Archaeological and Anthropological Sciences (2023) 15:194
https://doi.org/10.1007/s12520-023-01899-x

RESEARCH

Ancient DNA sheds light on the origin and migration patterns of the Xianbei confederation

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Received: 4 September 2023 / Accepted: 9 November 2023

Abstract
Xianbei was one of the most powerful nomadic groups in Eastern Eurasia since the collapse of the Xiongnu empire. However, owing to a lack of first-hand written records, the origins of Xianbei and their relationships with surrounding populations remain enigmatic. Here, we produce genomic data of nine Xianbei individuals (ca. 200 CE to 300 CE) from northern China. By combining the available genomes in the literature, we assemble a database that covers almost the entire period of Xianbei as well as samples pre- and post-dating them, allowing us to set the Xianbei in a temporal context. Our study decisively addresses a longstanding hypothesis and supports that the Xianbei was originated from the Amur River region, more specifically from far northeastern China around the Great Khingan Mountain ranges. We also provide direct genetic evidence that during their initial process of moving southward toward the Central Plains of China, Xianbei only received limited exogenous genetic contribution from the local population they encountered, but after settling in northern China, Xianbei not only transformed from nomadic tribes to sedentary agriculturalists but also genetically admixed into the local residents there. In sum, our study represents the inaugural genomic exploration into the origins of the Xianbei, affirms the profound historical connection between the Xianbei and ancient Han Chinese communities, and elucidates the dynamic population history of northern China.

Keywords Ancient DNA · Xianbei · Xiongnu · Northern China · Northern Wei dynasty

Introduction

Located at the intersection of the sedentary millet agriculturalists to the south and the nomadic pastoralists to the north, northern China was not only the birthplace of various confederations of ancient tribes but also a melting pot for the exchanges of material culture, agriculture, and technology between the two distinct societies (Di Cosmo 1999). Since the Iron Age, a series of pastoral nomadic groups in the eastern Eurasia steppe established political tribes that resided in and around northern China, such as Xiongnu, Xianbei, and Rouran. Many of them migrated southward into the Central Plains of China and even established their own dynasties. Among these nomadic confederations, Xianbei was perhaps

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the most prominent who established the Northern Wei Dynasty (386–534 AD), one of the longest-lived and most powerful dynasties in China's northern border (Holcombe 2013).

Xianbei first appeared in Chinese historical records, specifically the "*Hou Han Shu*," in 49 AD (*Hou han shu* 1959) and ruled over a vast territory covering the entire Mongolia Plateau and northern China at its peak. As a non-literate society, most of the information available on the Xianbei is known only from external inscriptions, particularly from Chinese historians; thus, the origins of Xianbei remain controversial. Historical accounts suggest that the Xianbei were descendants of the ancient nomadic Donghu tribe, the latter of which was defeated by Xiongnu around 206 BC and subsequently migrated northward to the "Great Xianbei Mountains," which is now considered as the Great Khingan ranges in the Amur River Region (*Hou han shu* 1959; Holcombe 2013; *Wei shu* 1975). Therefore, it is hypothesized that the vast Great Khingan regions were the original homeland of Xianbei (Fig. 1). Besides, other theories, including the Shanrong and Dongyi hypotheses, have been proposed by other scholars, tracing the Xianbei's origin to the eastern and northern China, respectively (Li 1993; Ma 2006). Anthropological evidence indicates that the Xianbei likely comprised multiple ethnic groups, suggesting a complex population structure (Chen et al. 2003; Chen 2002; Zeng 2009). Due to the absence of written records, little is known about the language spoken by Xianbei, and available evidence is mainly derived from a limited number of tribe names (e.g., Yuwen, Murong, and Tuyuhun) (*Wei shu* 1975), which is insufficient for definitively classifying the Xianbei language, despite that a large body of scholars believe it likely associated with the Pro-Mongolic language (Janhunen 2006).

Following the collapse of the Xiongnu empire, the Xianbei swiftly ascended in power, embarking on westward and southward migrations that eventually saw them dominating the entire Mongolian Steppe and vast regions in northern China. As a nomadic confederation, the Xianbei unified diverse tribes, including the remnants of the Xiongnu, the Dingling around the Lake Baikal region, as well as the Wuhuan in northern China. A notable example of this assimilation is found in historical records, where the Xianbei absorbed the lands previously held by the Xiongnu, incorporating over 100,000 Xiongnu warriors who adopted Xianbei identity in 91AD (*Hou han shu* 1959; Holcombe 2013). This is substantiated by archeological findings and anthropological evidence (Zhang 2022; Zhu 1989). Concurrently, the southward migration of the Xianbei is a pivotal aspect of their history. According to the historical record, Xianbei moved from their initial homeland, the "Great Xianbei Mountains," into the Hulunbuir grasslands, Inner Mongolia and soon migrated southward and eventually settled in the Central Plains of China. Although many archeological sites found so far provide important information on the southward migration of Xianbei, the lack of direct genomic data from the Xianbei population means that the detail pattern of these movement events and underlying population admixture event occurred are still unclear. The Northern Wei dynasty, established by the Tuoba clan, the dominant faction within the Xianbei confederations, held significant influence among the northern Chinese dynasties that predated the reunification of China under the Sui and Tang dynasties (Holcombe 2013). Despite their formidable military prowess, the leaders of the Northern

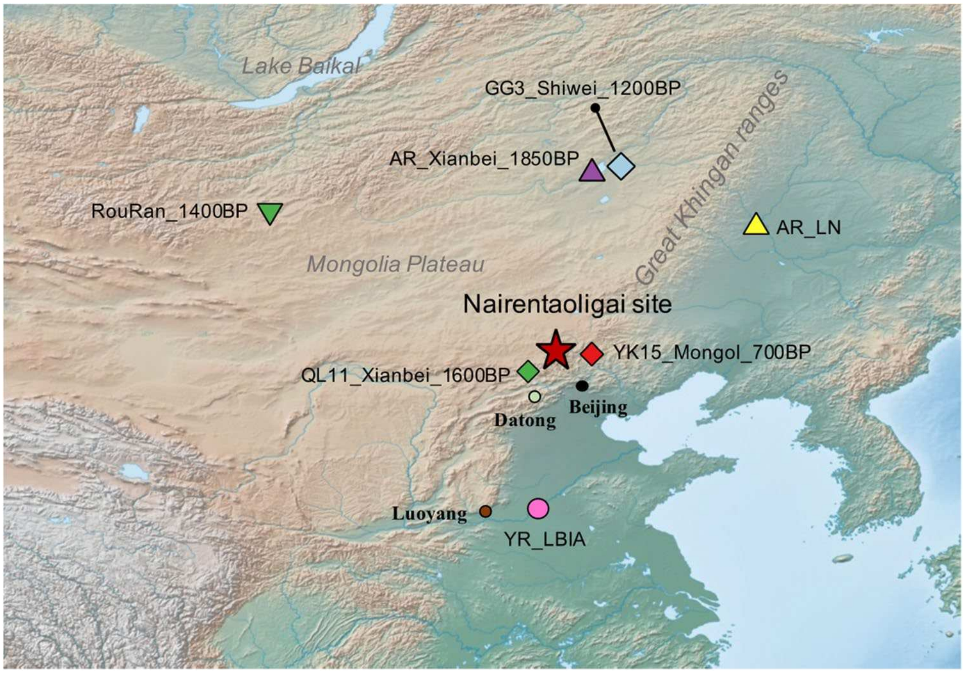


Fig. 1 Geographic location of the NRG site and published relevant sites

Wei implemented a series of policies aimed at ethnic tensions with the sedentary Chinese population. These policies included adopting Chinese names, promoting the Chinese language, and encouraging intermarriages between the Xianbei and elite Han families (Holcombe 2013). These changes accelerated the interaction between the Xianbei and ancient Chinese, yet the demographic shifts associated with this cultural transition inadequately understood.

Despite the profound significance of the Xianbei in Chinese history, our knowledge about them remains relatively limited, particularly with regard to their origins and whether the intricate population movement events were accompanied by genetic admixture during the sinicization process. Recent advances in archaeogenetic study have yielded a wealth of genome data from the Mongolia Steppe and the Lake Baikal region as well as time-series sample from Northern China over time, presenting a valuable opportunity to trace the roots of the Xianbei and reconstruct the genetic admixture between the Xianbei, neighboring nomadic tribes, and settled agricultural populations (Damgaard et al. 2018; Jeong et al. 2020; Ning et al. 2020). In this study, we have successfully retrieved ancient genome sequences of 9 individuals out of 21 individuals initially screened, from the Nairentaoligai (NRG) site in Inner Mongolia dating to the early Xianbei period. By combining these newly acquired genome sequences with the previously published Xianbei genomes, as well as those pre- and post-dating them from in and surrounding regions (J. Li et al. 2018; Ning et al. 2020) (Fig. 1), we have established a unique and comprehensive database. This database enables us to gain insights into the genetic origins of the Xianbei and unravel the scenarios of population genetic admixture amidst the intricate population movements of the Xianbei.

Materials and methods

Ethics statement

The tooth sample utilized in this study was sourced from the Institute of Cultural Relics and Archeology of Inner Mongolia Autonomous Region, as well as the Ulanqab Museum. We obtained permission from the principal investigator of this study, who had conducted the excavation at the site.

Ancient DNA extraction and sequencing

Ancient DNA was extracted from both teeth and bone samples from these remains following established protocols (Peltzer et al. 2016; Yang et al. 1998). Double-stranded libraries were prepared using the NEBNext® UltraII TMDNA Library Prep Kit for Illumina. In short, (1) we added 7 µl End Prep Reaction Buffer and 3 µl End Prep Enzyme Mix into 50 µl DNA extract and incubated 40 min at 20 °C, 30 min at 65 °C; (2) 2.5 µl Adaptor Mix, 1 µl Ligation Enhancer, and 30 µl Ligase Master Mix were combined with DNA from the first step and incubated for 20 min at 20 °C. We next added 3 µl USER Mix into the reaction mix and incubated for 15 min at 37 °C; (3) we then purified the mix using the MinElute PCR Purification Kit produced by QIAGEN and collected 18 µl DNA for the final PCR reaction; (4) finally, 30 µl Q5 PCR Master Mix, 1 µl Universal PCR Primer, and 1 µl Index Primer were added into the DNA, and the mix was amplified in the following cycles: 98 °C 30 s, 15 times 98 °C 10 s, and 65 °C 75 s, followed by 5 min extension at 65 °C. All libraries were then sequenced on an Illumina HiSeq X10 platform.

Data processing and genotyping

After sequencing, the data were processed using the EAGER software package (Peltzer et al. 2016). Specifically, we first removed Illumina adapters and merged the paired sequences using AdapterRemoval (Schubert et al. 2016). Then, single merged reads were mapped to the human reference genome hs37d5 using BWA "aln" and "samse" (H. Li and Durbin 2009). For individuals with more than one library, we merged the binary BAM files using SAMtools "merge." PCR duplicates were removed by Dedup software (Peltzer et al. 2016), and rates of misincorporation of DNA reads were calculated using MapDamage (Jonsson et al. 2013). Subsequently, the damaged bases on the 5′ and 3′ ends per read were trimmed with the trimBam function in bamUtils (Jun et al. 2015) to minimize the postmortem DNA damage. Finally, we used SAMtools mpileup and pileupCaller software (https://github.com/stschiff/sequenceTools) to call pseudodiploid genotypes by a random sampling strategy from each of the published '1240 K' SNPs (Mathieson et al. 2015).

Molecular sex estimation and ancient DNA authenticity

Molecular sex determination of individuals from the NRG was carried out by evaluating X and Y chromosome coverage. Individuals showing approximately equal X and Y chromosome coverage were categorized as males, while those with limited Y chromosome coverage were categorized as females. To validate the authenticity of the sequences generated in this study, we employed three methods. First, we observed increased frequency of G to A at the 3′ ends of all sequence data. Additionally, mitochondrial DNA contamination rates were assessed with Schmutzi (Renaud et al. 2015). Finally, we estimated nuclear contamination by comparing mismatch rates between polymorphic sites on the single-copy X chromosome for all male individuals, using ANGSD software (Korneliussen et al. 2014).

Uniparental markers, kinship estimation, and ROH analysis

Mitochondrial consensus sequence per individual was retrieved using Geneious (https://www.geneious.com/). Haplogroups were further determined using HaploGrep2 (Weissensteiner et al. 2016), and the Y chromosome haplogroups were assigned using the Yleaf package (Ralf et al. 2018), which used YFull (v10.01) (https://www.yfull.com/tree/) for the underlying tree structure of the haplogroups. The kinship relationships between NRG individuals were estimated using READ software (Monroy Kuhn et al. 2018). The runs of homozygosity (ROH) blocks of our ancient individuals were detected using the python package hapROH (https://pypi.org/project/hapROH/) with default parameters (Ringbauer et al. 2020).

Population genetic analysis

To characterize the genetic profile of NRG population, we first prepared a dataset by merging the newly generated data with previously published worldwide present-day and ancient populations (Supplementary Table 8, 9). The principal components analysis (PCA) was performed using smpartpca in EIGENSOFT package with the lsqproject: YES, and shrinkmode: YES parameters (Patterson et al. 2006). The *f*3- and *f*4-statistics were computed using the qp3Pop and qpDstat programs in ADMIXTOOLS (Patterson et al. 2012), and the Mbuti population from Africa was used as the outgroup. The potential source population and their proportions were estimated using the *qpAdm* software from the ADMIXTOOLS software packages (Patterson et al. 2012), a set of populations were used as the outgroups, including Mbuti. DG ($n = 5$), Israel_Natufian ($n = 6$), Villabruna ($n = 1$), Tianyuan ($n = 1$), Iran_Ganj_Dareh_Neolithic ($n = 5$), Mixe. DG ($n = 3$), Ami. DG (n = 2), Onge.DG ($n = 2$), Ust_Ishim_HG_published ($n = 1$), Kostenki14 ($n = 1$), Papuan. DG ($n = 16$), Anatolia_N ($n = 23$), and AfontovaGora3 ($n = 1$).

Results

Archeological background and ancient DNA data

The NRG cemetery is situated approximately 30 km southwest of the Xianghuang Banner of Inner Mongolia in northern China (42.5415° N, 114.0634° E) (Fig. 1). The site covers an area of 260 m in length from north to south and 300 m in length from east to west; the cemetery features a gentle slope and stands at an altitude of 1336 m. The site contains a total of 17 rectangular vertical pit tombs, which were popular burial forms of Xianbei. Four of them are joint burials (M4, M6, M9, and M17), ten are single burials, and three tombs do not yield any skeletal elements. Archeologists had uncovered a diverse array of burial objects, including pottery, bronze, bone, sheep bone, copper decoration plaques, Wu Zhu cash coin ("五铢钱" in Chinese), birch bark, agate, and turquoise beads. Of note, the Wu Zhu cash coin discovered in M4 has been traced back to the reign of Emperor Huan during the Eastern Han Dynasty, dating between 132 and 168 AD (Fig. 1). Further radiocarbon dating analysis of the NRG017 individual has yielded a date range of 1709–1819 BP (Supplementary Table 1). Based on a comprehensive evaluation of various archeological evidences, the tomb group is estimated to date back to the late second century AD in the late Eastern Han Dynasty, placing the NRG populations within the early period of Xianbei confederations (Dang 2021).

We extracted DNA from a total of 21 skeletal elements (teeth = 18 and bones = 3) at a dedicated laboratory that is specially designed for ancient DNA (aDNA) at the School of Archeology of Jilin University, China (Supplementary Table 1). After the initial shallow screening, nine individuals with better DNA preservation (endogenous human DNA > 4%) were further sequenced to low coverage (0.02–0.5 × per individual) for the subsequent genomics analysis. All samples displayed DNA damage patterns characteristic of ancient DNA and low levels of mitochondrial and nuclear contamination (< 4%) (Supplementary Fig. 1, Table 1). We then merged the newly generated genomes with two previously published reference datasets comprising ancient and modern individuals from Human Origins (HO) dataset and the '1240 k' dataset (https://reich.hms.harvard.edu/). The merged HO dataset was used for performing global population structure analyses, including principal component analysis (PCA) and ADMIXTURE, mainly because it contains more present-day worldwide populations. The 1240 K dataset was used for the rest of the analysis (e.g., *f*-statistics and *qpAdm*).

MtDNA and Y-DNA chromosome results

We detected 11 macro mtDNA haplogroups among the 21 Xianbei samples, including C (C4a1a4 and C5d1), D4 (D4, D4a1, D4c2b, D4e1, D4e5a, D4j + 16288, and D4m), M32′56, and N9a1 (Supplementary Table 2). Among them, the haplogroup C and D4 were prevalent in the Mongolian population from the Inner Mongolia (Bai et al. 2018), but the M32′56 and N9a1 have not been reported in modern local populations. The sub-clade D4 is prevalent in ancient northern East Asians, as well as Tungusic-speaking ethnic groups of the Amur River Basin such as Oroqen and Evenk (Miao et al. 2021; Ning et al. 2020, 2021; Xue et al. 2022). Haplogroup C is detected in northern East Asia (Miao et al. 2021; Ning et al. 2020; Xue et al. 2022). Haplogroup N9a1

Table 1 A summary of NRG samples reported in this study

ID	Analysis label	Number of reads sequenced	Endogenous DNA (%)	Bio. sex	Autosomal coverage	X chromosome contamination	mtDNA contamination (95% CI)	1240 k SNPs
NRG02	NRG_Xianbei_1750BP	38,083,191	80.23	Male	0.5011	0.0082 ± 0.0009	0.01 (0.01–0.02)	503,050
NRG04	NRG_Xianbei_o1	30,004,053	29.01	Female	0.153	-	0.01 (0.01–0.02)	178,098
NRG05	NRG_Xianbei_o2	41,095,250	6.82	Male	0.059	0.0315 ± 0.0192	0.01 (0.01–0.02)	38,125
NRG06	NRG_Xianbei_1750BP	26,739,754	16.94	Male	0.0626	0.0234 ± 0.0049	0.01 (0.00–0.02)	85,680
NRG07	NRG_Xianbei_1750BP	25,838,579	51.60	Female	0.1314	-	0.01 (0.00–0.02)	159,111
NRG09	NRG_Xianbei_1750BP	18,374,680	17.52	Male	0.055	0.0034 ± 0.0011	0.01 (0.00–0.02)	67,596
NRG12	NRG_Xianbei_1750BP	24,017,249	21.78	Male	0.1065	0.0056 ± 0.0015	0.01 (0.00–0.02)	132,359
NRG13	NRG_Xianbei_1750BP	7,297,840	12.12	Female	0.0153	-	0.01 (0.00–0.03)	20,259
NRG16	NRG_Xianbei_1750BP	13,242,219	4.13	Male	0.0083	0.0010 ± 0.0009	0.00 (0.00–0.01)	11,567

is found in two ancient individuals, including the Late Neolithic Lower Xiajiadian individual from West Liao River (WLR) and Iron Age Dacaozi individual from the Upper Yellow River region, suggesting that it might distribute predominantly in northern China in ancient times (Ning et al. 2020). Haplogroup M32′56 was only reported in Thailand and other southern East Asians (Kutanan et al. 2018), indicating that the NRG populations might share maternal genetic relationship with southern East Asians. Additionally, three previously published Xianbei individuals from the Amur River Basin in China carried C5a1, Z3a1, and C4a1a4a, respectively (Ning et al. 2020). One single Xianbei individual dating back to 1400BP from northern Mongolia (ZHS5_Xianbei_1400BP) was assigned to C4a2a1 (J. Li et al. 2020), and all these haplogroups were also found in the northern East Asians and North Asians. Thus, these results indicate that the Xianbei populations mainly harbored northern East Asian-associated haplogroups, but some individuals might share maternal genetic affinity with populations from southern East Asia.

The Y-DNA haplogroup diversity of the NRG population was lower than mtDNA haplogroup diversity. Except for two low-coverage samples, all six male NRG Xianbei individual carried haplogroup C, four of them belonged to the sub-clade C-F1756 (Supplementary Table 2). The haplogroup C-F1756 comprised only 1.96% of the local population in Inner Mongolia and was detected in the eastern Eurasians (Supplementary Fig. 2) (https://www.23mofang.com/gene-club/detail/1768930ac9e) (L. H. Wei et al. 2017), but was carried by three published Xianbei individuals, as well as subsequent ancient nomads, such as Rouran and Shiwei, who were thought to be the descendants of Xianbei (J. Li et al. 2020; J. Li et al. 2018; L. H. Wei et al. 2017), the other two individuals of them were only assigned to haplogroup C*(xM217) and C-F1699*(xM48) due to the limited available amounts of Y chromosome SNPs covered (Supplementary Table 2). Combined with earlier ancient DNA data, our results indicated that the haplogroup C-F1756 could be the primary paternal lineages of the ancient Donghu, Xianbei, Rouran, and Shiwei tribes. However, it is essential to emphasize that due to the limited number of male individuals in this study and the scarcity of published Y-chromosomal data related to Xianbei populations, further research on the paternal genetic structure of the Xianbei population would benefit greatly from additional ancient DNA data.

Kinship estimates and runs of homozygosity analyses

We estimated the kinship relatedness of all NRG individuals together with previously published early Xianbei individuals using READ software. The READ calculates the nonidentical allele ratio between the samples within non-overlapping 1 Mb segments of the genome (P0); lower P0 values mean more shared chromosomal segments. Through that, we first confirmed that none of the nine genomes are identical to each other or shared close relationships. Furthermore, we replicated one pair of first-degree kinship (MGS-M7L and MGS-M7R) Xianbei individuals previously published, which is consistent with the previous results (Supplementary Fig. 3). Coupled with uniparental markers, we identified they could be the father and son relationship (NC_2020). We further assessed runs of homozygosity (ROH) of Xianbei individuals by applying the program hapROH. ROH are contiguous regions lacking variation in the genome, the length of these long stretches of DNA segments potentially reflecting the pedigree inbreeding. Our results show that there is no genetic evidence of inbreeding in the Xianbei society (Supplementary Fig. 4).

The genetic origin of the NRG Xianbei population

To characterize the genetic profile of the NRG Xianbei individuals, we initially conducted principal component analysis (PCA) on their autosomal genomic data. We projected the NRG individuals, along with relevant ancient genomes from published sources, onto the background constructed from the present-day Eurasians. Our results revealed that all 9 genomes generated in our study fell within the genetic variations observed in present-day East Asian populations (Fig. 2A). With the exception of two outlier individuals, the majority of NRG individuals (NRG_Xianbei_1750BP, $n=7$) clustered with a group that includes the most recent published early Xianbei samples (AR_Xianbei_1850BP) and the Neolithic populations from the Amur River Basin region (AR_EN and AR_LN). All these individuals were previously identified as sharing a common ancestry, collectively termed Ancient Northeast Asians (ANA) gene pool. They were geographically distributed within the Amur River Basin region around Great Khingan ranges, suggesting that NRG Xianbei was most likely originated in these regions. We also observed that two nomadic individuals from Mongolia, one dating to the later Xianbei period (ZHS5_Xianbei_1400BP) and the other associated with the Rouran, who were considered descendants of Xianbei (Rouran_1400BP), both form a cluster with NRG_Xianbei_1750BP, indicating that some Xianbei might have migrated westward into the central and eastern parts of the Mongolian plateau before 1400 BP (Fig. 2A). Two NRG outlier individuals fall on the PC space markedly different from the main cluster: NRG04 (NRG_Xianbei_o1) shifted slightly southward toward the ancient millet farming community in the West Liao River Basin, such as the Bronze Age individuals associated with the Lower Xiajiadian culture (WLR_BA). NRG05 (NRG_Xianbei_o2) shifted toward the west along PC1 than the main group and fall within the variation of present-day Mongolic speaking populations (Fig. 2A).

We conducted further investigations into the genetic affinity between the NRG Xianbei clusters and worldwide populations using the outgroup f_3-statistics with present-day Mbuti from Central Africa as an outgroup. We noticed that the primary cluster of NRG (NRG_Xianbei_1750BP) exhibited the highest degree of allele sharing with ancient groups from Northeast Asia. These groups include AR_Xianbei_1850BP, AR_LN, GG3_Shiwei_1200BP, Rouran_1400BP, and WLR_BA_o, all of which possess comparable ANA-related ancestry (Fig. 2B, Supplementary Table 3). We then modeled the potential ancestry component of the NRG_Xianbei_1750BP using the *qpAdm*. We found that it can be adequately modeled with different one-way models when using ancient nomadic populations as a single source (p value > 0.05), such as AR_Xianbei_1850BP and AR_LN (Fig. 4, Supplementary Table 4A). We continued to try two-way models by using AR_Xianbei_1850BP as one sources and other potential Eurasian populations as another source. Although some models showed an adequate p value ($p > 0.05$), the observed ancestry proportion for the second ancestry was obviously smaller than the standard errors (Supplementary Table 4B), such an observation is consistent with PCA showing that the NRG_Xianbei_1750BP group from northern China shared a strong genetic affinity with the AR_Xianbei_1850BP and AR_LN, and others from the Amur River Basin around the Great Khingan ranges. These findings suggest a likely origin of the Xianbei from this region, providing further support for the Great Khingan hypothesis.

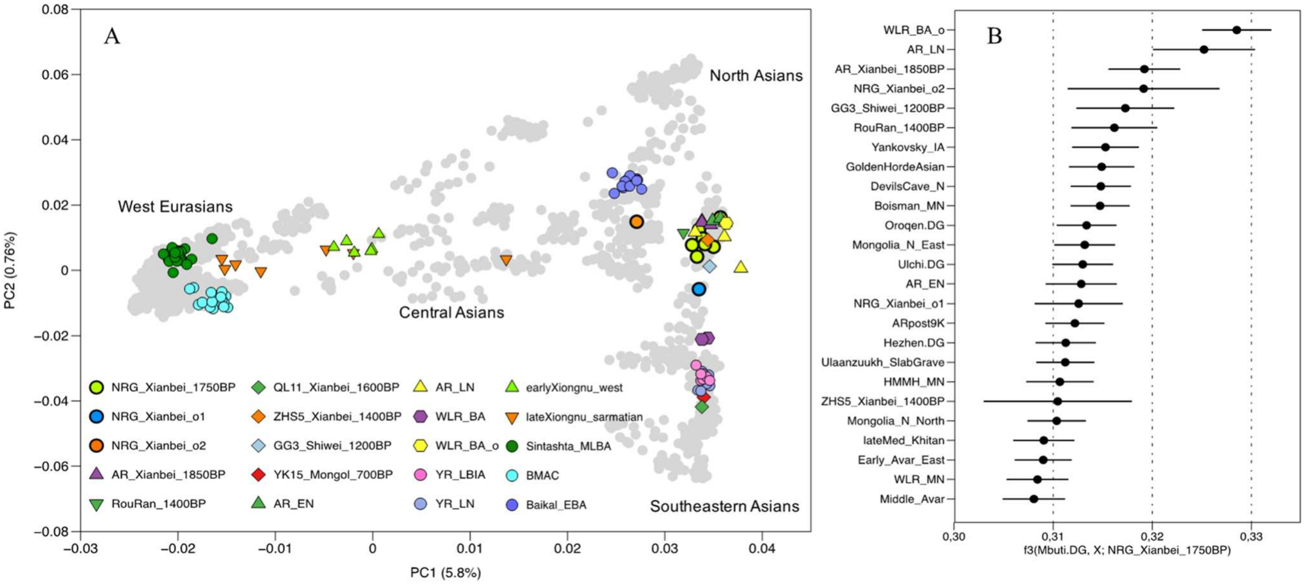


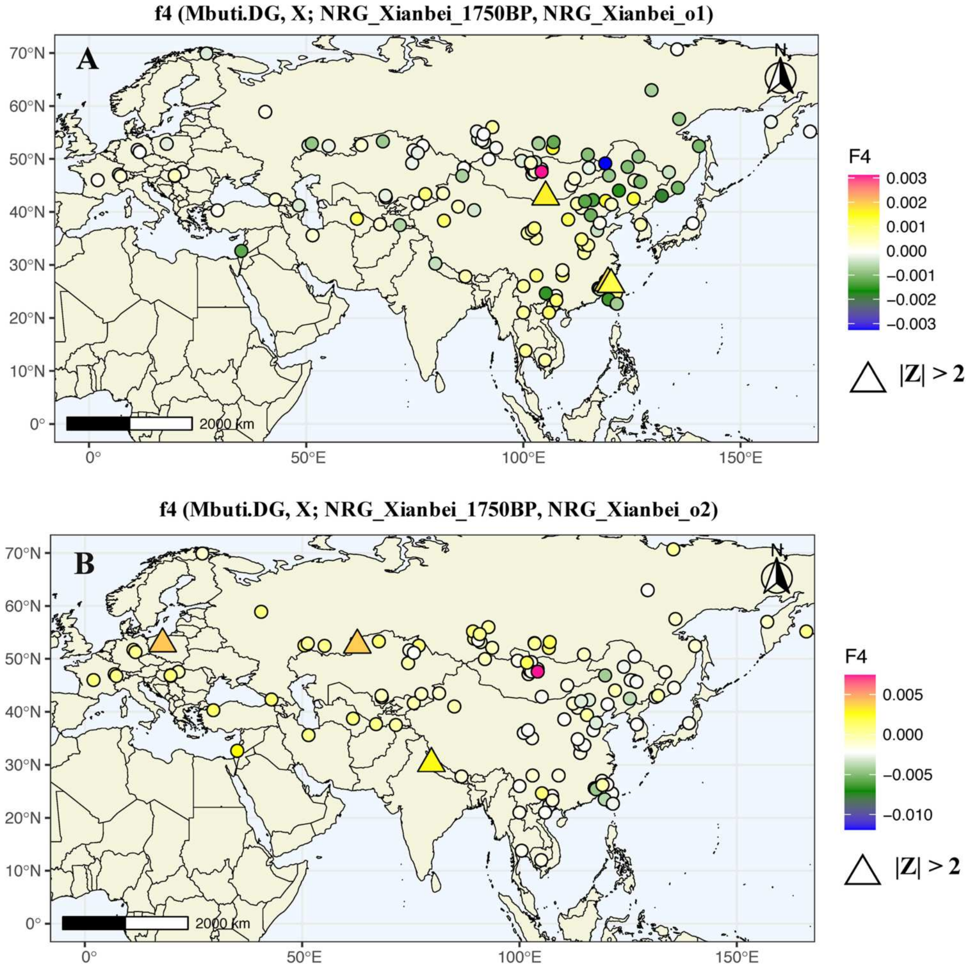
Fig. 2 Genetic structure of NRG individuals. **A** PCA constructed from present-day Eurasians, NRG individuals, and other ancient individuals in the literature are projected onto the top PCs. **B** The top 25 populations who shared the most genetic drift with NRG_Xianbei_1750BP across worldwide populations in the '1240k' dataset. Horizonal bars represent the point estimated ± 3 (thin) and ± 1 (thick) s.e.m., respectively, as estimated using 5 cM block jackknifing

Genetic admixture in the NRG Xianbei population

Apart from the main cluster, we also identified two genetic outliers within the NRG group. The PCA results revealed that these two individuals exhibited a subtle genetic affinity with the west Eurasians and other East Asian individuals further south of the AR, respectively. This suggests the possibility of gene flow from the surrounding regions into the NRG Xianbei population, as illustrated in (Fig. 2A). To delve deeper into these population movements and genetic admixture, we first employed the f_4-statistics of the form f_4 (NRG_Xianbei_o1/ NRG_Xianbei_o2, NRG_Xianbei_1750BP; X, Mbuti.DG). Regarding NRG_Xianbei_o1, in line with the findings from PCA analysis, we observe that many southern East Asians, such as Hanben population from Taiwan (Taiwan_Hanben), Liangdao2 from Fujian province, and two Han empire soldiers from Mongolia (Han_2200BP), yield positive f_4-statistics ($Z > 2$) (Fig. 3A, Supplementary Table 5A). These results suggest that, compared to NRG_Xianbei_1750BP, NRG_Xianbei_o1 possesses additional ancestry related to southern East Asian populations. Regarding the NRG_Xianbei_o2, which aligns with its position in the PCA analysis, the *f*4-statistic estimated increased allele sharing between NRG_Xianbei_o2 and ancient west Eurasian and Central Asian populations (such as Globular_Amphor, Lisakovskiy_MLBA_Alakul, and Rookpund_B) (Fig. 3B, Supplementary Table 5B). These findings suggest that, in comparison to the main cluster, additional gene flow from West Eurasia or the adjacent Eurasian Steppe regions contributed to the genetic makeup of the Xianbei populations, particularly in the case of NRG_Xianbei_o2.

To gain a more in-depth understanding of the potential ancestry sources and admixture proportions for these two genetic outliers, we modeled them using *qpAdm* method. We select the NRG_Xianbei_1750BP as one proxy source, 22 ancient populations potentially related across the Eurasia as the other. Overall, the *qpAdm* results demonstrated that these two outliers can be described as primarily ancestry related to Xianbei and subtle genetic admixture with surrounding populations. Specifically, the NRG_Xianbei_o2 was well fitted by two-way admixture models with NRG_Xianbei_1750BP (77.4~86.1%) and multiple populations from Mongolia Steppe and Central Asia, including lateXiongnu_sarmatian (18.3±6.7%), earlyXiongnu_west (22.6±9%), Sintashta_MLBA (13.9±5.3%), and

Fig. 3 The genetic between two outliers and the main Xianbei cluster. **A** The f_4 (Mbuti.DG, X; NRG_Xianbei_1750BP, NRG_Xianbei_o1). **B** The f_4 (Mbuti.DG, X; NRG_Xianbei_1750BP, NRG_Xianbei_o2)



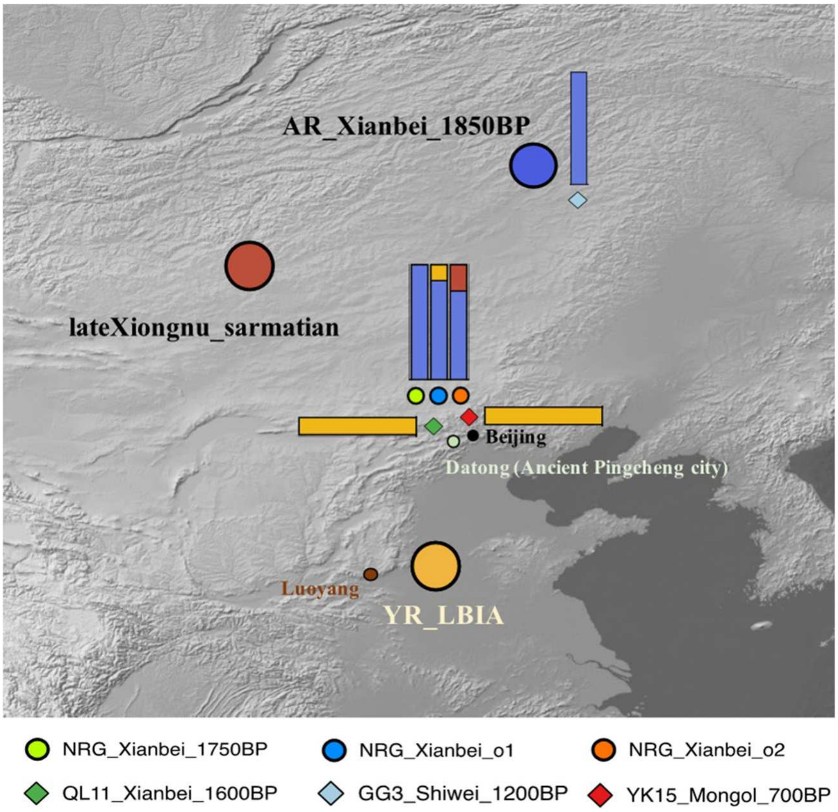


Fig. 4 The qpAdm results of NRG Xianbei populations and other relevant populations. Bar plots represent the genetic ancestry component of different ancient populations

BMAC (15.9 ± 5.6%) (Fig. 4, Supplementary Table 6A). The same as NRG_Xianbei_o2, NRG_Xianbei_o1 also derived a majority (86.1%) of their ancestry from NRG_Xianbei_1750BP with the remainder of their ancestry better characterized by YR_LBIA (13.9%) (Fig. 4, Supplementary Table 6B), in line with the f_4-statistics results. In summary, the comprehensive analyses consistently pointed to the presence of significant yet subtle ancestry contributions from both Central Asia and Southern East Asia in the genetic outliers of the NRG Xianbei. These findings suggest that there was indeed genetic admixture between the Xianbei and the surrounding populations during the time of the NRG. Importantly, these results are in alignment with archeological discoveries and historical records, providing further support for the notion of complex population interactions and migrations in the region during that era.

Increasing genetic admixture from the Central Plains of China since the early Xianbei period

Following the southward migration from their initial homeland near the Khingan ranges, the Tuoba clan, one of the most influential factions of the Xianbei, established the Northern Wei Dynasty in 386 CE, situated in central Inner Mongolia. Eventually, they settled in the city of Pingcheng, which corresponds to modern-day Datong in the Shanxi Province of China (*Wei shu* 1975). The period of the fifth century during the Northern Wei's rule in Pingcheng witnessed a significant series of cultural interactions between the Xianbei and the ancient Chinese (Holcombe 2013). During this time, the Xianbei experienced substantial changes in terms of economic practices, cultural characteristics, and funeral customs. These interactions and transformations marked a pivotal period in the history of the Xianbei people and their integration with the broader Chinese civilization (Li 2011; Liu 2020; Ni 2020). Following the collapse of the Northern Wei Dynasty in the mid-fifth century, northern China remained a region that witnessed the rise and fall of various historically recorded nomadic powers, such as Shiwei and the Mongol empire. During this period, goods, technologies, and ideas were exchanged between the settled agricultural populations and nomadic tribes, which had a far-reaching impact on the movement of people. To understand these momentous changes and their genetic impact, we re-analyzed previously published Xianbei genome (QL11_Xianbei_1600BP) dating back to the Pingcheng period together with post-Xianbei nomadic individuals (GG3_Shiwei_1200BP and YK15_Mongol_700BP) in northern China (Li et al. 2020).

We first observed that the single QL11_Xianbei_1600BP individual shows markedly different genetic profile from the early Xianbei populations (e.g., NRG individuals and AR_Xianbei_1850BP) and falls onto a cluster formed by the ancient Yellow River farmers in the Central Plains of China (e.g., YR_LBIA and YR_LN), suggesting an increasing ancestry influx from ancient farming communities from the

Central Plains of China (Fig. 2A). The strong affinity to the ancient Chinese farming communities in the Central Plains of China also mirrored by the outgroup f_3-statistics, where QL11_Xianbei_1600BP shared the most genetic affinity with southern East Asians, such as the present-day Miao, She, and ancient Miaozigou_MN, YR_LBIA and YR_LN from China (Supplementary Fig. 5A). The direct one-way *qpAdm* model also produced a valid fit when ancient farmers from the Central Plains of China was selected as the proxy sources ($p = 0.145 \sim 0.478$) (Fig. 4, Supplementary Table 7A). However, compared to the QL11_Xianbei_1600BP, the single Shiwei individual (GG3_Shiwei_1200BP) from east Mongolia shows a quite distinct genetic profile and clusters together with the NRG_Xianbei_1750BP and AR_Xianbei_1850BP in the PCA plot (Fig. 2A), indicating strong northeastern East Asian affinity. The f_4-statistics of the form f_4 (GG3_Shiwei_1200BP, NRG_Xianbei_1750BP; X, Mbuti.DG) showed that this single individual formed a clade with the main NRG group (Supplementary Fig. 5B), and further *qpAdm* analysis also suggested GG3_Shiwei_1200BP could be successfully modeled as a single source using the NRG_Xianbei_1750BP (Fig. 4, Supplementary Table 7B). The more recent YK15_Mongol_700BP individual who is associated with Mongolian culture in northern China showed a sharp discontinuity from the previous Shiwei individual, falls within the cluster formed by QL11_Xianbei_1600BP and YR_LBIA from Central Plain of China in the PCA (Fig. 2A), and could be modeled as one-way admixture using YR_LBIA as the proxy source in the *qpAdm* analysis (Fig. 4, Supplementary Table 7C), suggesting strong and continuing genetic influence from the Central Plains of China since the late Xianbei period documented by QL11_Xianbei_1600BP.

Discussion

The importance of Xianbei in the East Eurasian Steppe had been well attested by the historical record, particularly as the first nomadic confederation that established an empire in Northern China, Xianbei almost controlled the entire region of Northern China and the vast Mongolian Plateau at its height. However, the genetic origins of the Xianbei and their relationships with surrounding populations remain enigmatic. In the current study, we presented new genomic data from nine Xianbei individuals dating back to the early Xianbei period in Inner Mongolia and combined this data with previously published ancient genomes from the East Eurasian to assemble a dataset that includes populations pre- and post-dating Xianbei. Through our new study, we traced the genetic origins of the Xianbei, characterized their genetic structure, and more importantly to understand population genetic exchange with local populations during the southward migration of Xianbei as well as the interplay between cultural assimilation and genetic exchanges during the transformation from nomadic tribes to sedentary agriculturalists.

First, the origin of the Xianbei people has been a topic of uncertainty among researchers. Some researchers suggested they originated from the “Great Xianbei Mountains,” which is now identified as the Great Khingan ranges in the Amur River Region (*Hou han shu* 1959*; Wei shu* 1975). While others hypothesized that populations from eastern China or northern China were the ancestors of the Xianbei (Li 1993; Ma 2006). Previous genomic study shows that early Xianbei individuals dating to 1850 BP in Hulunbuir of northeastern China shared the homogeneous genetic profile of the Neolithic hunter-gatherers from the Amur River region (Ning et al. 2020). Our study also indicated that Xianbei populations show the closest genetic connections with the AR_Xianbei_1850BP and the earlier populations in the Neolithic period around Great Khingan ranges in the Amur region. These observations were also compatible with their highly homogenous paternal lineages. Thus, our genetic results show a better correspondence with the previously proposed Great Khingan hypothesis rather than other hypotheses.

Second, as attested by the Chinese historians, Xianbei moved south multiple times and eventually settled in close proximity to the Han society. During this long process of migration, Xianbei assimilated cultural influences from both the Eurasian steppe and the sedentary Han Chinese. They also united various populations from the surrounding regions, eventually forming a powerful nomadic confederation. However, the genetic interactions between the Xianbei and their neighbors remained unknown mainly due to the lack of direct evidence from ancient genomic data. By combining time series data associated with Xianbei, we demonstrate that during their southward migration, using NRG as an example, although the primary NRG group exhibits a genetic profile similar to earlier Xianbei individuals, genetic influences from neighboring regions began to manifest. Two genetic outliers who received small amounts of gene flow from surrounding regions in the NRG Xianbei possibly resulted from interactions between Xianbei and southern agricultural populations as well as pastoralists from the Mongolian Plateau. Additionally, we observed that, in contrast to the high diversity of mtDNA haplogroups in the Xianbei individuals, their paternal lineages appear to be relatively uniform. We speculate that Xianbei males may have intermarried with distinct maternal populations during their migration. Archeological evidence shows that the Xianbei had cultural and economic exchanges with surrounding regions as early as the first century CE. For instance, the lacquerware, bronze mirrors, and pottery characteristic of the Central Plains of China were frequently unearthed from the Xianbei cemetery in Hulunbuir (Wei 2004). Moreover, the pattern of the deer plaques of Xianbei might have been inspired by the Xiongnu animal art tradition, and the Wu Zhu cash coin from the Han empire discovered at the NRG suggest widespread cultural interactions between the Xianbei and the Han Chinese (Dang 2021; Zhang 2022). Nevertheless, despite profound cultural influence from the Han Chinese, we only discovered a small proportion of genetic into early Xianbei individuals. This suggests that in the early stages of Xianbei's history, influences from surrounding culture are mainly from the spread of ideas.

Finally, in contrast to early Xianbei populations, the 1600 BP Xianbei individual (QL11_Xianbei_1600BP) is genetically assigned to the Yellow River Basin gene pool, which was formed by the Late Neolithic Longshan as well as individuals dating from the Bronze Age to the Iron Age in Henan province, revealing direct migration from southern agricultural populations, particularly after Xianbei settled in northern China. This migrant from the south also supports historical records that document extensive cultural connections between the southern civilizations and the northern nomadic Xianbei. In 386 CE, the Tuoba clan, one of the most powerful clans of Xianbei, established the Northern Wei Dynasty and capitalized in Pingcheng (the modern Datong city of Shanxi Province, China). The emperor of Xianbei declared himself "Son of Heaven" ("天子" in Chinese), which was the supreme title for the highest rulers in traditional Chinese dynasties, and introduced various Chinese-style political institutions, such as the equal field system, Chinese-style bureaucracy, and legal and financial systems (Liu 2020; Wei 1975). In 494 CE, Emperor Xiaowen transferred most of the aristocrats, officials, and residents from Pingcheng to the new capital, Luoyang (the modern Datong city of Shanxi Province, China), and ordered the Tuoba aristocrats to adopt Chinese surnames, speak Chinese, wear Chinese clothes, intermarry with Chinese nobility, and follow Chinese customs and rites (Liu 2020; Wei 1975). This is consistent with the stable isotopic analysis of the Xianbei that early Xianbei consumed animal proteins as staple food and only engaged in minor millet consumption. But in the later period, millet agriculture developed quickly and became one of the most important economic activities for the Xianbei likely due to the influence of the economy and culture of the Han Chinese (G. Zhang et al. 2015). Our results show that, along with changes in the cultural customs and dietary habits, the Xianbei also began to accept immigrants from the southern agricultural civilizations.

Conclusion

We provide compelling evidence supporting the origin of the Xianbei people in the Great Khingan ranges within the Amur Region. This evidence contradicts the previously proposed hypothetical origins of "Shanrong" or "Dongyi." Furthermore, our research suggests that during their early southward migration, the Xianbei had limited genetic interactions with external populations. Nonetheless, as they established settlements in northern China, we have detected a substantial genetic impact from the Han Chinese population in the Central Plains. Particularly after their settlement in northern China, the genetic makeup of the Xianbei became increasingly similar to that of ancient Han Chinese. This observation suggests that the Xianbei underwent significant cultural and genetic admixture with the Han Chinese population. We also observed low diversity in the paternally inherited Y chromosome and high diversity in maternally inherited mtDNA, which may indicate a patrilocal marriage pattern. However, it is essential to acknowledge that our current analysis is based on a limited number of Xianbei genomes. Obtaining additional data from Xianbei populations would significantly contribute to our understanding of Xianbei society.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12520-023-01899-x.

Author contributions Dawei Cai, Fan Zhang and Chao Ning wrote the main manuscript text. Ying Zheng performed the experiment and data analysis. Qingchuan Bao, Xiaonong Hu, Wenhu Chen, and Jianen Cao provided the archaeological samples and information.

Funding This work was supported by the Major Projects of Key Research Bases of Humanities and Social Sciences of the Ministry of Education of China (Grant No. 22JJD780009), the National Key Research and Development Program of China (Grant No. 2020YFC1521606), the Fundamental Research Funds for the Central Universities (Grant No. 2022CXTD17), and the Special Major Foundation for Philosophy Social Science (2022JZDZ022); Chao Ning was supported by the National Natural Science Foundation of China (Grant No. T2192953) and Start-up Fund for Talent Introduction of School of History & Culture (Tourism) of Sichuan University (Grant No. 1082204112280).

Data availability The basemap used in Fig. 1 is in the public domain and accessible through the Natural Earth website (https://www.naturalearthdata.com/downloads/10m-raster-data/). The raw data for the newly sequenced individuals had been deposited to the Genome Sequence Archive (GSA) database (project no. PRJCA018315) and will be publicly available upon the publication of the manuscript.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no conflict of interest.

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