, respectively, than the upper limit of the aforementioned interval. Hence, the radiocarbon dating suggests that skeleton II/52 is probably Béla II.

2. *The importance of DNA sequence variations found via NGS.* The 1st allele of the D3S1358 marker of skeleton II/52 and Béla III has the following microvariant sequence: TCTA [TCTG]₂ [TCTA]₁₂, which is different from the consensus variation of the same allele types TCTA [TCTG]₃ [TCTA]₁₁, but the allele lengths are identical. At the 5'-end of marker D7S820, before the repeating sequences, we found a C>T sequence variation in the samples from both skeletons. The presence of both macrovariant alleles in both persons suggests that there may have been a close family relationship between person II/52 and King Béla III.

3. *Genetic investigations with PCR and NGS methods.* Comparison of the A-STR consensus marker data with PCR measurement data revealed 5 marker differences out of 20 between Béla III and skeleton II/52_3. This is too many marker differences for a probable Géza II (father) - Béla III (son) relationship. For this reason, we conducted next generation sequencing analysis on the five differing markers and could only confirm one difference (D2S441), while the rest seem to be PCR artifacts. The difference of only one marker is very much acceptable in the case of a Béla II (grandfather) - Béla III (grandson) relationship.

4. *Statistical analyses.* In our experience, simultaneous analyses of the A-STR markers of the ancient bones of several persons did not produce an acceptable result with traditional kinship statistical software, and a new statistical method became necessary (Zvénigoroszky et al. 2016). We conducted the statistical analyses with the negative log-likelihood method developed by Gábor Tusnády. In the course of the analysis, all of the available German and Hungarian A-STR marker data, as well as the next generation sequencing data were taken into account

separately. We considered various measurement error probabilities as we tried to establish the relationship between skeleton II/52 and Béla III. With a greater measurement error probability, the total likelihood difference suggested a father-son relationship, while with the realistic measurement errors of 0.06250, 0.03125 and 0.01563 the statistical analysis strongly suggests that skeleton II/52 is Béla II (Table 14 A/B/C/D).

5. *The mtDNA haplogroup of skeleton II/52_3.* At this point, the hereditary information of Predslava of Kiev and Béla II must be considered. Béla III's mitochondrial haplogroup is H1b, which is in line with his mother being Euphrosyne, the daughter of Mtsislav I, Grand Prince of Kiev, while his grandmother Helena was the daughter of Uroš I, Grand Prince of Serbia. This is why King Béla III's mtDNA haplogroup is of Kievan origin.

King Béla II's grandfather was Géza I, whose first wife was Princess Sofia of Loon and whose second wife was the Byzantine Princess Synadene. Their child was Álmos, Prince of the Árpád Dynasty (and King of Croatia), whose wife was Predslava of Kiev. Predslava's grandfather was Duke of Bohemia Spytihněv II (1031-1061), whose wife was of German origin. Their daughter, the grandmother of Béla II, married Sviatopolk II, Grand Prince of Kiev. The mother of person II/52_3 was Predslava, whose T2b mitochondrial haplogroup was inherited maternally all the way, which is why it is not surprising that person II/52_3's haplogroup points not to Serbian, but to western origin, which is strong evidence that this skeleton must be Béla II.

The mitochondrial (maternal) haplogroup analysis of Olasz et al. (2018) does not support the hypothesis that skeleton II/52 is Béla III's father, Géza II.

3. Genetic investigation of the fetus found in grave 4, and its family relation to person II/52_3, identified as Béla II (the Blind)

1. *Archaeological data.* In the immediate vicinity of the grave of Béla III and Anne of Antioch, at the feet of the royal couple, Érdy also found a fourth and a fifth grave (Figure 13). According to contemporary customs, only family members were buried next to royalty. In the fourth earth grave, in a wooden coffin sunk into the ground laid the skeletons of a woman and a fetus. The earth grave was around the same age as Béla III's grave. The female skeleton buried in the coffin was lost, but we still have the fetus found among the pelvic bones, it is registered under number II/52_4 (by Éry and her working group). According to archaeologists' opinions, it may have been Béla III's grandchild in grave 4, but the current genetic investigation suggests that it was Princess Predslava of Kiev. The fragmented bones excavated next to the earth grave from a wooden coffin in a brick-lined grave were lost. In view of the contemporary burial customs, we believe that the person in the 5th grave may have been Predslava's husband, Prince Álmos of the Árpád Dynasty.

2. *Genetic investigation of the fetus' skeleton.* Up to this point, the identities of the persons in the fourth and fifth grave were unknown, because only the skeleton of the fetus from the 4th grave was not lost, and thus genetic investigations had to be carried out on it alone. The fetus was identified as male in the Göttingen laboratory. We confirmed twice that that the gender determination was incorrect and that in fact the fetus was female (Olasz, J and Csernák, E). A-STR studies were conducted on the fetus' bones using PCR methods in Göttingen and

Budapest. In the Göttingen laboratory, the A1 and A2 alleles could only be determined in the case of a total of 3 markers (D5S818, FGA, TH01), each of these markers' alleles matched one of skeleton II/52_3's (maternal) alleles. In the other three cases (D10S, D18S51, D21S51), only one of the alleles of the A-STR markers could be determined, none of which were identical to skeleton II/52_3's A-STR markers. The A-STR marker investigation conducted in the Budapest-2 laboratory by Dr Erzsébet Csernák covered 15 markers, out of which in 11 cases we were able to detect both alleles, in two cases we were able to detect one allele, and in another 2 cases we did not arrive at any evaluable results. We evaluated the individual results obtained from the A-STR markers via PCR with next generation sequencing, and in the comparisons, we took into account the sequencing (NGS) data. After summarizing the results of the analyses conducted by the Göttingen and Budapest laboratories and supplementing them with the CSF1PO marker data examined by Dr Judit Olasz, it immediately becomes clear, that out of the 16 A-STR markers which can definitely be evaluated, only markers D10S1248, D21S11 and D22S1045 failed to match with either alleles of the II/52_3 consensus A-STR marker. Extremely long A-STR alleles can be found at the non-matching D21S11 marker, similarly to the SE33 marker which was excluded due to PCR artifacts (see Table 16). The possibility of a PCR artifact arises with a high probability for marker D21S11 as well, and thus going forward we do not take these data into account either. Thus, ultimately we only evaluated the results of fifteen A-STR markers. In a ratio of 13/15, the aggregate A-STR marker results were identical to one of skeleton II/52's alleles (the maternal one): this points to a close family relationship between the fetus and skeleton II/52 (Table 20).

	Árpád Dynasty consensus II/52 A-STR		Fetus, Göttingen		Fetus, Budapest				II/52 mtDNS haplogroup T2b2b1
			II/52_3, A-STR		II/52_3, A-STR				Fetus mtDNS haplogroup T2b
A-STR	A1	A2	A1	A2	A1	A2	A1	A2	
D1S1656	13	17.3	17.3	17.3	13	17.3	13	17.3	
D2S441	10	10	n.a.	n.a.	11.3	10**	sequencing		
D2S1338	20	18	n.a.	n.a.	17**	20**	sequencing		
D3S1358			n.a.	n.a.	15*	16*	15*	16*	
D3S1358	14	14	n.a.	n.a.	14**	14**	sequencing		
D5S818	10	12	12	13	n.a.	n.a.	n.a.	n.a.	
D7S820	8	9	n.a.	n.a.	8**	9**	sequencing		
D8S1179	12	14	n.a.	n.a.	n.a.	13*	n.a.	n.a.	
D9S1120	15	16	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
D10S1248	13	13	n.a.	n.a.	12	14	12	14	
D12S391	18	18	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
D13S317	8	13	12	n.a.	n.a.	n.a.	n.a.	n.a.	
D16S539	10	11	n.a.	n.a.	11	12	11	12	
D18S51	13	17	19	n.a.	17	19	17	19	
D19S433			30.2*	n.a.	14*	15*	n.a.	15	
D19S433	13	13	30.2	n.a.	13**	13**	sequencing		
D21S11	30	32.2	30.2	-	29.2*	30.2*	n.a.	30.2*	
D22S1045	15	17	n.a.	n.a.	11	16	11	16	
CSF1PO	9***	11***	9***	11***	n.a.	n.a.	n.a.	n.a.	
FGA	21	25	20	25	20	25	20	25	
TH01	9	9.3	9	9.3	6	9.3	6	9.3	
vWA	16	17	n.a.	n.a.	17	18	17	18	

*Table 20 Comparing the A-STR markers of the fetus with skeleton II/52_3's similar markers. Matching alleles of the A-STR investigation conducted on the fetus' skeleton in Göttingen and Budapest with the A-STR alleles of skeleton II/52_3 (highlighted in bold numbers and yellow). *: PCR-determined data; **: Sequencing data; ***: The A-STR marker CSF1PO was only investigated by Dr Olasz in the Budapest-1 laboratory, her data are displayed in the table; n.a.: no data; n.v.a.: no valid data; A1/2: allele.*

The mtDNA HVR 1/2 genetic investigation of the fetus' skeleton was conducted by Dr Olasz. She found that the fetus could not be Béla III's grandchild (one of his daughter's children), as its mitochondrial DNA differs from that of Anne of Antioch. The fetus does not have the 16240G specific to the Queen, and the Queen does not have 16294T and 16296T SNV found in the fetus.

According to Balázs Holczmann's hypothesis, if the maternal alleles of the fetus match with the maternal alleles of skeleton II/52_3 (they match in a ratio of 13/15), or its mtDNA haplogroup is T2b2b1, then the female skeleton is Predslava of Kiev, while skeleton II/52_3 is Béla II (the Blind) (Holczmann 2019a). The sequence variations 16194T and 16296T found in the mtDNA HVR-1 region of skeleton II/52 determine the fetus' T2b haplogroup by themselves (Pike et al. 2010). Thus, the fetus' maternal genetic markers and mitochondrial haplogroup match the maternal markers of skeleton II/52. This is possible only if the mother of the fetus is Predslava of Kiev, and the fetus is Béla II's sibling (see Figure 44). 16294T SNV can be found not only in the fetus, but in skeleton II/52 (Béla II) as well, which serves as further proof that the fetus is in fact Béla II's sibling.

The Ukrainians are proud of their royal connections and published a series of stamps on the Kievan wives of European Kings. Among these, the wives of Hungarian Kings were the following: Euphemia of Kiev, wife of Kálmán; Anastasia of Kiev, wife of András I; Predslava of Kiev, wife of Álmos; and Euphrosyne of Kiev, wife of Géza II (Figure 43). Predslava married Prince Álmos of the Árpád Dynasty, their later fate is still unknown.



Figure 43. Memorial stamp series of the Kievan wives of European Kings. Hungarian relations:

Column 1, row 2: Euphemia of Kiev, wife of Kálmán

Column 1, row 1: Anastazia of Kiev, wife of András I

Column 1, row 4: Predslava of Kiev, wife of Álmos

*Column 3, row 4: Euphrosyne of Kiev, wife of Géza II
(Holczmann, BO, personal communication)*

The lives of Predslava and Béla II were researched, organized and interpreted by Holczmann; furthermore, it was he who found the stamps (Holczmann 2019a; 2019b).

4. Evidence from archaeological, anthropological age determination, radiological morphological sequencing and genetic analyses

1. Every skeleton had been buried in the inner area of the Royal Basilica of Székesfehérvár. With the exception of Béla III and Anne of Antioch, the skeletons were moved to the sarcophagi in the crypt of the Matthias Church under the pretext of them being relatives of Árpád Dynasty Kings, Princes or highborn (*Reges Hungariae*). We quote three of the *Reges Hungariae* listed in the “*Codex Diplomaticus Hungariae Ecclesiasticus ac Civilis. Ab anno Christi 1367–1374*”: “*Relatio Ladislai Ducis Palatini per Blasium Notarium*”, “*Sigillum istud reformarunt Sigismundus, Ladislaus Posthumus et Wladistaus II*”, “*Paulus, filius Nicolai de Scephlak, pro quo Ladislaus de Koxo cum procuratoriis litteris*”. None of the persons marked II/52_3, II/53, II/54, II/55, I/3 G5 and I/4 H6 could be considered *Reges Hungariae* as described above.

2. Éry and her colleagues conducted anthropological dating on the skeleton, which was found in the 3rd grave and re-emerged in 1883 without its skull, and determined the age to be between 21 and 27 years. Based on this, and a comparison with Varsányi’s drawing displayed in Table 13, they did not accept that this skeleton could be the original one recovered by Érdy. The number II/52_3 intended to be temporary given by the working group to the person found in the third grave is flawed, because the persons buried in earth graves were assigned to group 2, but this skeleton was found in a stone-lined grave (Figure 13), which was noted at the time of excavation.

3. According to the polarization and two-photon microscopic surveys, the bone structure of skeleton II/52_3 had already sustained severe damage, the DNA was greatly fragmented and very few intact target sequences could be found in it. In the experience of the Göttingen laboratory, during the amplification of such degraded DNA samples, PCR artifacts such as stutters start appearing more and more!

4. A-STR analyses were carried out via PCR and NGS methods in Budapest and Göttingen. The A-STR surveys were conducted at the same time in the Budapest-1 and Göttingen laboratories, while the NGS data were obtained in the Budapest-2 laboratory. The A-STR data from these laboratories were compiled together, and a marker set with consensus alleles was created (Table 15). Using PCR investigation, 5 marker differences out of 20 could be detected between the bone samples of Béla III and skeleton II/52_3, which ruled out a father-son relationship.

5. The DNA sequencing analysis described in Point 5 conducted in the chromosome regions of five different markers showed that one of the markers shown to be different via PCR (D2S441) is indeed different in Béla III than in skeleton II/52_3, but a further 4 markers in the two skeletons are identical to each other. Out of 20 A-STR markers, 19 being identical suggests a close family relationship between the two skeletons.

6. Through NGS analysis, we detected microvariant allele variations that were present in person II/52 and King Béla III as well, which only occur, if the two persons are closely related.

7. All of the known anthropological, archaeological, radiocarbon dating and genetic data together point to person II/52_3 being Béla II. To confirm this, we compared the genetic data of Béla III with the rest of the skeletons in Table 21.

Skeletons	A-STR match	Y-STR match	Haplotype metapopulation	
			PCR	
Béla III and II/52_3	PCR:17/22 NGS:21/22	16/16	R1a	Middle East, West Asia vicinity
II/53	9/20	10/16	R1a	Africa, Southern Balkan, especially in Greece, Byzantine origin
II/54	11/20	11/16	R1a	Occurs in Northwestern Russia, Ukraine, Belarus, Northern Poland
II/55	13/20	4/16	R1b	France, Northern Italy, Northern England, Baltic region
I/3 G5	14/20	1/16	J1a	Frequent on the Arabian peninsula, west bank of the Caspian Sea
I/4 H6	13/20	4/16	R1b	Most frequent occurrence: France and Northern Italy

Table 21. Comparison of the male skeletons' genetic marker data.

There were difficulties with the evaluation of the other skeletons interred in the crypt at the Matthias Church. During his excavations in 1862 and 1874, Henszlmann found a female skeleton in one of the graves (later labelled II/109), while the other two graves contained male skeletons (II/53 and II/54). The grave of an additional skeleton, labelled II/55, had a resting place that has yet to be precisely identified; it was probably near persons II/53 and II/54. The fact that the skeletons were in the inner temple, in the southern aisle, in an earth grave does not mean that they could be royal persons, because in this period, not only royals were buried in the inner area of the Royal Basilica of Székesfehérvár. Henszlmann excavated another four stone-lined graves in the northern aisle in 1874, out of which only two remain today.

Directly above grave 4, a fifth grave with a wooden coffin and brick lining was excavated, with a skeleton inside that was so fragmented that it was not kept. With the help of the genetic investigation of the fetus, we were able to determine that the person found in the fourth grave next to it was Princess Predslava. We know that the remains of Princess Predslava's husband, Prince Álmos of the Árpád Dynasty, who died in Byzantine exile were brought home on the orders of his son, Béla II, and that he was subsequently buried in 1137 in the Royal Basilica of Székesfehérvár. The decade-long neglect of the bones of Álmos obviously damaged them severely, his skeleton had become just as fragile as the person's in grave 5. If we consider that, according to contemporary burial customs, close relatives were buried next to each other, in this case, next to his wife who died in 1116, and if we consider all of the above together, we can come to the conclusion that the person in grave 5 may have been Prince Álmos of the Árpád Dynasty.

SUMMARY: The radiocarbon dating conducted on person II/52_3's skeleton, the Y-STR surveys, the shared mutations shown through DNA sequence analysis, the statistical analysis and the mitochondrial DNA investigation all suggest that this person is indeed Béla II (the Blind).

We believe that person II/53 is a high-ranking person of Byzantine origin. Person II/54 is not related to the Árpáds, but his ethnic group is from the population of Northern Poland. Based on these, the outlines of an early Hungarian–Polish connection seem to emerge between the two populations. We know little about high-ranking persons II/55 and I/3 G5; they came to Hungary with an unknown purpose. The person labelled I/4 H6 came from the west as a high-ranking clergyman, probably sent by the Pope.

The mtDNA HVR 1/2 genetic investigation of the fetus' skeleton showed that the fetus could not have been Béla III's grandchild (the child of one of his daughters), as previously postulated, since the mitochondrial DNA of the fetus differs from the mtDNA pattern of Béla III and Anne of Antioch. The maternal alleles of the fetus' A-STR markers are identical in a ratio of 13/15 to the maternal alleles of skeleton II/52_3, while its mtDNA haplogroup matches with Predslava of Kiev's mitochondrial haplogroup. This is only possible if the fetus' mother is Predslava of Kiev, and the fetus is the sibling of Béla II (see Figure 44). The 16294T SNV can be found not only in the fetus, but in skeleton II/52 (Béla II) as well, which is further proof that the fetus is indeed Béla II's sibling.

CHAPTER ELEVEN

ZOLTÁN SZENTIRMAY

SUMMARY

1. Evidence supporting the identification of skeleton II/52 as Béla II

1. *Archaeological data.* The grave marked II/52 was 40 centimetres deeper than Béla III's grave, suggesting an earlier burial. The man resting in the grave laid in a coffin made of carved sandstone, which was placed on poles stuck into the muddy soil. The burial's circumstances point toward the grave being one of Hungary's 12th century Kings. The grave marked II/52_3 was located directly next to Béla III's grave, which, bearing contemporary burial customs in mind, suggests a close family relationship. In the 12th century, only Kings and their families were buried inside the Royal Basilica. So when determining family relations, only Kings that died earlier could be considered: these were Béla II (†1141), Géza II (†1162) and Béla III's brothers, László II (†1163), and István IV (†1165). Béla III's brothers were pretenders, and thus it is unlikely that Béla III would have been buried next to one

of them. We ruled out Géza II from the potential candidates via genetic and statistical investigations, and thus person II/52_3 can only be Béla II (the Blind).

2. *Radiocarbon dating.* Radiocarbon dating puts the age of skeleton II/52_3 between 1035 and 1155 with a 95.4% probability. This interval includes Béla II's death (1141), while the deaths of Géza II, László II and István IV are above the upper limit of the interval by 7, 8 and 10 years, respectively. Thus, the radiocarbon dating suggests person II/52 is probably Béla II.
3. *Consensus marker data.* Analysis of the consensus marker data based on the results from the Göttingen and Budapest laboratories and complemented by the next generation sequencing data detected only one marker difference out of 20 when comparing skeleton II/52_3 and Béla III's A-STR markers; this marker distribution suggests a grandfather-grandson relationship between Béla II (the Blind) and Béla III.
4. *Statistical analysis.* The statistical analysis conducted with the negative log-likelihood method and having considered all genetic data independently of each other suggests that skeleton II/52_3 is most likely Béla II.
5. *Y-STR marker surveys.* All of the alleles of the 16 paternal Y-STR markers of King Béla III and skeleton II_52_3 are completely identical. This find shows that skeleton II/52_3 belongs to the line of Árpád Dynasty Kings on the one hand, and on the other hand, it is also closely related to King Béla III.
6. *Mitochondrial DNA survey.* The grandfather of King Béla II (the Blind) was Géza I, whose first wife was Princess Sofia of Loon, and his second wife was the Byzantine Princess Synadene.

Their child was Prince Álmos of the Árpád Dynasty, whose wife was Predslava of Kiev. In the case of Béla II and Predslava, the mitochondrial haplogroup was inherited through the maternal line. Skeleton II/52's mitochondrial haplogroup is also not Serbian, but rather its western type, so according to this, skeleton II/52 must be King Béla II.

7. *Genetic investigation of the fetus.* The maternal A-STR markers of the fetus are identical in a ratio of 13/15 to the one of alleles (maternal) of skeleton II/52_3. The 16294T and 16296T SNV found in the mitochondrial gene's HRV1 region determined the fetus' T2b haplogroup. These data together prove that the mother of the fetus could not be anyone other than Predslava of Kiev, and skeleton II/52_3 is Béla II (the Blind), and thus, the fetus is Béla II's sibling.

2. New results obtained during the genetic investigation of the medieval skeletons interred in the Matthias Church

1. We synchronized the historical, palaeopathological, morphological, genetic and radiocarbon dating data with each other, allowing for successful completion of the task we had set for ourselves. There had previously been no example of such a widespread multidisciplinary summary analysis.
2. The anthropological survey conducted in 1984 did not consider person II/52_3 to be identical to the person originally found; instead, it was considered to be an unknown skeleton and was excluded from the Árpád Dynasty Kings and their close relatives. Thanks to the aforementioned multidisciplinary work, we were able to prove that person II/52_3 is Béla II (the Blind).
3. Up until this point, out of the persons in the five adjacent graves excavated by János Érdy we were only able to identify King Béla III and his wife, Queen Anne of Antioch, from the first and second graves. We have proven that it was Béla II (the Blind) who was buried in grave three. The fourth grave contained Predslava of Kiev and her fetus, who was the sibling of Béla II (the Blind). In the fifth grave, it was probably Prince Álmos of the Árpád Dynasty who was laid to rest.
4. The identities of King Béla III and his wife were disputed by historian Endre Tóth, who believed that they were instead Kálmán the Learned and his wife. Using archaeological, palaeopathological and computed tomographic (CT)

investigations, we have proven that Endre Tóth's theory does not hold up to scrutiny.

5. We were the first to investigate the histological preservation of several hundred year old ancient bones with polarization and two-photon microscopic methods. This investigation helped us to determine which bones the subsequent genetic investigations should be based upon.
6. In order to investigate the ancient bones, we devised a DNA isolation method which was suitable for A-STR and Y-STR, as well as next generation sequencing analyses.
7. We applied a new statistical approach devised by Mr Tusnády, which – unlike the generally used, so-called kinship analysis – was suitable for comparing the DNA samples of several skeletons at the same time.
8. With the help of A-STR marker data and next generation sequencing, we determined the Árpád Dynasty Kings' paternal consensus genotype. Using this, we were able to draw realistic conclusions regarding the family relationships of the skeletons.
9. In 1862, in the inner area of the Royal Basilica of Székesfehérvár, Imre Henszlmann removed four skeletons thought to be Árpád Dynasty kings or their close relatives; then, in 1874, in the northern aisle, he excavated another four skeletons found in stone-lined graves as well. Of these skeletons, we have proven that none are related to the Árpád Dynasty; their probable identities are discussed under Point 3 in Chapter 11.
10. We determined the R1a haplogroup of the Árpáds, which points towards an Indo-Iranian origin, i.e. a Middle Eastern, West Asian region.

11. The Árpáds' R1a haplogroup differs from the Finno-Ugric peoples' most common N1b and N1c haplogroups, which suggests that there is no genetic relation between them and the Finno-Ugric peoples.
12. Doctor Józsa reported degenerative bone diseases on Béla III and Anne of Antioch, and skeletons I/3_G and I/4_H, which seem unusual today. These can be compared to the frequency of similar diseases of the same age group in today's population in order for us to get an idea of the quality of life of the people in the Árpád age.

3. List of identified unknown bone samples kept in the Matthias Church

- Skeleton II/52_3:** King Béla II (the Blind), grandfather of Béla III
- Skeleton II/53:** Most likely a high-ranking person of Byzantine origin
- Skeleton II/54:** A person originating from the Middle East, belongs to haplotype R1a, not related to the Árpáds, people belonging to this haplotype populated Northern Poland
- Skeleton II/55:** A person who came to Hungary with an unknown purpose, possibly sent by the Pope
- Skeleton I/3 G5:** Not related to the Árpád Dynasty, unknown high-ranking person from the east
- Skeleton I/4 H6:** A very high-ranking clergyman probably sent by the Pope

Female skeleton found in grave 4: Predslava of Kiev, mother of Béla II

Fetus of the female skeleton found in grave 4: Child of Prince Álmos of the Árpád Dynasty, and Predslava of Kiev, a sibling of Béla II

Supplemental information: The bones found in grave 5 were so fragmented that they were not kept. We also know that Predslava of Kiev's husband, Prince Álmos of the Árpád Dynasty, was buried in the Royal Basilica: his bones were neglected for ten years before he found his final resting place. If we consider that, according to contemporary burial customs, close relatives are buried next to each other, and put all this knowledge together, we may believe that **the**

person in grave five may have been Prince Álmos of the Árpád Dynasty, who was buried next to his wife in grave 4, Predslava of Kiev, who died in 1116.

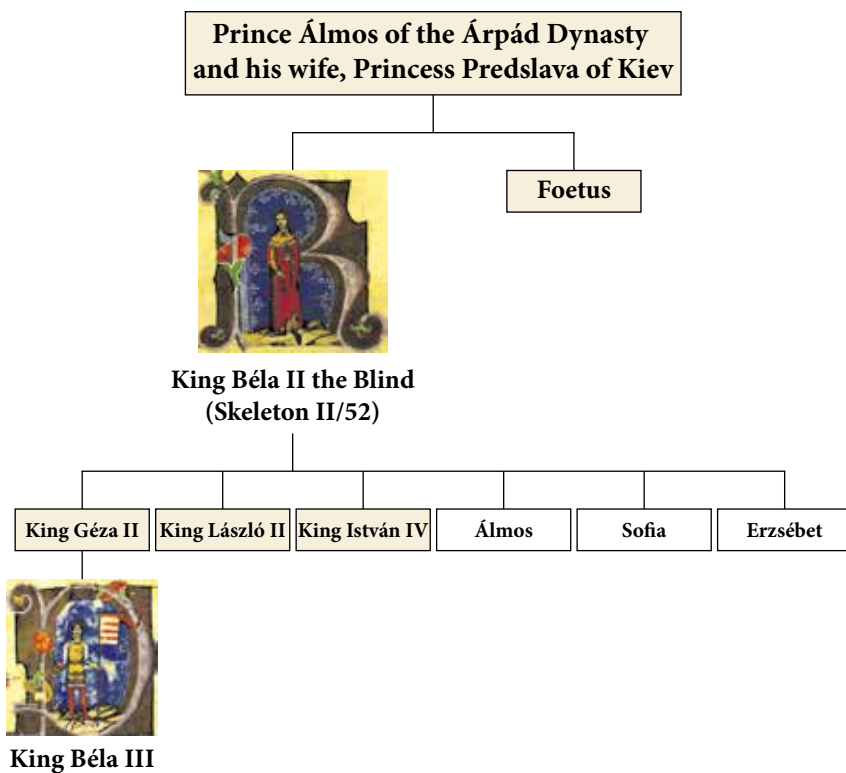


Figure 44. Descendants of Predslava of Kiev and Prince Álmos. King Béla III is the grandson of King Béla II (the Blind) (skeleton II/52_3), the female fetus is the sister of Béla II. Each of the persons was buried in the Virgin Mary Basilica of Székesfehérvár, from which they were brought to the Matthias Church. The portraits of Béla II and Béla III are from the *Chronicon Pictum*

EPILOGUE

MIKLÓS KÁSLER, GÁBOR NYÁRI

“After him, Béla the Blind reigned, the son of Prince Álmos the Blind; he avoided many evils and humbly practiced good deeds. He did not seek help from the strength of his arms, he did not place his trust in men, but sought refuge in the Highest Highness, the Lord became his guardian, directed him through his immense mercy, and put the fruit of his loins in his seat. In his hands, the land became strong, his enemies were led to disgrace by the Lord until this day. [...] Béla the Blind ruled for nine years, eleven months and twelve days. In the Year of the Lord 1141, on the ides (13th) of February, Thursday, he went to the Lord, his body lies at Fehérvár” – wrote the author of the *Chronicon Pictum* of Béla II in the 14th century: this was probably the same Márk Kálti, who – as the canon guardian of the Virgin Mary Basilica – could have seen the grave of the King, who had died two centuries earlier.

One of the most important results of the research conducted between 2012 and 2017 is that we may now know for certain what Márk Kálti and his contemporaries knew back then: the skeleton labelled II/52_3 is indeed the earthly remains of Hungary’s King Béla II. The participants of the research trust that this discovery will

contribute to a better understanding of Hungary's past, that they are writing a new chapter in the history of research on the Árpád Dynasty, and that it will allow posterity to pay its respects before one of Hungary's medieval Kings.

The research, however, has a much greater benefit, even if it may not be immediately clear to the outside observer. The research team demonstrated that it is possible to achieve new results with the means and institutional background available, even if several professionals expressed their doubts about that previously. It is also possible for the representatives of several disciplines from several nations to conduct outstanding work together for a common goal, devising and applying completely new or underused methods of investigation.

Thanks to these investigations, in addition to the identification of King Béla II, through the genetic investigation of the remains found in the graves excavated at the Virgin Mary Basilica of Székesfehérvár, we have become more familiar with the Árpád Dynasty royals and their families, and by extension, the history of all Hungarians. The participants of the research trust that their work will inspire historians, archaeologists and geneticists interested in this subject, and allow for a better understanding of some aspects of the Hungarian people's distant past.

We hope that this book, although it summarizes the results of multidisciplinary research that lasted for several years, is not the beginning of the end, but rather the start of a new scientific process.

GLOSSARY

Allele: The length of a repeating sequence within a gene expressed in base number.

AMELX and AMELY: Ameologenin is a single copy gene; it is located in chromosome regions Xp22.1-Xp22.3 and Yp11.2 in a homologous manner. It is called AMELX on the X chromosome, and AMELY on the Y chromosome. Gender determination is based on the fact that in the first intron of AMELX there exists a 6-base long deletion, which does not occur in the AMELY gene.

Amplification: Multiplication of a given nucleic acid sequence.

Annealing: Interconnection of complementary single-stranded nucleic acid molecules (the opposite of denaturation).

Antisense: Single stranded DNA or RNA segment complementary with sense nucleic acid.

bp: Basepair, complementary nucleotide pair.

cDNA: DNA generated from a specific mRNA-template via reverse transcription.

Complementer: Basepairs in double-stranded nucleic acids which connect to each other specifically via hydrogen bridges.

Consensus sequence: Take a certain kind of virus, such as HPV, as an example. The various types of viruses that belong here do not have the same base order, but they do have shorter or longer

nucleic acid segments which are identical to each other in the various types. These are consensus sequences.

Coverage: This shows how many synthesized DNA strands detect a certain DNA sequence in the course of next generation sequencing.

Degenerate primer: Short nucleic acid segment used at polymerase chain reactions, not all its bases are complementary with the sequence they bind to.

Denaturation: The macromolecules lose their native configuration due to heat, chemical or other effects; in the case of a DNA this means the two strands will untwist.

DISH: (Diffuse Idiopathic Skeletal Hyperostosis) Forestier's disease, the ossification of the ligaments in front of the vertebra.

DNA cloning: The technique with which a specific DNA sequence is put into elements capable of self-replication (plasmid, virus) in order to multiply itself, so that a large volume of copies can be obtained.

Dot/slot blot: Bringing the DNA or RNA isolated from cells to a nitrocellulose or nylon filter, which is made visible with a marked probe. The result is called "dot" or "slot blot".

Downstream: The position which follows the gene's 3'-end (its opposite is upstream).

Epitope: Antigen determinant (a few for each molecule).

Formamide: A small, organic molecule that denatures the DNA; it connects to the adenine's free NH₂ groups and prevents the formation of A-T basepairs.

Gene: The DNA sequence responsible for coding an RNA and regulating the transcription process.

Genome: The entire set of genetic information of an organism.

Genotype: The genetic makeup of an organism.

Haplogroup: The group of haplotypes originated from the same recent ancestor.

Haplotype: A continuous segment of the genome which is inherited from a given parent.

Heteroduplex: DNA/DNA or DNA/RNA double helix of nucleic acid molecules originated from different sources.

Hybridization: Conjoining of complementary DNA or RNA segments.

Intron: A segment of pre-mRNA which is eliminated during the maturation of the mRNA. Intron is not responsible for protein encoding.

Karyotype: Chromosomal makeup of a cell.

Locus: A position/region on a chromosome.

Melting temperature (T_m): The temperature at which the DNA's double strand is halfway untwisted.

Mismatching: Non-canonical basepairs connecting in a flawed manner during hybridization.

Motif: Repeating unit, pattern.

mtDNA: Mitochondrial DNA.

Mutation: Any kind of change in the DNA sequence (point mutation, deletion, translocation, etc.).

NGS: Next generation sequencing, the most current method of determining the DNA base order.

Non-coding DNA: Accounts for over 90% of the cell's DNA stock, does not code peptides or RNA.

OPLL: Ossification of the posterior longitudinal ligament.

P, q: Short (p) and long (q) arms of the chromosome.

PCR: Polymerase chain reaction, the *in vitro* amplification of a given segment of a DNA molecule.

Plasmid: Two-stranded, circular DNA, which is capable of autonomous reproduction inside bacteria; used for cloning DNA.

Point mutation: Swapping a base with another in the nucleic acids.

Polarization microscopy: A process aimed at detecting organized biological structures, which makes use of the phenomenon of double refraction. The polarized light means that the light waves vibrate on one plane, parallel to each other. The polarized light which enters the organized molecular structure splits into two beams, one of them goes through the material slower (collagen fibre, cell membrane for example), while the other goes through it faster, and the two beams unite again upon exit, but the polar light's plane of vibration swivels. The birefringent biological structure appears glowing brightly in the microscope calibrated to a dark field of vision, which can be further emphasized through various staining methods.

Polymerases: Enzymes, which catalyze the integration of nucleotides at the DNA- or RNA-chain's 3'-end during DNA replication or RNA transcription.

Polymorphism: Alternative forms of protein, DNA or RNA sequences, which are also present in the population under normal circumstances.

Primer: Short, usually 20-30 bases long, single-strand DNA sequence, which is complementary with one of the DNA strands. On the free 3'-OH end the DNA polymerase starts synthesizing a deoxyribonucleotide chain.

Probe: DNA or RNA fragment, which is used to determine whether the unknown DNA or RNA under investigation contains the complementary nucleotide sequence in question.

Read: The DNA sequence generated and displayed during the course of next generation sequencing.

Repeating (repetitive) sequences: 30% of the cell's DNA stock consists of non-coding, repeating sequences (tandem repeats, satellite DNA), the function of which is unknown.

Reverse transcriptase: Enzyme of retroviruses, which creates a cDNA from the RNA template via reverse transcription.

Sense: Nucleic acid sequence that codes an amino acid chain.

SNP or SNV: (Single nucleotide polymorphism or variation) Changing one base on a DNA sequence on a given point. Its pattern of incidence is used to determine the haplotype.

STR: (Short tandem repeat). Short repeating (microsatellite) sequence. It can be used for unique personal identification.

Transcription: The process of RNA synthesis on the DNA-template.

Translation: Protein synthesis from the mRNA template.

Two-photon microscopy: Two-photon absorption fluorescent microscopy is a new type of fluorescent microscopy, which operates under the theory of nonlinear optics. Essentially, with this method, the exciting laser releases its energy in short impulses, thus, the excitement and the fluorescence can only occur under the duration of the impulses in the focus spot.

Upstream: The position preceding the DNA's 5'-end.

INVESTIGATED BONE SAMPLES AND METHODS

Bone samples used

	Göttingen	Budapest-1	Budapest-2
Persons	PCR	PCR	sequencing
Béla III	metatarsal tarsus femur vertebra	metatarsal tarsus femur vertebra	tarsus tarsus
Anne of Antioch	rib vertebra femur	rib	-
Fetus	vertebra	n.a.	-
II/52_3	tarsus-1 tarsus-2 femur	tarsus-1 rib	tarsus-2 tarsus-2
II/53_7	sternum rib	vertebra rib	-
II/54_9	rib femur	rib femur	-
II/55_10	rib femur	rib	-
II/109_8	rib vertebra	rib	-
I/3 G5	rib tarsus femur	rib	-
I/4 H6	metatarsal tarsus	metatarsal tarsus	-
Total samples	25	16	4

Table 22: Processed bone samples and their respective skeletons; the bone samples which were investigated by at least two laboratories are highlighted in bold. n.a.: not analyzed.

The STR-marker investigations were conducted simultaneously in three laboratories:

1. Historical Anthropology and Human Ecology, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, University of Göttingen, Göttingen, Germany (Verena Seidenberg, Dr Susanne Hummel)
2. National Institute of Oncology, Pathogenetics Department, Budapest-1, Hungary (Judit Olasz, Dr Orsolya Csuka)
3. National Institute of Oncology, Tumour Pathology Centre, Department of Surgical and Molecular Pathology, Budapest-2 (Dr Erzsébet Csernák, Dr Zoltán Szentirmay)

DNA isolation

- The template DNA rules out of the possibility of the researchers' own DNA becoming contaminants:
- Opening of the sarcophagi at the Matthias Church, as well as the metal and glass caskets within them and their transportation to the National Institute of Oncology occurred under sterile conditions.
- Pulverization of the bone samples prior to DNA isolation was conducted in a special room where air could be sucked out of the room, with the researcher handling the sample wearing a special suit.
- All genetic investigations occurred in areas already set up for the specific purpose of genetic investigations according to "good laboratory practices".

With regards to the fact that the DNA that can be isolated from ancient bones is severely fragmented, during the DNA isolation, each laboratory took steps to optimize their methods.

*DNA isolation, Göttingen laboratory (Verena Seidenberg, Dr
Susanne Hummel)*

We would like to mention in advance, that the DNA isolated from various bone samples had degraded to various degrees, and that the bone samples of Béla III and Anne of Antioch were treated with some sort of resin, and this made PCR analyses almost impossible. For this reason, Béla III's metatarsal sample was investigated with four special DNA extraction kits (EZ1, QiaVac MinElute Standard, QiaVac MinElute Short, QiaVac MinElute Organic) and six different DNA extraction methods, out of which method four, five and six were used successfully to isolate PCR DNA templates from various bones.

Initially, the DNA of all ten samples was extracted by two different extraction methods ("***QiaVac MinElute Standard***" and "***EZ1***"; see below). Many samples revealed promising amplification results following these extraction methods. However, the samples of Bela III (HU 3B Mt) and Anne (HU AA Co) in particular were found to contain too many inhibiting substances to enable successful amplification. Therefore, two new extraction methods ("***QiaVac MinElute Short***" and "***QiaVac MinElute Organic***"; see below) were developed for optimizing the DNA extraction process.

Sample preparation (for all DNA extraction methods)

The surface of each bone fragment was decontaminated by incubation for 15 min in a commercially available bleach (6% NaOCl) followed by 15 min rinsing in bi-distilled water. The samples were dried overnight at 37°C and then crushed in a steel mortar and powdered in a ball mill (Retsch). 0.25 g of bone powder was

incubated rotating with 3,900 μ l EDTA (0.5 M; pH 8) and 100 μ l Proteinase K at 37°C for 18h.

EZ1

Subsequently, further 50 μ l of Proteinase K was added and the samples were rotated for another 2 h at 56°C. Subsequently, 50 μ l of SDS was added followed by incubation for 5 min at 65°C. The lysate was centrifuged for 3 min at 3,300 rcf.

The supernatant was transferred to Amicon® Ultra-4 Centrifugal Filter Devices 30 K (Millipore) and concentrated to approx. 250 ml by centrifugation at 5,000 rcf. The remaining lysate was purified in the BioRobot EZ1 using the Trace Protocol on the Forensic Card and the EZ1 DNA Tissue Kit (all components, soft- and hardware Qiagen). The elution volume was 50 μ l. The extracts were stored at -20°C.

QiaVac MinElute Standard

The duration of the EDTA/Proteinase K incubation was 18 h. Subsequently, a further 50 μ l of Proteinase K was added and the samples were rotated for another 2 h at 56°C. Then 50 μ l of SDS was added followed by incubation for 5 min at 65°C. The lysate was centrifuged for 3 min at 3,300 rcf.

The supernatant was mixed with 16 ml of PB buffer (Qiagen) and 100 μ l sodium acetate buffer (pH 5.2), centrifuged for 3 min at 3,300 rcf again and transferred to MinElute columns with large volume funnels on a QIAvac 24 Plus vacuum system (both Qiagen). The lysate was pulled through by vacuum, followed by three washing steps with 700 μ l PE buffer waiting each time for 5 min before

opening the VacValves. The MinElutes were inserted into collection tubes and centrifuged for 1 min at 15,700 rcf and then placed at room temperature with open lids for 20 min to remove any remaining ethanol from the PE buffer. For elution of the DNA 20 µl of warm RNase free water (Qiagen) were pipetted to the membranes and after waiting for 5 min the columns were centrifuged for 1 min at 15,700 rcf. The elution step was repeated three times for each sample. The extracts were stored at -20°C.

QiaVac MinElute Short

The duration of the EDTA/Proteinase K incubation was 1 h. Subsequently, a further 50 µl of Proteinase K was added and the samples were rotated for another hour at 56°C. The lysate was centrifuged for 3 min at 3,300 rcf.

The supernatant was purified on MinElute columns with large volume funnels using a QIAvac 24 Plus vacuum system (see above). The extracts were stored at -20°C.

QiaVAc MinElute Organic

The duration of the EDTA/Proteinase K incubation was 18 h. Subsequently, a further 50 µl of Proteinase K was added and the samples were rotated for 1 h at 56°C. The lysate was centrifuged for 3 min at 3,300 rcf.

The supernatant was mixed with 3 ml Phenol by inverting for 6 min. For phase separation the samples were placed for 10 min at 56°C. The organic phase was removed, and the samples were mixed with 4.5 ml chloroform by inverting for 6 min. Again, the phases were separated as described above. The aqueous phase was purified

on MinElute columns with large volume funnels using a QIAvac 24 Plus vacuum system (see above). The extracts were stored at -20°C.

DNA isolation at Budapest-1 laboratory (Dr Judit Olasz, Dr Orsolya Csuka)

1. Soaking the bone samples in a 0.5% NaOCl solution for 15 minutes
2. Three washes with ultrapure distilled water, overnight drying
3. UV radiating every side for 10 minutes
4. Pulverization in a Spex Freezer Mill
5. Decalcination of 0.15-0.20 g bone dust in a solution of 5 ml 0.5 M EDTA (pH8.0) at 4°C for 72 hours (The EDTA solution was changed every 24 hours after prior centrifugation.)
6. DNA isolation using DNA IQ system (Promega) Kit.

DNA isolation at Budapest-2 Laboratory (Dr Erzsébet Csernák, Dr Zoltán Szentirmay)

1. We devised the process for sequencing and the creation of the DNA library. The steps are as follows:
2. Preparation of bone samples (decontamination in order to decrease the amount of inhibitors present):
3. Incubation of bone samples in a 0.5% NaOCl solution, and UV radiation for 10 minutes.
4. Pulverizing in a Spex Freezer Mill.
5. Washing 0.15-0.3g bone dust at room temperature in a 1ml 0.5M EDTA (pH8.0) with 15 minutes of incubation.
6. Decalcination of 0.15-0.3g bone dust at room temperature with adding 1ml 0.5M EDTA for at least 48h (with shaking: 600rpm)

7. Digestion of supernatant in Proteinase K (200µg/ml) solution at 56°C for a whole night (with shaking: 300rpm). A second analysis on the remaining bone dust, the same manner as with the first isolation.
8. DNA isolation with a Qiagen MinElute Gel Extraction Kit in an Elutio 40 µl 1xTE puffer.
9. Control PCR amelogenin on DNA with controls of known concentration.

STR-marker detection with PCR method

According to experience, the success of the detection of A-STR markers depends partly on the *method of detection applied*, thus the detection kit or DNA sequencing method, and how the results are read.

The Göttingen and Budapest-1 laboratories used the following detection kits to detect A-STR and Y-STR markers:

Göttingen laboratory (Seidenberg, Dr Hummel)

For the ***autosomal STR-marker*** investigations the following, generally available detection kits were used: (1) Heptaplex miniSTR assay, Seidenberg et al. (2012); (2) a Decaplex miniSTR assay. Fehren-Schmitz et al. (2015); (3) Investigator ESSplex SE plus and Investigator ESSplex SE QS (Qiagen) a 0.5-5 µl with prior PCR amplification of the DNA sample.

The ***Y-chromosome STR-marker*** analyses were performed using the following kits: Powerplex Y kit (Promega) and the lab-internal decaplex Y-miniSTR kit (we do not show primer sequences at this time).

Budapest -1 laboratory (Dr Judit Olasz, Dr Orsolya Csuka)

Autosomal STR-marker investigations: AmpFI STR MiniFiler (Applied Biosystems)

Y-chromosome STR-marker investigations: AmpFI STR Yfiler kit (Applied Biosystems). The results were evaluated with GeneMapper Software v.4.0 (Applied Biosystems).

Next generation sequencing (NGS)

Budapest-2 laboratory (Dr Erzsébet Csenák, Dr Zoltán Szentirmay)

Analysis of five A-STR markers with sequence capture and subsequent DNA sequencing on the DNA samples of Béla III and skeleton II/52. Out of the investigated 20 A-STR markers, the following 5 were different for the two skeletons, and we wished to decide if these are real differences or artifacts occurring in the degraded DNA samples. The markers were the following: D2S441, D2S1338, D3S1258, D7S820 and D19S433. The process is the following:

- a. Creation of a PCR target panel using Generead DNAseq Custom Panel v2 kit (Qiagen). A change was necessary in the makeup of the PCR mix due to sample degradation: the reaction's final volume is 50µl, in which 25 µl Kapa Hifi HotStar Uracil+ enzyme mix (Kapa Biosystems), 10 µl primer mix, 2.5 µl BSA (1mg/ml) and 12.5 µl template can be found. (Note: additional volume of the primer mix / template depends on DNA concentration).
- b. Creation of amplicon library via QIAseq Ultralow Input Library Kit and GeneRead Adapter I Set A 12-plex (Qiagen) kit.
- c. Sequencing via MiSeq Reagent Nano Kit v2 kit on MiSeq Illumina platform using the following parameters (Table 23).

- d. The length of individual reads (sequenced DNA strands) are the following: D2S441, D2S1338, D19S1338 = 151 bp, D3S1358, D7S820 = 142 bp.

Chr	A-STR	Genome region (bp)	PCR primer forward	PCR primer reverse
2p13.3	D2S441	2:68239063-68239103	AGGAACTGTGGCTCAT- CTATG	TTCACTCTCCTTCC- CAAATGTTTA
2q35	D2S1338	2:218879568-218879718	CATAATCATGAGTTATT- CAGTAAGTTAAAGG	GAGCCAGTGGATTT- GGAAAC
3p21.32	D3S1358	3:45582186-45582336	GGCATCTCTTATACTCAT- GAAATCA	CCCACTGCAGTCCA- ATCTG
7q21.11	D7S820	7:83789519-83789619	GTAATTAAATGTTTACTA- TAGACTATTTAGTGAGAT	GGTATGATAGAA- CACTTGTTCATAGTT
19q12	D19S433	19:30417112-30417212	GCACCCATTACCCGAA- TAAAAATC	GGCTGCAAAAAGCTA- TAATTGTAC

Table 23. Chromosome regions and PCR primers.

Evaluation of the data was performed using the Burrows-Wheeler Alignment Tool (BWA) software. Description of the software: “BWA is a software package for mapping low-divergent sequences against a large reference genome, such as the human genome (HG19). It consists of three algorithms: BWA-backtrack, BWA-SW and BWA-MEM. The first algorithm is designed for Illumina sequence reads up to 100 bp, while the remaining two for longer sequences ranged from 70 bp to 1 Mbp (Lee and Durbin, 2010)”.

We compared the sequence-order of alleles determined during sequencing to data gathered from the following database: STRbase: tandem repeat DNA internet database (Butler JM and Reeder DJ 1997; Ruitberg et al. 2001).

Autosomal STR-marker frequencies:

The allele frequencies are the Hungarian data from the AllSTR Autosomal Database (<http://allstr.de/allstr/home.seam>) with the exception of the D9S1120 marker data, which are from the article of Phillips et al. (2008), and the SE33 marker data, which are from the book of Butler (2015) (the data gathered are stored in a separate Excel file).

Y-STR frequencies and rate of mutation:

International Society of Genetic Genealogy (ISOGG) database, from the chapter “Y-DNA STR frequencies”. <HTTP://ISOGG.ORG/WIKI/Y-STR>

Y-STR mutation database: http://isogg.org/wiki/Mutation_rates

Subchapter: A table of mutation rates for 111 markers provided by Marko Heinila

Low molecular weight DNA (<300bps) enrichment from all samples was performed using AMPure XP beads (NEB). Library preparation was performed using the TruSeq Nano DNA LT kit (Illumina) according to the manufacturer’s recommendations. Library size and quality was confirmed with Fragment Analyzer (Advanced Analytical) and quantitative PCR (Biorad S1000; CFX96 Real Time System). Paired-end sequencing (2X125 bps; 250 Cycles) was performed on the Illumina HiSeq 2500 System (Illumina) using high output flow cells in multiple runs following the manufacturer’s recommendations.

Y-chromosomal haplogroup analyses

Y-chromosome haplogroups were determined using the SNV markers described in Karmin, Saag et al. (2015), Poznik, Xue et al. (2016) and Rootsi, Behar et al. (2013)

Mitochondrial haplogroup analyses

Because of the higher copy number of mitochondrial genomes present in cells, we obtained good coverage depth and percent of mitochondrial genome covered for all samples. The same data were used to determine the percentage of mitochondrial genome with less than ten-fold coverage. We used the PhyloTree_{MT} (Build16) to infer the mitochondrial haplogroups (Van Oven and Kayser 2009). Each marker was individually visualized and visually verified to avoid the possibility of a variant calling error.

Statistical analysis of the relationship between Béla III and skeleton II/52_3 based on A-STR markers

With regards to the A-STR marker alleles, allele frequencies and the allele's European and Hungarian mutation frequency, we gave the *probability of family relations* with the following method (Dr Gábor Tusnády):

Investigated persons are supposedly members of the same royal family, but membership is not necessary. We know from other sources that persons B and A are couples, B is the husband, a King, A is the wife, a Queen. C is an infant, other persons are men but 9 are female. The persons C,G,H,3,7,9,0,8 are supposed to be descendants of the couple B, A.

Let us denote by $p(w; v; k)$ the population frequency of the allele for the

w -th locus ($w = 1; 2; \dots; 7$);

v -th allele ($v = 1$ for maternal, $v = 2$ for paternal allele); k -th person ($k = 1; 2; \dots; 10$).

For practical reasons, the frequency of missing data is set to 1. For example $p(3; 2; 4) = 0.052$; it is

the population frequency of the third locus of the fourth person's paternal allele.

Neglog likelihood: In our analysis the basic concept is the logarithm of the likelihood of the whole sample. To avoid negative numbers, we multiply the log likelihood by (-1) , and refer the number as NL (negative log-likelihood). If all of the alleles were independent, then $NL = 216.330451$ In the general case

$$NL = - \sum_{w=1}^m \sum_{v=1}^2 \sum_{k=1}^n \log(p(w, v, k)).$$

Thanks to the negative multiplier, any improvement in NL refers to a better family structure of the investigated persons. It may occur that for some structure NL is larger than the above cornerstone number, and such a situation tells us that the structure is out of question. We shall seek the appropriate family structure by trial and error method for the number of all possible structure is astronomically large. We do not present all of the details in our research, but it will be motivated solely on corresponding NL numbers.

In relation to father, grandfather and uncles $NL=205.639!$

G	G	B	B	S	S			G	G	B	B	S	S	
1	2	1	2	1	2			1	2	1	2	1	2	
130	173	130	173	0	0	D1S1656		120	173	120	173	0	0	D1S1656
110	113	110	113	110	113	D2S441		0	0	100	100	100	100	D2S441
170	0	170	170	170	190	D2S1338		0	0	200	250	170	200	D2S1338
150	170	150	170	140	150	D3S1358		140	0	140	140	140	140	D3S1358
100	120	0	0	0	0	D5S818		100	120	0	0	0	0	D5S818
100	110	100	110	80	110	D7S820		0	90	80	90	80	90	D7S820
130	140	130	0	0	0	D8S1179		120	140	0	0	0	0	D8S1179
150	160	0	0	0	0	D9S1120		150	160	0	0	0	0	D9S1120
130	130	130	130	0	0	D10S1248		130	0	130	130	0	0	D10S1248
180	190	180	190	0	0	D12S39		0	0	170	180	0	0	D12S39
90	130	90	130	0	0	D13S313		80	130	80	130	0	0	D13S313
110	120	110	120	0	0	D16S539		100	110	100	110	0	0	D16S539
130	160	130	160	130	130	D18S51		130	170	130	170	120	130	D18S51
150	162	150	162	130	130	D19S433		130	0	130	130	120	130	D19S433
300	322	310	322	0	0	D21S11		300	322	300	322	0	0	D21S11
150	160	150	160	0	0	D22S1045		0	0	150	170	0	0	D22S1045
0	0	110	120	0	0	CSF1PO		0	0	90	110	0	0	CSF1PO
210	210	210	210	0	0	FGA		210	250	210	250	0	0	FGA
200	272	0	0	0	0	SE33		0	0	0	0	0	0	SE33
70	90	70	90	0	0	TH01		90	93	90	93	0	0	TH01
170	170	170	170	0	0	VWA		0	0	160	170	0	0	VWA

Table 24. Final validated statistical database, which separately contains the Göttingen and Budapest-1 laboratories' A-STR marker allele length data determined by PCR. Under G-G and B B, the paternal and maternal alleles from Göttingen and Budapest are displayed, while paternal and maternal alleles obtained with NGS are under S-S. Tusnády: February 5, 2017

LITERATURE

- *150 éve történt. III. Béla és Antiochiai Anna sírjának fellelése.* (1999): A Szent István Király Múzeum közleményei B. sorozat 49. szám. Cserményi V (ed.).
- Athey TW (2005): *Haplogroup prediction from Y-STR values using a Bayesian-allele-frequency approach.* J. Genet. Geneol. 1:1–7.
- Athey TW (2006): *Haplogroup prediction from Y-STR values using an allele-frequency approach.* J. Genet. Geneol. 2:34–39.
- Bernert Zs: *Pogány szertartás az Árpád-házi királyok udvarában?* https://sirasok.blog.hu/2011/02/22/pogany_szertartas_az_arpad_hazi_kiralyok_udvaraban (accessed: 2019.08.25.)
- Biczó P (1999): *Érdy János leletmentésének tudományos jelentősége.* In: *150 éve történt. III. Béla és Antiochiai Anna sírjának fellelése.* A Szent István Király Múzeum közleményei B. sorozat 49. szám. 16–24.
- Biczó P (2016): *Az Árpád-házi királlysírok – A magyar történelem rejtélyei.* Kossuth, Budapest.
- Brinkmann B, Klintschar M, Neuhuber F, Hühne J, Rolf B (1998): *Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat.* Am. J. Hum. Genet. 62:1408–1415.
- Budowle B, van Daal A (2008): *Forensically relevant SNP classes.* 44:603–610.

- Butler JM, Reeder DJ (1997): *STRbase: A short tandem repeat DNA database*. GenPrint.
- Butler JM (2005): *Forensic DNA typing. Biology, technology, and genetics of STR markers*. Elsevier Academic Press. Second edition, Y-STR loci.
- Butler JM (2012): *Advanced topics in forensic DNA typing: Methodology*. Elsevier Academic Press.
- Butler JM (2015): *Advanced topics in forensic DNA typing: Interpretation*. Elsevier Academic Press.
- Chandler FJ (2006): *Estimating per locus mutation rates*. J. Genet. Geneal., 2:27–33.
- Crouse C, Rogers S, Amriott E, Gibson S, Masibay A (1999): *Analysis and interpretation of short tandem repeat microvariants and three-banded allele patterns using multiple allele detection systems*. J. Forensic Sci. 44:87–94.
- Decker AE, Kline MC, Redman JW, Reid JW, Bottler JM (2009): *Analysis of mutations in father-son pairs with 17 Y-STR loci*. Anal. Bioanal. Chem. 394:1183–1192.
- Demeter Zs (1999): *Az 1848-as királysír-leletek megtalálásának körülményei és visszhangja Székesfehérvárott*. In: 150 éve történt. III. Béla és Antiochiai Anna sírjának fellelése. A Szent István Király Múzeum közleményei B. sorozat 49. szám. 25–35.
- Engel P (1987): *Temetkezések a középkori székesfehérvári bazilikában*. Századok, 121:613–637.
- Éry K (1999): *Embertani vizsgálatok III. Béla és Antiochiai Anna földi maradványán*. In: 150 éve történt. III. Béla és Antiochiai Anna sírjának fellelése. A Szent István Király Múzeum közleményei B. sorozat 49. szám. 9–15.

- Éry K (ed.) (2008): *A székesfehérvári királyi bazilika embertani leletei. 1848–2002.* Balassi, Budapest.
- Érdy J (1853): *III. Béla király és nejének Székes-Fehérvárott talált síremlékei.* In: Kubinyi F, Vachot I: *Magyarország és Erdély képekben.* I. Pest. 42–48.
- Fehren-Schmitz L, Llamas B, Lindauer S, Tomasto-Cagigao E, Kuzminsky S, Rohland N, Santos FR, Kaulicke P, Valverde G, Richards SM, Nordenfelt S, Seidenberg V, Mallick S, Cooper A, Reich D, Haak W (2015): *A Re-Appraisal of the Early Andean Human Remains from Lauricocha in Peru.* PLoS One 10 (6):e0127141. doi: 10.1371/journal.pone.0127141
- Fornaciari G, Giuffra V (2013): *The “gout of the Medici”: making the modern diagnosis using paleopathology.* Gene. 1:528(1):46–50.
- Gansauge M-T, Meyer M (2013): *Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA.* Nature Protocols, 8:737-748.
- Gombos AF (ed.) (1937–1938): *Catalogus Fontium Historiae Hungaricae* I–III. Budapestini, Academiae Litterarum de Sancto Stepheno rege nominatae necnon Academiae Litterarum Hungaricae socius ordinarius.
- Guo Y et al. (2013): *MitoSeek: extracting mitochondria information and performing high-throughput mitochondria sequencing analysis.* Bioinformatics, 29(9):1210–1211.
- Henke J, Henke L (1999): *Mutation rate in human microsatellites.* Am. J. Hum. Genet., 64:1473–4.
- Henszlmann I (1864): *A székesfehérvári ásatások eredménye.* 9 rajzzal. (Ivr. 226 l.) Heckenast G, Pest. (Akadémia) 2.
- Holczmann BO (2019a): *Identification of Bela II (the Blind).*

- ResearchGate GmbH (Ed) https://www.researchgate.net/publication/331834431_Identification_of_Bela_II_The_Blind
- Holczmann BO (2019b): <https://www.researchgate.net> & <https://hu.wikipedia.org>
 - Hóman B (1931): *Külpolitikai irányok a magyar történetben*. Franklin, Budapest.
 - Hutai G (1999): *III. Béla király és Antiochiai Anna sírleleteinek restaurálásáról*. In: 150 éve történt. III. Béla és Antiochiai Anna sírjának fellelése. A Szent István Király Múzeum közleményei B. sorozat 49. szám. 36–59.
 - Janssen HAM, Maat GJR (1999): *Canons buried in the stiftskapel of the Saint Servaas Basilica*. Barger's Anthropologica. Vol. 5. Springer Verl. Berlin. 1–43.
 - Józsa L, Forgács S (2009): *A Forestier betegség története*. Osteologiai Közlemények, 4:174–179.
 - Józsa L (2010): *A Forestier betegség pathomorphológiája*. Osteologiai Közlemények, 1:11–14.
 - Józsa L (2014): Personal communication.
 - Karmin M et al. (2015): *A recent bottleneck of Y chromosome diversity coincides with a global change in culture*. Genome Res, 25(4): 459– 466.
 - Kayser M, Roewer L, Hedman M et al. (2000): *Characteristics and frequency of germline mutations at microsatellite loci from the human Y chromosome, as revealed by direct observation in father/son pairs*. Am. J. Hum. Genet. 66:1580–1588.
 - Kásler M, Szabados Gy (2015): *A középkori magyar nagyhatalom*. In: Kásler M (ed.): Nemzeti Nagyvizit, 2. köt. Pallas Athéné Domus Animae Alapítvány, Budapest. 172–180.

- *Képes Krónika* (1986): Translated by Bellus I, Európa, Budapest.
- King TE, Fortes GG, Balaesque P et al. (2014): *Identification of the remains of King Richard III*. *Nature Communications*, 5:5631.
- Kristó Gy (ed.) (1999): *Az államalapítás korának írott forrásai*. Szegedi Középkorász Műhely, Szeged.
- Kristó Gy, Makk F (1995): *Az Árpád-ház uralkodói*. I.P.C. Könyvek, Budapest.
- Kubinyi A (1999): *Királyi kancellária és udvari kápolna Magyarországon a XII. század közepén*. In: Kubinyi A: *Főpapok, egyházi intézmények és vallásosság a középkori Magyarországon*. METEM, Budapest. 7:67.
- László Gy (1965): *Szent László Győri ereklyetartó mellszobráról*. *Arrabona Múzeumi közlemények* 7. 157–209.
- Li H, Durbin R (2010): *Fast and accurate long-read alignment with Burrows-Wheeler Transform*. *Bioinformatics*, 25:1754–60.
- Loogváli EL, Roostalu U, Malyarchuk BA, Derenko MV, Kivisild T et al. (2004): *Disuniting uniformity: a pied cladistic canvas of mtDNA haplogroup H in Eurasia*. *Mol. Biol. Evol.* 21(11):2012–2021.
- Luzsa Gy, Gáspárdy G, Nemeskéri J, Éry K (1988): *Paleoradiológiai tanulmány a székesfehérvári bazilika 15 csontváz maradványról*. *Magyar Rad.*, 62:39–50.
- Luzsa Gy, Gáspárdy G, Nemeskéri J, Éry K (1989): *Occurrence of Hahn's vertebral vascular channels on medieval skeleton*. *Hung. Rheumat. Suppl.* 99–101.
- Mader R, Verlaan J-J, Eshed I, Jacome B-A, Puttini PS, Atzeni F, Buskila D, Reinshtein E, Novofastovski I, Abdallah Fawaz A, Kurt de V, Baraliakos X (2017): *Diffuse idiopathic skeletal*

hyperostosis(DISH): where we are now and where to go next. RMD Open, 3:e000472.

- Makk F (1981): *III. Béla emlékezete.* Eds.: Zolnay L, Klaniczky T, Stoll B, Dercsényi D, Szűcs J, Kristó Gy. Magyar Helikon, Budapest.
- Makk F (1989): *The Árpáds and the Comneni: Political Relations between Hungary and Byzantium in the 12th century.* Akadémiai Kiadó, Budapest.
- Makk F (1996): *Magyar külpolitika (896–1196).* Ed.: Kristó Gy. Szegedi Középkorász Műhely, Szeged. 243.
- Mende BG (2012): *Hogyan ne azonosítsuk az Árpád-házi királyokat?* In: Liska A, Szatmári I (eds.): *Sötét idők rejtélyei.* 6–11. századi régészeti emlékek a Kárpát-medencében és környékén. Tempora Obscura 3. Békéscsaba. 561–571.
- Nakajima M, Kou I, Ohashi H et al. (2016): *Identification and functional-characterization of RSPO2 as a susceptibility gene for ossification of the posterior longitudinal ligament of the spine.* Am. J. Hum. Genet., 99:202–207.
- Olasz J, Seidenberg V, Hummel S, Szentirmay Z, Szabados Gy, Melegh B, Kásler M (2018): *DNA profiling of Hungarian King Béla III and other skeletal remains originating from the Royal Basilica of Székesfehérvár.* Archaeol. Anthropol. Sci. 4(11):1345–1357.
- Pala M, Olivieri A, Achilli A, Accetturo M, Metspalu E et al. (2012): *Mitochondrial DNA signals of late glacial recolonization of Europe from near eastern refugia.* Am J Hum Genet 4 90(5):915–924.
- Pauer J (1849): *A Székesfehérvárott földfedezett királyi sirboltról.* Székesfehérvár.
- Phillips CA, Rodriguez A, Mosquera-Miguel A, Fondevila W, Porras-Hurtado LF, Rondon F, Salas A, Carracedo A, Lareu MV

(2008): *D9S1120, a simple STR with a common Native American-specific allele: Forensic optimization, locus characterization and allele frequency studies*. *Forensic Science International: Genetics*, 3:7– 13.

- Pike DA, Barton TJ, Bauer SL, Kipp EB (2010): *Haplogroup T phylogeny based on full mitochondrial sequences*. *J. Genetic Geneal.* 6(1).
- Pohl W (2003): *A non-Roman empire in Central Europe*. In: Goetz H-W, Jarnut J, Pohl W (eds.): *Regna and Gentes. The Relationship between Late Antique and Early Medieval Peoples and Kingdoms in the Transformation of the Roman World*. Brill, Leiden–Boston. 571–595.
- Poznik GD et al. (2013): *Sequencing Y chromosomes resolves discrepancy in time to common ancestor of males versus females*. *Science*, 341 (6145):562–565.
- Regöly-Mérei Gy (1968): *III. Béla magyar király és hitvese, Anna királynő hamvainak palaeopathologiai vizsgálata*. *Orv. Hetil.*, 109(8):423– 427.
- Richards M, Macaulay V, Hickey E, Vega E, Sykes B, Guida V, Rengo C, Sellitto D, Cruciani F, Kivisild T, Villems R, Thomas M, Rychkov S, Rychkov O, Rychkov Y, Gölge M, Dimitrov D, Hill E, Bradley D, Romano V, Cali F, Vona G, Demaine A, Papiha S, Triantaphyllidis C, Stefanescu G, Hatina J, Belledi M, di Rienzo A, Novelletto A, Oppenheim A, Nørby S, al-Zaheri N, Santa-chiara-Benerecetti S, Scozzari R, Torroni A, Bandelt HJ (2000): *Tracing European founder lineages in the near eastern mtDNA pool*. *Am. J. Hum. Genet.* 67(5):1251–1276.
- Rootsi S et al. (2013): *Phylogenetic applications of whole*

Y-chromosome sequences and the Near Eastern origin of Ashkenazi Levites. Nat. Commun. 4: 2928.

- Ruitberg CM, Reeder DJ, Butler JM (2001): *STRBase: a short tandem repeat DNA database for the human identity testing community.* Nucleic Acid Res. 1(29):320–322.
- Sajantila A, Lukka M, Syvanen A-C (1999): *Experimentally observed germline mutations at human micro- and minisatellite loci.* Eur. J. Human Genet. 7:263–266.
- *Sancti Stephani regis primi Hungariae Libellus de institutione morum sive Admonitio spiritualis – Szent István: Erkölcsstanító könyvecske avagy Intelmek* (2004): Edited and translated by: Havas L. Debrecini.
- Seidenberg V, Schilz F, Pfister D, Georges L, Fehren-Schmitz L, Hummel S (2012): *A new miniSTR heptaplex system for genetic fingerprinting of ancient DNA from archaeological human bone.* Journal of Archaeological Science 39:3224–3229.
- Szabados Gy (2011): *Magyar államalapítások a IX–XI. században. Előtanulmány a korai magyar állam történelmének fordulópontjairól.* Szegedi Középkorász Műhely, Szeged. 416.
- Szabados Gy (2016): *Könyves Béla király? Egy székesfehérvári királysír azonosításáról.* Alba Regia. Szent István Király Múzeum Közleményei. (Annales Musei Stephani Regis). Székesfehérvár, C sorozat, 44:193–204.
- Szabados Gy (2018): *Folytonosság és/vagy találkozás? „Avar” és „magyar” a 9. századi Kárpát-medencében.* Hága TK, Kolozsi B (eds.): *Sötét idők túlélői. A kontinuitás fogalma, kutatásának módszerei az 5–11. századi Kárpát-medencében.* *Tempora Obscura* 4. Debrecen. 227–253.

- Szabados Gy (2016): *Szent István Király Múzeum Közleményei*. (Anneles Musei Stephani Regis). Székesfehérvár, C sorozat 44. szám.
- Szentpétery E (ed.) (1937–1938): *Scriptores Rerum Hungaricarum* I–II. Budapestini.
- Szőke BM (2014): *A Karoling-kor a Kárpát-medencében*. Magyar Nemzeti Múzeum, Budapest.
- Thoroczky G (ed.) (2018): Írott források az 1116–1205 közötti magyar történelemről. Szegedi Középkortörténeti Könyvtár, 28. Szegedi Középkorász Műhely, Szeged.
- Tóth E (2005–2006): *III. Béla vagy Kálmán?* Folia Archeologica LII, 52:141–146.
- Török A (1894): *Jelentés III-ik Béla magyar király és neje testereklyéiről*. MTA Értekezések a Term. Tud. köréből, 23, 175–355.
- Török A (1900): *III. Béla és első hitvese földi maradványai*. In: Forster Gy (ed.): *III. Béla magyar király emlékezet*. 200–206.
- Török A (1994): *Adatok az Árpádok testereklyéinek embertani bűvárlatához*. MTA Értekezések a Term. Tud. köréből 23. 565–630.
- Török A (2008): *1883. évi antropológiai vizsgált*. Quote from Éry's book. 19.
- Uzsoki A (1984): *I. András király sírja Tihanyban és a sírlap ikonográfiai vonatkozásai*. A Veszprém Magyar Múzeumok Közleményei 17:145–188.
- Vanek D, Saskova L, Koch H (2009): *Kinship and Y-chromosome analysis of 7th century human remains: novel DNA extraction and typing procedure for ancient material*. Forensic Sci., 50:286–294.

- Van Oven M, Kayser M (2009): *Updated Comprehensive Phylogenetic Tree of Global Human Mitochondrial DNA Variation*. Human Mutation. 30.
- Váradi OA, Horváth O, Marcsik A, Molnár E, Pálfi Gy, Bereczki Zs (2015): *Különleges formájú jelképes trepanációk a Dél-Alföldről*. Anthropologiai Közlemények 56:91–104.
- Waldron T (1985): *DISH at Merton Priory: evidence for a “new” occupational disease*. Brit. Med. J. 291:1762–1763.
- Walsh B (2001): *Estimating the time to the most recent common ancestor for the Y chromosome or mitochondrial DNA for a pair of individuals*. Genetics, 158:897–912.
- Weber JL, Wong C (1993): *Mutation of human short tandem repeats*. Human Molec. Genet. 2:1123–1128.
- Zvánigorosky V, Crubézy E, Gibert M, Thèves C, Hollarda C, Gonzalez A, Fedorovac AS, Alexeev AN, Rozalia I, Bravinae BI, Ludesb B, Keysera C (2016): *The genetics of kinship in remote human groups*. Genetics 25:52–62.

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In this book, we provide a detailed description of the joint work, the goal of which was to carry out the genetic identification of the skeletons originally interred in the Basilica of the Assumption of the Blessed Virgin Mary in Székesfehérvár and preserved in the crypt of the Matthias Church in Budapest. To this end, we had to perform the genetic identification of the skeletons of King Béla III and Queen Anne of Antioch whose remains were preserved in the marble sarcophagi in the Royal Chapel of the Matthias Church. The individual identification of the skeletons preserved in the crypt of the Matthias Church could be carried out on the basis of the Árpád-Dynasty genotype thus identified and we could also identify a formerly unknown king from the Árpád- Dynasty, which in turn has led us to investigate the origins of the Árpáds.

We recommend this book for those who are interested in this glorious era of the Hungarian history, who would like to know more about the most significant Hungarian kings and who would like to pay tribute to their identified remains in the place where their eternal sleep was disturbed by history. We would also like to recommend this volume for those who would like to gain a glimpse of the application of modern genetics.