ORIGINAL INVESTIGATION

Genetic evidence suggests a sense of family, parity and conquest in the Xiongnu Iron Age nomads of Mongolia

Christine Keyser[1,2] · Vincent Zvénigorosky[1,2] · Angéla Gonzalez[1] · Jean-Luc Fausser[1] · Florence Jagorel[3] ·
Patrice Gérard[4] · Turbat Tsagaan[5] · Sylvie Duchesne[4,6] · Eric Crubézy[4] · Bertrand Ludes[2]

Abstract

In an effort to characterize the people who composed the groups known as the Xiongnu, nuclear and whole mitochondrial DNA data were generated from the skeletal remains of 52 individuals excavated from the Tamir Ulaan Khoshuu (TUK) cemetery in Central Mongolia. This burial site, attributed to the Xiongnu period, was used from the first century BC to the first century AD. Kinship analyses were conducted using autosomal and Y-chromosomal DNA markers along with complete sequences of the mitochondrial genome. These analyses suggested close kin relationships between many individuals. Nineteen such individuals composed a large family spanning five generations. Within this family, we determined that a woman was of especially high status; this is a novel insight into the structure and hierarchy of societies from the Xiongnu period. Moreover, our findings confirmed that the Xiongnu had a strongly admixed mitochondrial and Y-chromosome gene pools and revealed a significant western component in the Xiongnu group studied. Using a fine-scale approach (haplotype instead of haplogroup-level information), we propose Scytho-Siberians as ancestors of the Xiongnu and Huns as their descendants.

Introduction

The Xiongnu were a confederation of nomadic pastoral tribes during Mongolian prehistory that founded the First Steppe Empire in Inner Asia (Giscard et al. 2013). From the third century BC to the second century AD, they ruled over a territory that extended, at its peak, from Lake Baikal in the north to the Gobi desert in the south and from western Manchuria in the east to the Pamirs in the west. Their relations with the neighboring Chinese during the reign of the Han dynasty (206 BC–220 AD) were complex, with alternating periods of conflict (the construction of the Great Wall was the Chinese response to Xiongnu incursions) as well as trade, gifts, and tributes, along with arranged marriages (the policy of "harmonious relations" adopted by the Han) (Giscard et al. 2013). Around 130 BC, the Chinese began implementing more aggressive policies, leading to the decline of the Xiongnu, who disappeared from Chinese records after the second century AD.

Since they were non-literate, most of the information available about the Xiongnu is, in fact, found in Chinese historical texts (e.g. lifeways; social, economic and political systems; military practices) but archeological excavations have been another important source of data (e.g. grave goods, burial types, technological skills) (Wright et al. 2009; Park et al. 2010). However, some key aspects of Xiongnu culture are still unknown, namely their biological structure, their ethnic or linguistic identity and their relationship with other Iron Age nomads. The term "Xiongnu" designated both a people and a political entity, but it is uncertain whether it could be assigned to an ethnicity, a biological population, an archeological culture or, more likely, some combination of those. Results from physical

✉ Christine Keyser
  ckeyser@unistra.fr

1. Institut de Médecine Légale, Université de Strasbourg, Strasbourg, France

2. Université de Paris, BABEL, CNRS, Paris, France

3. Institut National de la Transfusion Sanguine (INTS), Paris, France

4. Université de Toulouse, AMIS, CNRS, Toulouse, France

5. Institute of Archaeology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia

6. Institut National de Recherches Archéologiques Préventives (INRAP), Paris, France

anthropological studies and notably comparative craniofacial analysis revealed a complex population structure for the Xiongnu people (Schmidt and Seguchi 2016). Genetic analyses of skeletal remains unearthed from burials attributed to the Xiongnu era revealed features characteristic of East Asian populations, with low-level admixture from West Eurasian populations (Keyser-Tracqui et al. 2003; Kim et al. 2010). More recent paleogenomic studies confirmed that the Xiongnu were genetically heterogeneous and suggested that they belonged to two distinct groups, one being of East Asian origin and the other presenting high levels of admixture with West Eurasian sources (Damgaard et al. 2018).

To attain a more comprehensive understanding of the peoples that composed the groups known as the Xiongnu and gain insight into their origins and potential biological relationships with other nomadic groups, we investigated the genetic structure and affinities of people buried in a large Mongolian necropolis from the Xiongnu period, the Tamir Ulaan Khoshuu cemetery. Through the combined analysis of DNA markers located on autosomes, the Y-chromosome and whole mitochondrial genomes, we reconstructed an unprecedented ancient Xiongnu family structure, probably that of an elite family. Moreover, following maternal and paternal lineages characterized using a fine-scale approach (haplotype instead of haplogroup-level information), we attempted to disentangle Xiongnu genetic history.

Materials and methods

Site and bodies

The Tamir Ulaan Khoshuu (TUK) cemetery is located near the confluence of the Tamir River and the Orkhon River in the Arkhangai Aimag (Central Mongolia), about four hundred kilometers west of the capital of Mongolia, Ulaanbaatar (Fig. 1). It encompasses an area of 22 hectares located on a prominent granitic outcrop and comprises a total of 397 graves, delimited by stone circles. The site is divided by a gully into a western area consisting of 344 graves and an eastern area composed of 53 larger graves (up to 20 m in diameter), reflecting a tomb style typical of the Xiongnu era and possibly belonging to an elite (Brosseder 2011). Between 2013 and 2018, 48 of these eastern stone ring burials were excavated by a French–Mongolian joint research team (the remaining 5 had been investigated in 2005 by a Mongolian-American Expedition but no DNA data were published). Although robbers have looted all tombs, many grave goods were found in the burials, including gold earrings, glass beads, ceramic jars, iron knives, Chinese lacquer as well as Chinese imported artifacts, such as bronze mirror fragments and circular nephrite stones. Quatrefoil decorations, specific to the Xiongnu culture, were recovered on several coffins. Like in other Xiongnu tombs, animal deposits (only the heads and legs of sheep, horses and oxen) were found close to the deceased, outside the coffins. Radiocarbon (^{14}C) dates to the cemetery between the first century BC and the first century AD (Poznan Radiocarbon

Fig. 1 Location of the Tamir Ulaan Khoshuu (TUK) cemetery and map of the necropolis. Colors indicate members of the same family

Laboratory), in the middle of the Xiongnu period. The 48 excavated graves yielded the remains of 57 individuals, specifically 21 males, 17 females and 19 of undetermined sex. Although most of the graves were single (individual) graves, seven contained the remains of two or three individuals. Moreover, four of them (07, 13A, 14A and 14B) were attributed to culturally Turkic individuals. Detailed information about the samples is presented in Table S1.

DNA extraction and quantification

DNA was extracted from bone fragments and/or teeth in a dedicated clean room facility as previously described (Mendisco et al. 2011). Two to three independent DNA extractions were completed from each sample. Quantification of DNA extracts was performed on a 7500 Real-Time PCR system (Applied Biosystems) using the Quantifiler™ Trio DNA quantification Kit (Thermo Fischer Scientific) which permits the quantification of autosomal and Y-chromosomal human DNA and the estimation of DNA degradation (Table S2).

Autosomal STR typing and kinship determination

Twenty-one autosomal STR (aSTR), one Y-chromosomal STR (Y-STR), one Y-chromosomal indel polymorphism and the marker amelogenin were amplified using the GlobalFiler® kit (Thermo Fischer Scientific) according to the manufacturer's protocol except that 32 PCR cycles were used instead of 29. Independent extracts from each sample were amplified at least twice. Capillary electrophoresis was performed on a 3500 Genetic Analyzer (Applied Biosystems) and data analysis was performed using the GeneMapper v.4.1 software (Applied Biosystems). Consensus aSTR profiles were determined by retaining the alleles that had been typed at least twice for each sample. Genetic relationships between individuals were tested by pairwise comparison of the aSTR profiles using the ML-Relate computer program (Kalinowski et al. 2006). ML-Relate implements an estimation of the likelihood of four levels of kinship (unrelated, half-siblings, full-siblings and parent-offspring) using modeled Identity-by-Descent levels in each pair of individuals given the allelic frequencies (estimated from the ancient sample set under study). It is used as a first approach to the population, to highlight possibly significant links. Likelihood Ratios (LR) were calculated using the Familias 3 software (https://familias.no) (Kling et al. 2014) which allows us to compute the LR of each level of kinship against the likelihood that individuals are unrelated. This permits the estimation of the reliability of kinship calls and the analysis of larger pedigrees.

Y-chromosomal STR and SNP typing

The DNA of the male individuals was analyzed at 17 and/or 27 Y-STR loci using the AmpFLSTR Y-Filer® and Y-Filer-Plus® kits (Thermo Fisher Scientific) according to the manufacturer's protocols except for the number of PCR cycles which was increased from 30 to 34 (Y-Filer®) and from 27 to 30 (Y-FilerPlus®). PCR products were analyzed on the ABI Prism 3500 Automatic Sequencer using the GeneMapper software as previously noted. The Y-STR haplotypes were individually compared to an in-house world Y-STR database containing ~216,000 haplotypes retrieved from the literature. Y-haplogroups (Y-Hg) were determined from consensus Y-haplotypes using Y-Haplogroup Predictor (https://www.hprg.com/hapest5/?hapest5). To increase resolution for the R and Q haplogroups, two sets of Y-chromosomal SNP (Y-SNP) were selected. The first set was composed of 15 Y-SNP defining the R haplogroup and some R subclades (M207, M173, M420, M516, M17, Z280, M458, Z93, M746, Z95, Z2125, M434, M560, M582 and M780). The second set was composed of 14 Y-SNP characterizing the main Asian Y haplogroups (M214, M175, M231, Tat, M128, P43, M242, M346, L54, M3, M120, M130, M217 and M174) (Hollard et al. 2018). SNP typing was performed on the Mass Array platform using the iPLEX® gold technology (Agena Bioscience) as described in Mendisco et al. (2011). The Y-SNP haplogroup nomenclature followed the ISOGG-Y DNA Haplogroup Tree 2019.

Whole mitochondrial genome sequencing

Complete mitochondrial genome analysis was performed on the Ion Torrent Personal Genome Machine™ (PGM™, Thermo Fisher Scientific) using the Precision ID mtDNA Whole Genome Panel (Applied Biosystems). DNA libraries were constructed using the Ion Ampliseq™ Library kit (Thermo Fisher Scientific) with 2 separate primer pools amplifying 162 amplicons that target the entire human mitochondrial genome. To sequence multiple samples simultaneously, Ion Xpress™ Barcode Adapters were used. Using emulsion PCR, the generated libraries were attached to beads and further amplified. The concentration of each of the libraries was determined with qPCR using the Ion Library TaqMan™ Quantitation Kit (Thermo Fischer Scientific). High-quality templated Ion Sphere™ particles containing clonally amplified DNA were prepared using the Ion PGM™ Template OT2 kit and the IonTouch™ 2 instrument (Thermo Fisher Scientific). The template-positive Ion Sphere™ particles were enriched with the Ion One Touch Enrichment System (Thermo Fisher Scientific) as per the manufacturer's guidelines. Semiconductor sequencing was conducted on the Ion PGM using Ion 316 or 318 Chips using the IonPGM Hi-Q Sequencing kit. The Torrent Suite software was used to

analyze all the sequences generated that were aligned to the mitochondrial DNA (mtDNA) revised Cambridge Reference Sequence (Andrews et al. 1999) and searched against an in-house database of ancient and present-day human whole mitochondrial genomes containing more than 40,000 publicly available sequences. Mitochondrial DNA haplogroups (mt-Hg) were assigned using HaploGrep2 (Weissensteiner et al. 2016), based on the latest version of PhyloTree mt (https://www.phylotree.org/).

Phenotype- and biogeography-informative SNP typing

Twenty-four phenotypic SNP and 56 biogeographical ancestry-informative SNP included in the ForenSeq™ DNA Signature Prep Kit (Verogen) were typed on a set of seven Xiongnu individuals from the great family (TUK-01,-03,-18,-19,-23,-25,-26). The samples were kindly processed by Verogen according to their own recommended protocols and analyzed on a MiSeq FGx System using the ForenSeq Universal Analysis Software (Verogen).

Results

An exceptional genealogy detected by autosomal STR profiles

The overall preservation of DNA in the TUK samples was rather good since complete or near complete aSTR profiles were obtained for 52 of the 57 excavated individuals (consensus aSTR profiles are reported in Table S3). DNA from five individuals (TUK-02A,-02B,-27,-30C and -41) appeared severely degraded since no or few amplified products were obtained. The sex-linked loci (amelogenin, Y-STR, Y-indel) indicated that 26 of the 52 successfully genotyped individuals were male and 26 were female (Table S3). The results of the molecular-based sex identification were consistent with available physical anthropological data and permitted the determination of the sex of the 17 individuals for whom morphological indicators of sex were not well defined or absent (Table S1).

Visual comparison of the profiles in pairs and LR analyses revealed the presence of first-degree (parent–offspring and full-sibling) and second-degree relatives in the TUK cemetery. As parent–offspring relationships have been shown to be the only relationships systematically reliably detected by the LR method applied to this number of STR (Zvénigorosky et al. 2020), we focused on these relationships and identified five nuclear families (composed of the parents and one or two children) (TUK-01,-02C,-03,-26; TUK23,-19,-18; TUK-36,-34,-48,-25; TUK-25,-13B,-40; TUK-40,-32,-11). All of them were part of a five-generation familial structure composed of

19 individuals and represented in Fig. 2. The reliability of this ancient family tree (where only 3 members were missing) was confirmed by concordant LR values calculated for full-sibling and second-degree relationships (Table S4a and S4b) as well as by the paternal and maternal lineage analysis (see below).

Outside this multi-generational family, 5 parent/offspring pairs were also identified (TUK-06A,-08; TUK-9A,-10; TUK-14A,-14B; TUK-20,-22 and TUK-43,-44) (Fig. S1). At this stage, it was not possible to determine which individual was the parent or the child in each pair, except for the pair TUK-14A,-14B; since TUK14A was a 12–18 year-old female, and because she was buried together with TUK 14B, an adult female, in a double burial (from the Turkic period), we assumed that she was the child. Note that all of these parent/offspring pairs were found buried together or close to each other (Fig. 1).

Y-chromosomal haplotypes confirm detected relationships

To identify male lineages, an analysis of polymorphic STR systems located on the male-specific part of the Y-chromosome was performed. Y-haplotypes consisting of 19 to 27 STR were obtained for all the 26 male individuals studied (Table S5). Sixteen different Y-haplotypes could be identified, 6 of which were shared by 2 to 5 individuals. A 26-marker Y-haplotype was shared not only by TUK01 and his putative son TUK26 (confirming the aSTR typing result) but also by the sub-adult TUK20, making him a putative paternal relative of the father–son pair. While TUK23 and TUK18 shared the same 24-Y-STR haplotype, as expected for a father–son relationship, pairs TUK-09A,-10 and TUK-43,-44, also identified as father–son pairs, did not share exactly the same Y-STR-haplotype; a single repeat difference was observed at locus DYS570 (alleles 18 vs 19) for the first and at locus DYS576 (alleles 17 vs 18) for the second. Since these markers have been described as rapidly mutating Y-STR (Ballantyne et al. 2012), we considered that a mutation could have occurred in one generation. Similarly, while TUK25 and his son TUK40 shared the same 27-Y-STR haplotype, TUK36 (the father of TUK 25 and grandfather of TUK40) carried a Y-haplotype differing at one locus (DYS19) by a one-step mutation (allele 16 vs 15). Finally, one 20-Y-STR haplotype was shared between TUK12 and TUK35, not considered closely related to our LR calculations but possibly sharing a more distant paternal relationship.

Y-chromosomal lineages reveal the mixed origin of the Xiongnu

Haplogroup (Hg) assignments revealed that Xiongnu Y-haplotypes belonged to at least 5 (R, Q, N, J and G) of

Fig. 2 Description of the five-generation familial structure. A first nuclear family is composed of a father (TUK01), a mother (TUK02C) and two children (TUK03 and TUK26). This latter appeared to be the father of four females (TUK-19,-21,-28 and -29) buried a few meters from each other. One of them (TUK19) was the mother of a male (TUK18) whose father was TUK23 (second family). TUK01, the maternal great grandfather of TUK18, could be considered the putative brother of a female individual (TUK15) who was the mother of TUK34. She formed a third nuclear family with the male TUK36 whose sons are TUK48 and TUK25. The adult male TUK25 was found to form with the adult female individual TUK13B and their son TUK40 a fourth nuclear family. This son was buried in the north of the necropolis not far from TUK32. Both were considered as the parents of a female child (TUK11) interred close to her putative paternal great-great-grand mother TUK15 (fifth nuclear family)

MtDNA haplogroups

- J2a1a1
- T2b3
- H47
- J2b1a2a
- H2a1
- X2b4a

Y haplogroups

- R1a
- Q1a1a
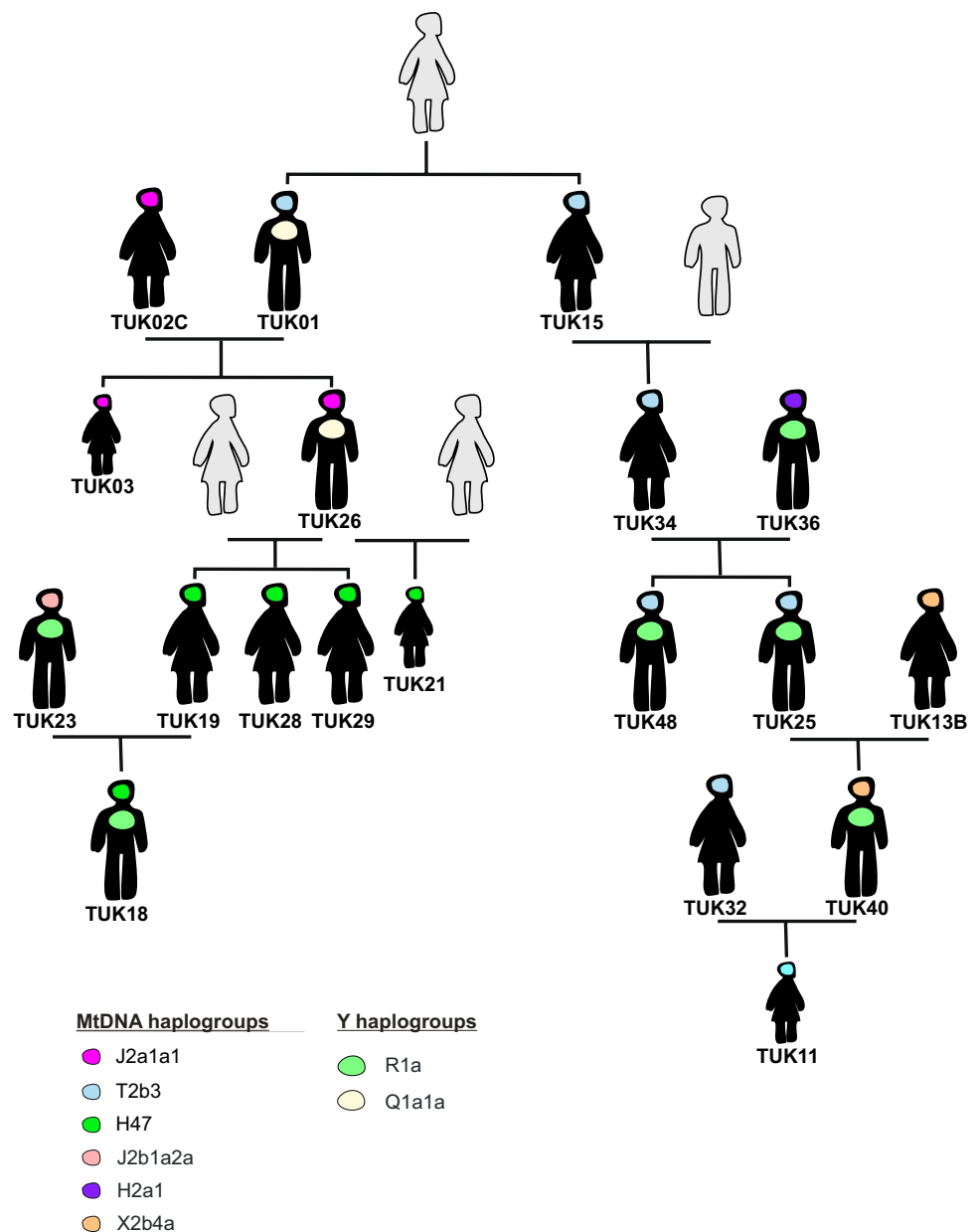
the 20 major clades. Sixteen individuals (some closely related to one another) were affiliated to sub-haplogroup (sub-Hg) R1a, among which 10 (TUK-04,-09A,-09B,-10,-12,-25,-35,-36,-40 and -48) belonged to Hg R1a-Z2125 and 4 (TUK-08,-18,-23 and -37) to Hg R1a-Z95. Five individuals were affiliated to sub-Hg Q1a-M120. Two individuals belonged to Hg N, one to sub-Hg J1 and one to sub-Hg G1. The last one could not be assessed with certainty (Table S5).

Y-haplotypes were individually compared to an in-house Y-STR database maintained by our laboratory containing ancient and modern data retrieved from the literature. Although comparisons with modern-day individuals provided us with a clear geographic distribution of the Y-haplotypes characterized, only the matches with ancient samples reaching 80% of shared alleles have been considered and were reported in Table S6. The highest scores were obtained for the Xiongnu individuals affiliated to Hg R1a. Most of them showed Y-haplotypes close to those of Iron Age male individuals from the Krasnoyarsk region (Keyser et al. 2009). The closest haplotypes (a single difference on 16 or 17 Y-STR loci) were observed between TUK09A and TUK10 and individuals S26 and S28 from the Tagar culture (Keyser et al. 2009). Matches were also observed with Middle Bronze Age male individuals of the same area (*eg.* TUK10 shared a 14 Y-STR haplotype with individuals S10 and S16 from the Andronovo culture). Close matches were also found with 2 Scytho-Siberians from the Tuva Republic

(ARZ-T1,-T28) (Mary et al. 2019). The database search also revealed a shared haplotype (14 Y-STR) between TUK-09A,-10 and King Béla III (1172–1196), one of the most significant rulers of the first Hungarian dynasty (Olasz et al. 2019) as well as a matching haplotype (15 Y-STR) between TUK-12,-35 and another male individual found in the Royal Basilica where King Béla III was buried (II/54). Individuals TUK-9A,-10,-18 and -23 also carried haplotypes similar to those carried by the tenth century Hungarian Conquerors (KEII/61 and NF/2) (Fóthi et al. 2020), whereas TUK-36 and -48 could be linked to a warrior (a commoner) found in the Tavan Tolgoi graves (1130–1250 AD) of the Mongol imperial family (MN376) (Lkhagvasuren et al. 2016). Finally, TUK12 was found to share at least eight Y-STR with a Xiongnu individual from the Egyin Gol Valley (Keyser-Tracqui et al. 2003). Interestingly, TUK-13A (considered to be Turkic), carried a Y-haplotype (Hg N) shared with the eighteenth century Yakut individuals (up to a single difference on 17 Y-STR loci) (Table S6), seventh–eighth century Avar individuals (up to a single difference on 14 Y-STR loci) (Csáky et al. 2020) and a tenth century Hungarian Conqueror (on 13 Y-STR) (Fóthi et al. 2020). No close matches were found for the other Xiongnu males, possibly due to the small number of ancient Y-STR haplotypes included in the database ($n = 369$).

Mitochondrial haplotypes support first- and second-degree relationships

We obtained 52 complete mitochondrial genomes (for the 57 excavated individuals) with an average coverage depth between 21 and 2846 (samples TUK-02A,-02B,-30C,-33 and -42B were dismissed because of insufficient DNA or data quality). Twenty-eight distinct mtDNA-haplotypes were identified (Ht1 to Ht28 in Table S7), ten of which were shared by two to eight individuals.

All first-degree relationships identified through aSTR-typing were supported by shared mitochondrial haplotypes. This was relevant for the six mother/child relationships of the five-generation familial structure (TUK02C and her two children TUK03 and TUK26; TUK19 and her son TUK18; TUK15 and her daughter TUK34 as well as her grandson TUK25; TUK13B and her son TUK40 and finally TUK32 and her son TUK11). This was also true for the TUK-14A,-14B mother–daughter Turkic pair and particularly informative for the TUK-06A,-08 parent–offspring pair; since both shared the same mtDNA haplotype, we assumed that TUK06A was the mother and TUK08 the son and not the opposite (Fig. S1). Their mtDNA haplotype was also shared by the infant TUK06B. The possibility that she was the daughter of TUK06A was dismissed, but they could share a half-sibling level relationship (Table S4a). The most parsimonious interpretation of this result is that TUK06A was

the grandmother of TUK06B. Consequently, TUK08 would have been the uncle of TUK06, a conclusion also compatible with LR calculations (Table S4a, Fig. S1). Full- or half-sibling relationships were also confirmed by the analysis of mitochondrial lineages (TUK-01 and -15; TUK-03 and -26; TUK-19,-21,-28 and -29). Other individuals, not involved in first-degree relationships but possibly in second-degree relationships (TUK-12,-17) or more distant ones (TUK-04,-10; TUK-07,-09B), were also found to share identical mtDNA haplotypes (see Table S7). The fact that the parent–child pair TUK-20,-22 shared the same mtDNA sequence allowed us to establish who the parent was and who the child was; since TUK20 was a 12–18-year-old male, and because he appeared to carry the same mtDNA haplotype as TUK22, we assumed that he was her child.

Mitochondrial lineages reveal European ancestry in the largest family

The 28 distinct haplotypes identified were affiliated to 11 different main haplogroups of European (H, J, T, U, X) or Asian (A, B, C, D, F, G) origin. To assess the geographical and temporal distribution of these maternal lineages, whole mitochondrial DNA sequences were compared to those of ancient and modern-day individuals. As matches (limited to 5 differences) were usually numerous, only comparisons with ancient whole mtDNA genomes have been considered and reported in Table S8.

The most represented haplotype (Ht1) shared by 7 of the 19 members of the great family in addition to one unrelated individuals (TUK35) was affiliated to Hg T2b3 + 151 and produced the closest matches (3 to 4 differences) with individuals associated with the Beaker complex and found in France, Italy or Spain (Olalde et al. 2018). Two other haplotypes (Ht3 and Ht16) both belonging to Hg T1a1, were also very close (2 differences) to Beaker complex-associated individuals from the Czech Republic, Germany and Great Britain as well as to a Hungarian early-medieval individual associated with the Longobard culture (Vai et al. 2019). The second most represented haplotype (Ht13), found in five members of the multi-generational familial structure and affiliated to Hg H47, could be connected (5 differences) to an individual from a Neolithic eastern Hungarian site (Lipson et al. 2017). Three other Xiongnu haplotypes (Ht7, Ht21 and Ht23) were also affiliated to Hg H (H5a1, H2a1 et H7b). Two of them (Ht7 and Ht23) were close (4 differences) to those of individuals associated with Neolithic to Bronze Age cultures in Europe (Gamba et al. 2014; Brotherton 2013; Haak et al. 2015; Saag 2017; Lipson 2017; Mathieson 2018; Mittnick 2018; Olade et al. 2018; Matisoo-Smith 2018). The other maternal lineages, appearing in the largest family, belong to Hg J2a1a1 (Ht2), J2b1a2a (Ht15) or X2b4a (Ht10). Again, these lineages are close to those found in individuals

associated with the Beaker-complex (Olalde et al. 2018) or the Srubnaya culture (Mathieson 2018). Therefore, all the maternal lineages found in the multi-generational family were of Western European ancestry. Other European lineages, outside this great family, are J1d5 (Ht12), U2e2a1 (Ht14), U5a1a1 (Ht24) and U5a1 + a1 (Ht27). Only the last two were closely related (2 to 3 differences) to those of Bell Beaker culture individuals (Olalde et al. 2018). Moreover, Ht27 was present (with 4 differences) in one individual from the Baden culture (Lipson et al. 2017) as well as in early–medieval individuals associated with the sixth-seventh century Longobard (Vai et al. 2019) and Avar cultures (Csáky et al. 2020).

Regarding the Asian lineages, few closely related (less than 5 mismatches) haplotypes were found in our ancient mitochondrial genome database (probably due to the lack of published ancient genomic data from Asian archeological sites). Nevertheless, the mtDNA haplotype Ht22 carried by TUK37 and affiliated to Hg D4j8 was close (4 differences) to that of a Yakut individual unearthed by our team from a nineteenth century grave (unpublished data, YAKa96, Table S7). Moreover, the mtDNA haplotype carried by TUK46 and affiliated to Hg D4j7 was closely related (4 differences) to the haplotypes carried by 3 Scytho-Siberians from the Tuva Republic in Russia, affiliated to the same haplogroup (Mary et al. 2019). Finally, the haplotype of TUK30B (Ht19, Hg D4q) showed only 3 differences with the haplotype of a Late Sarmatian individual (Krzewińska et al. 2018) and the haplotype of TUK-43,-45 (Ht26, Hg G2a1d2) a single difference with the haplotype of a tenth century Hungarian Conqueror (Neparáczki et al. 2018).

SNP typing added phenotypic and biogeographical information

Seven out of the 19 members of the great family were subjected to phenotypic and ancestry-informative SNP typing, allowing estimation of their hair and eye color as well as of their biogeographical ancestry. TUK01, who shared a European mtDNA sequence (Hg T2b3 + 151) with his (half-)sister TUK15 and her offspring but carried an Asian Y-haplotype (Hg Q1a-M120), was found to have black hair and brown eyes as well as a mixed Asian-European ancestry, as expected. He married TUK02 who was of European maternal ancestry (Hg J2a1a1). Their children, TUK03 and TUK26, showed an admixed ancestry but the color of their hair appears to have been slightly lighter (Table S9). TUK26 married a woman (or two) carrying a European mtDNA sequence, which was transmitted to their offspring. One of the daughters, TUK19, married TUK23 of paternal (R1a-Z95) and maternal (J2b1a2a) European ancestry. Interestingly, their son, TUK18, seems to have had lighter hair and was found to be of European ancestry. The genetic ancestry

prediction (European) for TUK25, the grandson of TUK15, is also consistent with his paternal (R1a-Z2125) and maternal (T2b3) European haplotypes.

Discussion

Through the joint analysis of autosomal, Y-chromosomal and mitochondrial DNA markers, we established that the Tamir Ulaan Khoshuu site was a familial cemetery with no evidence of gender-based burial preference (the sex ratio was 1:1, females and males were buried in the same types of tombs, grave goods seemed equally abundant). Such practices, where men and women were treated equally at death, have apparently been observed in another cemetery attributed to the Xiongnu (at Daodunzi). It has been suggested that those practices were likely inherent to mobile pastoralists, whose sustenance depends on men, women and children (Linduff 2008). This contrasts with other cemeteries that lack adult female descendants, a phenomenon attributed to female exogamy (Amorim et al. 2018; Mittnik et al. 2019).

Our multi-marker approach permitted the reconstruction of a family tree spanning five generations with two branches. The first branch was composed of the descendants of a middle-aged male (TUK01) and the second branch of the descendants of his (half-)sister (TUK15), an older woman found in one of the largest and deepest graves in the southern part of the cemetery (Figs. 1, 2). Both had inherited a Western Eurasian maternal lineage (T2b3) and the male (TUK01) had inherited an Eastern Eurasian paternal lineage (Q1a-M120). This Y-haplogroup seems to have migrated from Mongolia to China during the Neolithic period and spread over China with the ancestors of Han Chinese (Huang et al. 2018); it has previously been observed at a high frequency in 3000-year-old ancient individuals from the Central Plain region (Zhao et al. 2014). We can, therefore, suppose that our five-generation family originates in European/Asian Xiongnu intermarriage. More interestingly, the descendants of this union have married men or women of Western Eurasian ancestry. In fact, except for TUK01 and his son TUK26, all members of this presumed elite family carried West Eurasian maternal (H, J, T, X) and paternal (R1a) lineages. This European genetic contribution is mirrored in the hair pigmentation of some members of the family: as expected for an Asian individual, TUK01's hair appeared to have been darker than the hair of his children (TUK-03 and -26) who themselves seemed to have had darker hair than TUK26's descendants (TUK-23 and -18). This small-scale admixture process was also observed in the biogeographical ancestry characterization with a "mixed European-East Asian" ancestry changing over the generations to a "European" ancestry (Table S9).

Another unexpected result of our study was that the descendants of the woman TUK15 and their spouses (TUK-15,-34,-48,-25,-13B,-40) were found in the largest tombs, a marker of high social status. This could suggest that after the death of her (half-)brother TUK01 (or due to his incompetence or even his Asian paternal ancestry), TUK15 could have become the leader of this family. The description of a Xiongnu tomb from a female elite leader at the Xigoupan cemetery (Linduff 2008) supports this hypothesis and suggests, as previous studies have (Davis-Kimball 2000), that during the nomadic Early Iron Age, a woman could also attain a position of prestige. By conferring power to their daughters, Mongolian leaders were assured to keep full control over their territories (Lkhagvasuren et al. 2016). It is notable that TUK24, also buried in one of the largest tombs of the necropolis, was highlighted as a possible half-sibling of TUK34 (Table S4a), possibly her paternal uncle or grandfather, reinforcing the view that this branch of the genealogy is associated with especially high status.

Analysis of burial locations showed funeral decisions based on a combination of family ties and matrimonial status. Husbands and wives were buried close to each other and to their infant or sub-adult children, contrary to adult children with a family of their own, who occupied more distant graves. When a child died before their parents, they could be buried close to an ancestor (it is possibly the case for TUK11 and her great-great-grand mother TUK15). Such burial practices, organized around biological kinship, have also been described (although to a lesser extent) in the Xiongnu Egyin Gol necropolis (Northern Mongolia) (Keyser-Tracqui et al. 2003).

Genetic comparisons of our TUK samples set with other Xiongnu groups did not reveal great similarities in Y-haplogroup composition, even if Y-Hg R1a1a has previously been reported in one Xiongnu individual from the Duurling Nards graveyard (North Eastern Mongolia) (Kim et al. 2010) along with three individuals from the Egyin Gol Valley (graves 70, 72 and 73) (Keyser-Tracqui et al. 2003). All R1a1a haplotypes among our TUK samples are closely related and were attributed to sub-Hgs R1a1a1b2a-Z95 or R1a1a1b2a2-Z2125. Both are subclades of R1a1a1b2-Z93, considered to be the Asian branch of the R1a1a haplogroup that would have originated in the Eurasian steppes (Pamjav et al. 2012; Haak et al. 2015). Most of these haplotypes were very close to those of Southern Siberian Middle Bronze or Iron Age individuals from the Krasnoyarsk region (Keyser et al. 2009) and the Tuva Republic (Mary et al. 2019) confirming the presence of Andronovo or Scytho-Siberian ancestry in the Xiongnu, as previously suggested (Damgaard 2018) and supporting the Xiongnu–Yenisseian hypothesis (Huang and Li 2016). Moreover, these haplotypes also matched those of ancient Hungarian rulers or invaders (Olasz et al. 2019; Fóthi et al. 2020), which might indicate the persistence of

some Xiongnu paternal lineages in the gene pool of early Hungarian conquerors. Some further haplotype-level matches were observed between the Turkic carrier of the Y-Hg N (TUK13A) and Avars as well as Yakuts from the seventh and fifteenth–nineteenth centuries, respectively. This Turkic haplotype, affiliated to Hg N1a1 or N-Tat (Keyser et al. 2015; Csáky et al. 2020), could indicate that the Avars originated from people living in the Mongolian and Baikal area as previously suggested (Csáky et al. 2020). It could also provide the first direct evidence that the Turkic-speaking Yakuts originated from the same area.

The investigated Xiongnu elite group shows higher maternal than paternal lineage diversity, as expected in populations practicing patrilocality (where newlywed women leave their place of birth to rejoin their husbands) (Oota et al. 2001). This was likely the case in the TUK community (it is the case in all nomadic societies), since the only adult women in the genealogy were wives buried next to their husbands, with the notable exceptions of female leader TUK15, who was buried in the same cemetery as her brother, and her daughter TUK34, who was buried in the same cemetery as her mother, despite marrying and having children. The diversified mitochondrial gene pool of Tamir Ulaan Khoshuu contains equal proportions of Western and Eastern components, contrasting with the Egyin Gol necropolis, in which most mtDNA haplogroups were of Asian origin (Keyser-Tracqui et al. 2003). This East–West balance in TUK mtDNA lineages is mainly due to the genetic input from the large family which carried Western Eurasian maternal lineages exclusively, while two-thirds of individuals outside this family are of Eastern genetic ancestry. This suggests the inclusion of an ethnically Asian individual within a group of western origin.

Most matching mtDNA haplotypes where found west of the Ural Mountains, mainly in individuals associated with the Bell Beaker complex. This is an expected result, given that most of them derive a considerable portion of their ancestry from steppe populations (Olalde et al. 2018). Some matches were also observed with Avar and Hungarian early-medieval individuals (Csàky et al. 2020; Neparáczki et al., 2018), as noted above for the Y-haplotypes. In their study, Neparáczki et al. (2019) showed that east Eurasian R1a subclades R1a1a1b2a-Z94 and R1a1a1b2a2-Z2124 were a common element of the Hun, Avar and Hungarian Conqueror elite and very likely belonged to the branch that was observed in our Xiongnu samples. Moreover, haplogroups Q1a and N1a were also major components of these nomadic groups, reinforcing the view that Huns (and thus Avars and Hungarian invaders) might derive from the Xiongnu as was proposed until the eighteenth century but strongly disputed since (De la Vaissière 2005). After they were defeated by the Chinese Han dynasty, the Northern Xiongnu had fled northwestward; their descendants may have migrated through Eurasia and conquered the Carpathian Basin. Finally, one

mitochondrial haplotype matched an ancient Yakut individual, confirming their southern origin. Taken together, these results show that genetic links may exist between consecutive nomadic populations, especially between elite groups.

In conclusion, this extensive study of a funerary ensemble allowed us to reconstruct a large, five-generation pedigree, and show that the Xiongnu tribes of Central Mongolia buried their dead according to familial ties. Results also revealed that at least some women could have been social equals to the men. Some Xiongnu paternal and maternal haplotypes could be found in the gene pool of the Huns, the Avars, as well as Mongolian and Hungarian conquerors. This supports the existence of a penchant for conquest in these peoples of Scytho-Siberian ancestry.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest or competing interests.

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