Multi-disciplinary analysis of an elite Xiongnu tomb from Belkhin Am cemetery, Ulaanbaatar

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ABSTRACT

The Xiongnu Empire was the first integrated political formation of Eastern Steppe nomads, whose history is known from the contemporaneous Chinese written sources and the archaeological findings of their burials. Here we present a complex

multidisciplinary study of a tomb from the Xiongnu cemetery of Belkhin Am. The archaeological features characterized the grave as an elite Xiongnu burial. By radiocarbon analysis the tomb could be dated to the middle of the Xiongnu era, between the 1st century BCE and 1st century CE. The phylogenetic connections of the human remains found in the grave were in concordance with its Inner East Asian location.

KEYWORDS: radiocarbon dating, archaeogenetics, ancient DNA, phylogenetic analysis, mitochondrial DNA

1. Introduction

The Xiongnu Empire was the first documented nomadic empire in the Eastern Steppe located in modern-day Mongolia which was established at the end of the 3rd century BCE and existed for three centuries. The borders of the empire at its greatest expansion spanned from the Korean Peninsula in the East to Lake Balkhash in the West, from Lake Baykal in the north to Ordos and the Great Wall of China in the south (Danilov 2011; Miller 2011; Brosseder and Miller 2011; Honeychurch 2015). Around 55 CE, the empire was torn apart by power struggles among crown princes; the southern tribes led by Huhanye integrated into China, while the northern Xiongnus remained independent. After serial struggles between the Northern Xiongnu and the joint forces of Chinese and Southern Xiongnu during the 1st–2nd centuries CE, leading groups of the northern Xiongnu migrated westward (Brosseder and Miller 2011; Obrusánszky 2021).

Due to the lack of Xiongnu literacy as they had no written language of their own, the written sources of their history and polity were limited to Chinese historiography (Pan Ku 1938; Sima 1993; Watson 1973). On the other hand, a great abundance of archaeological findings has been recovered, of which the most important sources are the Xiongnu cemeteries of Mongolia, in the heartland of the empire (Honeychurch 2003; Ochir 2007). The most typical burial style in the Xiongnu era were the stone terraced graves often containing a stone or wooden coffin (e.g., Figure 1B). Differences in the burial constructions (e.g., size and structure), burial customs, and grave good types have been explained by the social status of the buried. Aristocratic tombs had greater size, square shaped, tens of meters wide and deep, while commoners were buried under smaller stacks of stones or in unmarked graves. (Wright 2006; Brosseder 2009; Miller 2014).

Archaeogenetic and comparative anthropological studies suggested multiple origin and heterogenous ethnical composition of the Xiongnu people with significant West Eurasian contribution (Keyser-Traqui et al. 2003; Kim et al. 2010; Schmidt and Seguchi 2014; Keyser et al. 2021). Archaeogenomic studies have shown that the Xiongnu developed by an admixture of the descendants of two deeply diverged populations that had previously lived in the western and eastern parts of Mongolia: peoples of the Deer Stone Kirighsur Complex-related Siberian Scythian Uyuk culture and of the East Asian-type Ulaanzuukh/

Slab Grave culture. During the existence of the empire, further genomic influx was detected from the Central Steppe and China, resulting in a very heterogeneous population with great genomic diversity (Damgaard et al. 2018; Jeong et al. 2020).

The aristocratic cemetery at Belkhin Am (Ulaanbaatar) is likely to be one of the cemeteries of the central Xiongnu rulers and aristocrats. In 1960, the Institute of History of the Mongolian Academy of Sciences and the Academy of Sciences of the People's Republic of China agreed to excavate some human graves at the request of T. Horvát, director of the Hungarian Association for Asian Studies and director of the Asian Art Museum in Budapest. A joint Mongolian-Hungarian field research team conducted the excavations and identified six large rectangular aristocratic tombs and four small circular satellite tombs. Among them, the aristocratic tomb Nr. 4 and two satellite tombs, Nr. 7 and 9 were excavated. However, due to the looting of the graves between the 2nd and 3rd centuries CE, no artifacts were found, except few remains of wooden coffins and textiles and iron rust in tomb Nr. 7 (Erdélyi and Sugár 1982).

The main goal of the present project was the excavation and multi-disciplinary analysis of the aristocratic tomb Nr. 3, which started in 2021 with the joint conduction of the Xiongnu and Ancient Countries Research Institute of the Institute of Archeology of the Mongolian Academy of Sciences and the Institution of Hungarian Research.

2. Materials and Methods

2.1. Excavation site and archaeological methods

The site is located north of Ulaanbaatar (Lat.: 48,01; Lon.: 106,94), on the right bank of Belch River flowing down from the mountains, on a plateau in a valley formed by a rising foothill and mountain ridge. The excavation began with the cleaning of the stone covering of the tomb Nr. 3. The area of the tomb was divided into four excavation sections - so the stone-loaded layering of the tomb could be well documented both on the east-west and the north-south section walls. After the documentation and subsequent tearing of the stone loading, as well as the demolition of the section walls left as witness walls, it became clear that the tomb is a rectangular burial pit, deepening gradually. A satellite grave was also excavated and the stone covering of a third larger grave was cleaned. After the excavation, the graves were backfilled to the original ground level and the stone covering of the noble grave was reconstructed from the hundreds of cubic meters of excavated stone material.

2.2. Radiocarbon dating

Radiocarbon analysis was performed on the sampled bone fragments to confirm the archaeological dating of the remains. The measurements were done by accelerator

mass spectrometry (AMS) in the AMS laboratory of the Institute for Nuclear Research, Hungarian Academy of Sciences, Debrecen, Hungary (AMS Lab ID: DeA-37107; technical details concerning the sample preparation and measurement: Molnár et al. 2013). The conventional radiocarbon date was calibrated with the OxCal 4.4 software (https://c14. arch.ox.ac.uk/oxcal/OxCal.html, date of calibration: 1st of July 2022) with IntCal 20 settings (Reimer et al. 2020).

2.3. Sample preparation and DNA extraction

A bone sample was taken *in situ* from the upper half of the tibia with Dremel tools treated with bleach in the building of the Mongolian Academy of Sciences. DNA extraction and library preparation were carried out in clean-room conditions in the common ancient DNA laboratory of the Department of Archaeogenetics, Institute of Hungarian Research and the Department of Genetics, University of Szeged. The DNA was extracted two times from the upper half of the tibia fragment. DNA extraction included a pre-digestion step for 30 min at 48 °C in 0.5 M EDTA (pH 8) supplemented with Proteinase K with 100 µg/ml final concentration, and a digestion for 72 h at 48 °C in 0.45 M EDTA (pH 8) supplemented with 250 µg/ml Proteinase K and 1 % Triton-X 100. After digestion the DNA was purified from the extraction buffer on MinElute column (Qiagen) and eluted in 50 µl Elution Buffer. The DNA concentration was measured with Qubit system (Invitrogen). During the secondary extraction only the digestion and purification steps were repeated.

2.4. Library preparation and mitochondrial enrichment

Libraries were generated from the primary and the secondary DNA extracts as well. We applied the double stranded library protocol of (Meyer and Kircher 2010)target capture from sequencing libraries has largely replaced polymerase chain reaction (PCR with double indexing (Kircher *et al.*, 2012)sample multiplexing is necessary for making economical use of available sequencing capacities. A widely used multiplexing strategy for the Illumina Genome Analyzer utilizes sample-specific indexes, which are embedded in one of the library adapters. However, this and similar multiplex approaches come with a risk of sample misidentification. By introducing indexes into both library adapters (double indexing). Libraries were generated from partial uracil-DNA glycosylase (UDG)-treated DNA extracts (Rohland et al. 2015)ancient DNA protocols have not, in general, focused on reducing the time required to screen samples. We present an adaptation of a popular ancient library preparation method that makes screening more efficient. First, the DNA extract is treated using a protocol that causes characteristic ancient DNA damage to be restricted to the terminal nucleotides, while nearly eliminating it in the interior of the DNA molecules, allowing a single library to be used both to test for ancient DNA authenticity and to carry out population genetic analysis. Second, the DNA molecules are ligated to a unique pair

of barcodes, which eliminates undetected cross-contamination from this step onwards. Third, the barcoded library molecules include incomplete adapters of short length that can increase the specificity of hybridization-based genomic target enrichment. The adapters are completed just before sequencing, so the same DNA library can be used in multiple experiments, and the sequences distinguished. We demonstrate this protocol on 60 ancient human samples.","container-title":"Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences","DOI":"10.1098/rstb.2013.0624","ISSN":"1471-2970","issue":"1660","journalAbbreviation":"Philos Trans R Soc Lond B Biol Sci","language":"eng","note":"PMID: 25487342\nPMCID: PMC4275898","page":"20130624","source":"PubMed","title":"Partial uracil-DNA-glycosylase treatment for screening of ancient DNA","volume":"370","author":[{„family":"Rohland","given":"Nadin"},{„family":"Harney","given":"Eadaoin"},{„family":"Mallick","given":"Swapan"},{„family":"Nordenfelt","given":"Susanne"},{„family":"Reich","given":"David"}],"issued":{"date-parts":[[„2015",1,19]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} and were purified on MinElute columns (Qiagen). Quantity and quality measurements were performed with the Qubit fluorometric quantification system (Invitrogen) and the TapeStation automated electrophoresis system (Agilent). Additionally, the endogenous human DNA content of the libraries was estimated with shallow shotgun sequencing on iSeq 100 platform (Illumina). Mitochondrial genome enrichment with hybridization capture was performed according to Maár et al. 2021. Whole mitogenome sequencing was carried out on iSeq 100 platform (Illumina) in paired-end mode.

2.5. Raw data handling

Raw sequence data was handled according to (Maár et al., 2021). The raw nucleotide sequence data of the sample was deposited to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under accession number: PRJEBxxxxx.

2.6. Mitochondrial Hg assignment and phylogenetic analysis

Mitochondrial haplogroup (Hg) determination was performed using HaploGrep v2.1.25 (Weissensteiner et al., 2016). All the available ancient mitochondrial genomes belonging to the C4a1a and C4a1a+195 Hgs were downloaded (Damgaard et al. 2018; Jeong et al. 2020; Keyser et al. 2021; Veeramah et al. 2018; Yu et al. 2020), and the Median-Joining network phylogenetic tree (Bandelt et al. 1999) was reconstructed according to (Neparáczki et al. 2018).

3. Results

3.1. Archeological findings

Tomb Nr. 3 was a square-shaped, stone-covered burial place of nearly 20x20 meters size, with a grave exit (dromos) facing south (Figure 1 A-B). After the cleaning of the stone covering of the grave, it became clear that it was a large, rectangular Xiongnu grave. The stone layers of the grave and the position of the central robber pit could be documented clearly on the east-west and north-south section walls. After dismantling the section walls, it was already verified that the tomb is a typical Xiongnu tomb with a square burial pit deepening gradually and narrowing in a step-like manner (Figure 1 B). The carved wooden burial chamber and the disturbed remains of a noble burial were found at the bottom (Figure 1 D).

Figure 1. The location of the tomb and the recovered grave goods. A) The map of the cemetery. B) Tomb Nr 3. C) The excavated grave goods. D) The wooden burial chamber.

Despite the substantial degree of grave robbery, beautiful bronze coffin curbings depicting a four-leafed flower, coffin nails, the remains of a Xiongnu-type two-wheeled chariot and fragments of ceramic vessels could be recovered. The remains of textile and organic matter found around the metal objects, the imported amber beads, and a clothes ornament made of openwork bronze plate depicting a galloping horse, of which there could be many in the grave, are also worth mentioning (Figure 1 C).

Unfortunately, no human remains were found in this noble tomb among the disturbed grave goods, due to the thorough activity of the grave robbers, except a fragment of a human tibia in the robber pit, from which genetic and radiocarbon analysis could be carried out. Overall, even though the burial was heavily robbed, typical Xiongnu objects were found in the grave, which, even in their fragments, prove the nobility of the dead Xiongnu resting in the grave.

From the other two investigated graves no valuable anthropological or archaeological findings were found.

3.2. C[14] dating

The radiocarbon analysis of the Belkh burial (conventional radiocarbon age is 2040 ± 21; δ13C value is −15.9 (‰, VPDB, ±0,1 ‰)) dated the sample between the 1st century BCE and 1st century CE. In particular, from 103 BCE to 26 CE with 94.9% probability, and between 50−55 CE with 0.5% probability (Fig. 2). Thus, the analysis confirmed the preliminary archaeological data, and the burial can be dated, indeed, to the late Xiongnu period.
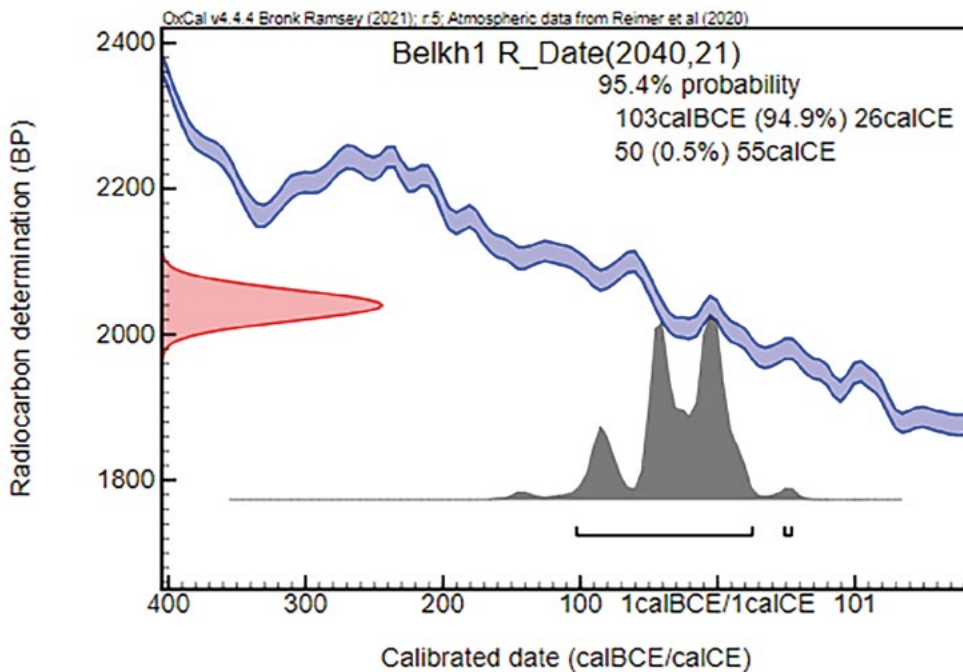

Figure 2. Calibration graph of the Belkh1 sample radiocarbon data (Oxcal 4.4 software with IntCal20 settings)

3.3. Sequencing results and assigned Hg

We successfully extracted DNA from the tibia fragment two times and generated double stranded libraries for Illumina sequencing. Shallow shotgun sequencing detected 0.0% human DNA content in the primary library, but the repeated approach resulted in 0.3% human reads, thus we enriched the mitochondrial genome from the secondary library. We could obtain 94x average coverage over the whole mitochondrial genome, while 99.9% of the bases were covered at least 5x (for sequencing statistics see Table 1).

ID	All reads	Endogen reads	Endogen percent	MT reads	Mt reads after dup-lication removal	Insert size	Insert standard deviation	Average depth of coverage	Bases covered at least 5x	Bases covered 0x
Belkh1	2753414	286630	0.104	216668	53162	58.64	19.71	93.964	99.9%	6

Table 1. Sequencing statistics

Schmutzi estimated negligible contamination for the sample (1 %). On the grounds of haplogroup determination by HaploGrep 2.0, the sample belongs to Hg C4a1a+195 (Table 2). Genetical sex of the individual (Skoglund et al. 2013) was ambiguously determined according to the primary library as male (consistent with XY, but not XX), and according to the secondary library as female (consistent with XX) due to the low endogenous DNA content of the libraries.

Sample identifier	haplogroup defining SNP-s found	haplogroup defining SNP-s not found (**due to lack of coverage**)	private SNP-s	haplogroup (HaploGrep)	overall rank (HaploGrep)
Belkh1	73G 195C 263G 489C 750G 1438G 1715T 2706G 3552A 4715G 4769G 6026A 7028T 7196A 7999C 8584A 8701G 9540C 9545G 10398G 10400T 10873C 11719A 11914A 11969A 12672G 12705T 13263G 14318C 14766T 14783C 15043A 15204C 15301A 15326G 15487T 15968C 16129A 16223T 16298C 16327T	**249d 2232.1A 8860G** 16093C	2226.1A 3107C 8855N 8856N 8857N 8858N 8859N 8860N 16519C	C4a1a+195	0.9625

Table 2. Mitochondrial Hg assignment data

3.4. Phylogenetic analysis

The Median-Joining network phylogenetic tree was constructed from all available archaic mitogenomes belonging to C4a1a and C4a1a+195 Hgs (Figure 3). The C4a1a+195 Hg was widespread in Central-Inner Asia from the Bronze Age. The most similar mitogenomes to Belkh1 were from a Late Xiongnu individual and a Middle-Late Bronze Age sample from

Mongolia connected to Dear Stone – Kirighsur Complex (DSKC_MLBA). This Hg additionally includes other mitogenomes from Xiongnu remains and peoples of Middle-Late Bronze Age Mongolia (Damgaard et al. 2018; Jeong et al. 2020; Keyser et al. 2021; Veeramah et al. 2018; Yu et al. 2020).
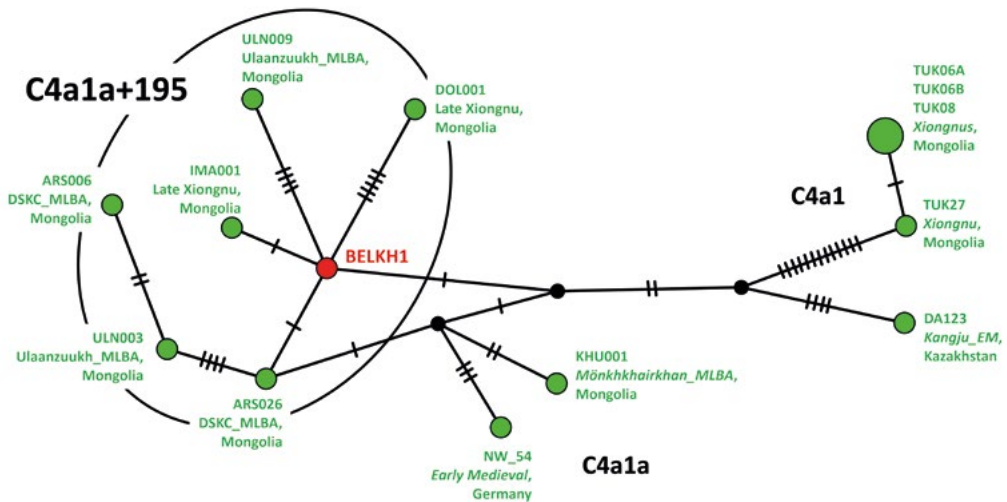

Figure 3. The M-J network of Hg C4a1a+195. On this phylogenetic tree, bubbles indicate individual mitogenomes and the striae crossing the line linking two individuals refer to the number of divergent bases between the linked mitogenomes. Red bubble – this study; green bubble – archaic samples (Damgaard et al. 2018; Jeong et al. 2020; Keyser et al. 2021; Veeramah et al. 2018; Yu et al. 2020).

4. Discussion

The archeological aspects of Tomb Nr. 3 were typical for the Xiongnu period. The large, square, terrace tombs were general among the Xiongnu elite (Brosseder 2009; Shan 2012). The grave goods, which were found in the tomb despite the obvious traces of grave robbery, also referred to an elite Xiongnu burial (Shan 2012).

The radiocarbon dating of the human bone found in the robber pit underpinned the archaeological observations: the tibia dated to the middle Xiongnu period (Miller et al. 2014). The mitochondrial haplogroup of the individual belongs to a haplogroup typical in Inner-East Asia, including Mongolia (Damgaard et al. 2018; Jeong et al. 2020; Keyser et al. 2021; Yu et al. 2020). Extraordinarily, this haplogroup was also found in an early Medieval female from Bavaria (Germany) with artificially deformed skull (Veeramah et al. 2018), showing that the haplogroup reached Western Eurasia in the 4–5th centuries CE,

probably with the westward migration of peoples originated in the territory of present-day Mongolia.

Thus, the excavation can be considered successful from an archaeological point of view, and further exploration of the cemetery is reasonable.

Acknowledgement

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KIVONAT

Egy xiongnu elit sír multidiszciplináris elemzése Belkhin Am (Ulaanbaatar) temetőjéből

Az eurázsiai sztyeppe keleti részén élő nomád népek első egységes politikai formációja az Ázsiai Hun Birodalom volt, melynek történetéről elsősorban a sírjaikban talált régészeti leletekből és a korabeli kínai írott forrásokból tájékozódhatunk. Jelen tanulmányban a Belkhin Am ázsiai hun kori temető egyik sírjának komplex multidiszciplináris vizsgálatát mutatjuk be. A régészeti karakterek tanúsága szerint a sír egy elit rétegbe tartozó egyénhez tartozott. Radiokarbon kormeghatározással a temetkezést az ázsiai hun korszak közepére datáltuk, a Kr. e. I. és a Kr. u. I. század közé. A sírban talált emberi maradvány filogenetikai kapcsolatai jól korreláltak a belső-ázsiai lelőhelyével.