Report

Current Biology

Ancient genome of the Chinese Emperor Wu of Northern Zhou

Highlights

- Wudi had a typical East or Northeast Asian facial appearance
- Pathogenic SNPs suggest an increased susceptibility of Wudi to stroke
- Wudi derived ancestry from Northeast Asians but also has Han-related admixture

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In brief

Du et al. report the ancient genome of Emperor Wu of Northern Zhou. The Emperor is suggested to have had increased susceptibilities to certain diseases, such as stroke. He derived ancestry from Ancient Northeast Asians and Yellow River farmers, which is consistent with his typical East or Northeast Asian appearance, inferred from facial reconstruction.

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Report

Ancient genome of the Chinese Emperor Wu of Northern Zhou

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SUMMARY

Emperor Wu (武帝, Wudi) of the Xianbei-led Northern Zhou dynasty, named Yuwen Yong (宇文邕, 543–578 CE), was a highly influential emperor who reformed the system of regional troops, pacified the Turks, and unified the northern part of the country. His genetic profile and physical characteristics, including his appearance and potential diseases, have garnered significant interest from the academic community and the public. In this study, we have successfully generated a 0.343×-coverage genome of Wudi with 1,011,419 single-nucleotide polymorphisms (SNPs) on the 1240k panel. By analyzing pigmentation-relevant SNPs and conducting cranial CT-based facial reconstruction, we have determined that Wudi possessed a typical East or Northeast Asian appearance. Furthermore, pathogenic SNPs suggest Wudi faced an increased susceptibility to certain diseases, such as stroke. Wudi shared the closest genetic relationship with ancient Khitan and Heishui Mohe samples and modern Daur and Mongolian populations but also showed additional affinity with Yellow River (YR) farmers. We estimated that Wudi derived 61% of his ancestry from ancient Northeast Asians (ANAs) and nearly one-third from YR farmer-related groups. This can likely be attributed to continuous intermarriage between Xianbei royal families, and local Han aristocrats.[1,2] Furthermore, our study has revealed genetic diversities among available ancient Xianbei individuals from different regions, suggesting that the formation of the Xianbei was a dynamic process influenced by admixture with surrounding populations.

RESULTS

The emperor of China was endowed with symbolic significance for over 2,000 years, considered the "Son of Heaven" bestowed with the "Mandate of Heaven" and, as such, enjoyed what was perceived as a divinely ordained rule over the state.[3] The title of "Emperor" first appeared in 221 BC, beginning with Ying Zheng's self-proclamation as the "First Emperor." The position lasted until the abdication of the last emperor of the Qing dynasty, Aisin-Gioro Puyi, marking a total of 2,132 years and 83 feudal dynasties.[4] Among them, the Wei (220–266 CE), Jin (266–420 CE), and the Northern and Southern dynasties (420–589 CE) comprised a time of frequent changes in political power, with separate emperors ruling in the north and south of China. In the north, historians have pointed out that integration between nomadic and farming groups reached a zenith during this period, with the sinification of nomadic groups, and vice versa. Emperors were also frequently replaced during these centuries as dynasties rose and fell. The imperial families, as representatives of core tribes or ethnic groups of this period, have attracted

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considerable interest in historical genealogy and genomics, as well as widespread public attention within China. However, currently, only a handful of genetic studies exist, for example, the 3rd-century Emperor Cao Cao,[5] the Qing dynasty Aisin-Gioro family[6] and the Mongol Genghis Khan.[7–9] These studies have often been considered controversial, with possible paternal genetic lineages being inferred through the distribution frequency of modern populations. Ancient genomic data related to these imperial families are still lacking.

The Xianbei (鲜卑) were an extremely important minority group in Chinese history,[10,11] playing an active role in Chinese history for at least 7 centuries. The Xianbei established various dynasties, including the Yan, Southern Liang, Northern Wei, Northern Qi, and Northern Zhou. Although lasting for only 24 years, the Northern Zhou regime unified the northern part of China and laid a solid foundation for further unification. The Northern Zhou regime was the precursor to the second Chinese empire (Sui and Tang empires). Wudi (武帝) of Northern Zhou, the subject of this study, was the third emperor of the Northern Zhou dynasty. According to Beishi (*History of Northern Dynasties*, 北史), he reformed the system of regional troops, pacified the Turks, and unified the northern part of the country, laying the foundation for Emperor Wen of Sui to unify all of China. His remarkable talents and strategies had a profound influence on Chinese history. As the representative of the Xianbei people after their domination in the Central Plains, is there any difference between the genetic makeup of Emperor Wu and the Xianbei people studied previously? In addition, what were his physical features and susceptibility to diseases, considering Wudi passed away quite suddenly at the age of 36, and his son died at the age of 21?

Ancient genome-wide data from Emperor Wu

Because the low level of endogenous DNA (0.16%) was evaluated from a subset of the libraries via shallow shotgun sequencing (Table S1), we used two Twist Bioscience genome-wide in-solution hybridization capture panels (Twist 1.4M and Twist 1240K) to enrich the endogenous DNA. First, we generated hybridization capture data using the twist 1.4M panel from two sequencing platforms: Illumina NovaSeq-6000 and MGI DNBSEQ-G99. We validated the sequencing quality and consistency among these two platforms and found a comparable performance between the two platforms. In addition, we generated genome-wide data through a twist 1240K panel. After merging all data, we finally generated a 0.343×-coverage genome in total. We checked the reliability of ancient DNA (aDNA) using several measures. We verified the postmortem pattern characteristic of aDNA (Table S1; Figure S1). Following this, three methods were used to estimate modern human contamination. We observed a low level of contamination, <1% for mitochondrial DNA (mtDNA) contamination,[12] and <0.6% for autosomal contamination based on the X chromosome (Table S1).[13] This individual was determined as male, and his Y chromosome was assigned as a haplogroup C2a1a1b1a-F3830+, F8497− (Data S1A) by PCR-based targeted amplification covering 485 Y chromosome SNPs, which was further confirmed by hybridization capture data as a downstream haplogroup C2a1a1b1a2a1-FGC28857 × (FGC28846, Z44095, FGC31362, Z45818) (Data S1B). Due to the higher copy number of mtDNA versus nuclear DNA,[14] we generated an mtDNA genome with coverage of 507.08 X and determined his haplogroup as C4a1a + 195.[15,16] His paternal and maternal lineages can both be traced back to Northeast Asia and still reach moderate frequency at present.[17–19] We then generated pseudo-haploid data using two SNP panels: Affymetrix Human Origin (HO)[20] and Illumina "1240k",[21–23] which covered a total of 511,109 and 1,011,419 SNPs, respectively (Table S1).

Facial reconstruction and phenotypic prediction

The face of Xianbei_Wudi was reconstructed using the open-source 3D environment of Blender software based on the soft tissue depth average of modern Chinese (Figures 1A and 1B). To reconstruct more facial details, we leveraged the HIrisPlex-S system (https://hirisplex.erasmusmc.nl/), which predicts externally visible human traits using 41 SNPs. Following these instructions, we set the two missing SNPs in our data to NA (Data S2A). This system predicted that Xianbei_Wudi had brown eyes, most likely dark black hair, and fell between dark and intermediate skin (Figure 1C). We completed the facial reconstruction by adding these features (Figure 1D). We noticed a strong similarity between the virtual model and present-day northern and eastern Asians.

Next, we used Promethease to clarify further the personalized information concerning Xianbei_Wudi. This genetic analysis tool provides information about ancestry, health risks, and potential genetic pre-dispositions to certain conditions or traits. The results are shown in Data S2B. In total, 698 single-nucleotide mutations were found, 42 of which were related to pathogenic variants, such as the increased risk of gout, stroke, and chronic lymphocytic leukemia. As a caveat, these results were derived from relatively rare data, and further study with higher sequencing depth is needed to confirm these observations.

Overall qualitative genomic structure shows the Northern East Asian origin of Emperor Wu

Overall genetic structure was preliminarily investigated through a principal component analysis (PCA) (Figure 2A). PCA results showed a pattern with Northeast Asian (NEA)-related, Southeast Asian-related, and Tibetan-related populations at each corner of the PCA triangle. The Xianbei_Wudi individual clustered with NEA-related populations, showing the greatest proximity with modern Mongolic-speaking Daur and modern Tungusic-speaking Oroqen and Hezhen individuals (Figure S2A). This individual was also close to ancient individuals from the eastern Mongolian Plateau, such as lateMed_Khitan and lateXiongnu Han, previously modeled as deriving 20%–30% ancestry from Han (Figure 2A).[24] The Xianbei_Wudi individual projected closer to the ancient Yellow River (YR) farmers compared with other representative Xianbei-related ancient individuals. In the model-based ADMIXTURE analysis, we observed the minimum cross-validation error k = 3, where Xianbei_Wudi consists of the blue component maximized in Amur River-related ancient and modern populations. The yellow component is maximized in Atayal, and the orange component is maximized in Eurasian steppe populations. We observed a close ancestry composition of Xianbei_Wudi with lateMed_Khitan (Figure S2B).

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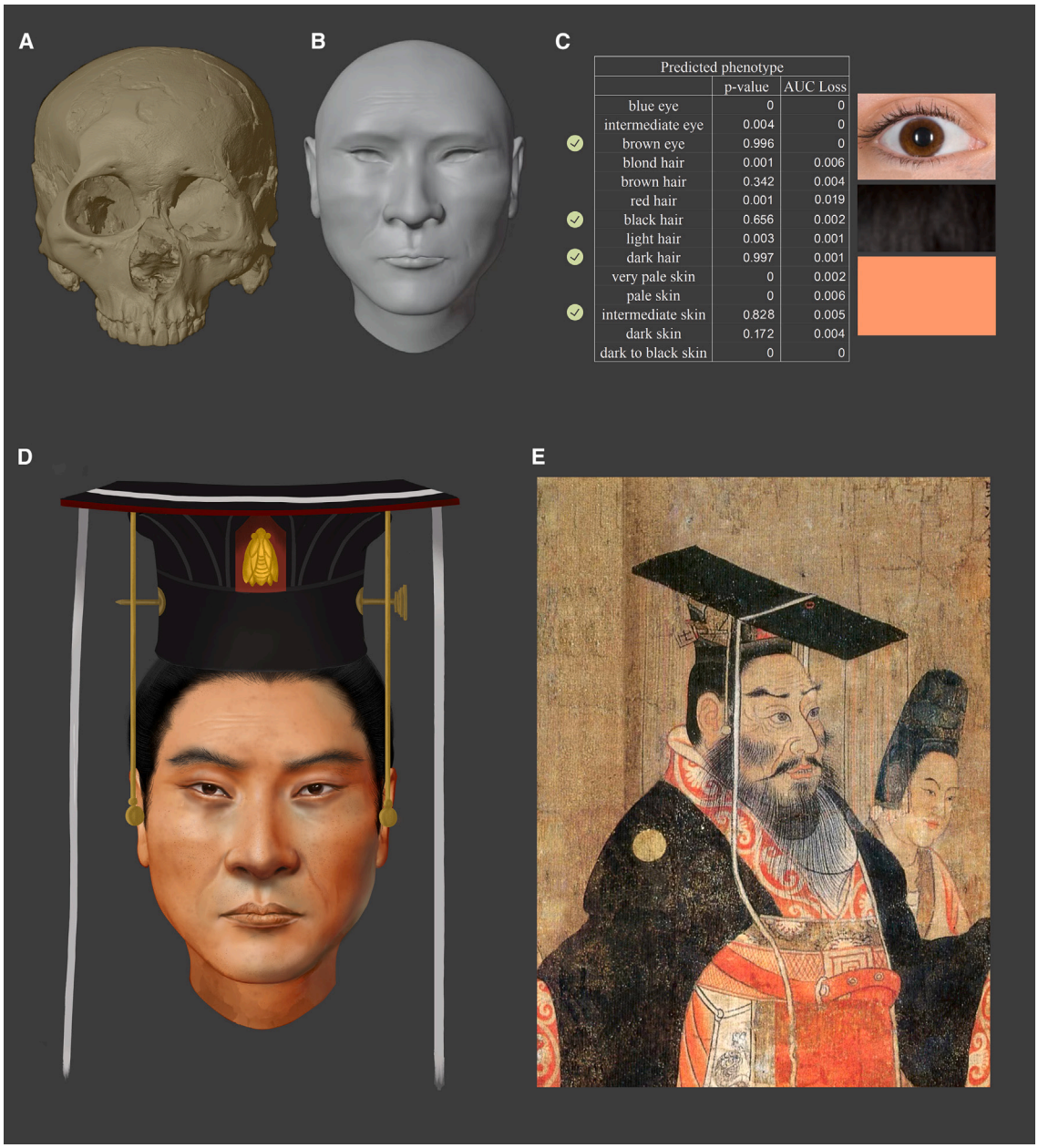

Figure 1. Facial reconstruction and phenotypic prediction of Wudi

(A) The 3D virtual digital model of the cranium.
(B) The original virtual reconstruction.
(C) The appearance prediction using SNPs in the HIrisPlex-S system.
(D) The final facial reconstruction.
(E) The portrait of Wudi from the *Thirteen Emperors Scroll*.
See also Data S2.

Close inspection of the genomic profiles within Emperor Wu

We employed quantitative f_4-statistics and *qpAdm* analyses to examine the genomic profiles of the Xianbei_Wudi individual. Symmetric f_4-statistics in the form of f_4(Mbuti, Reference; X, Xianbei_Wudi) directly compared the genetic profiles of Xianbei_Wudi with population X, where X includes 96 modern and ancient populations from East Asia and Eurasian steppe, and Reference contains 84 populations from various places (Figure 2B and Data S1C). We observed that most of the populations in X exhibiting lower *Z* scores are from NEA, showing the genetic affinity of Xianbei_Wudi with NEA-related populations. We observed that ancient individuals from Iron Age Amur River[25] and late Medieval Khitan[24] exhibited the closest genetic affinity

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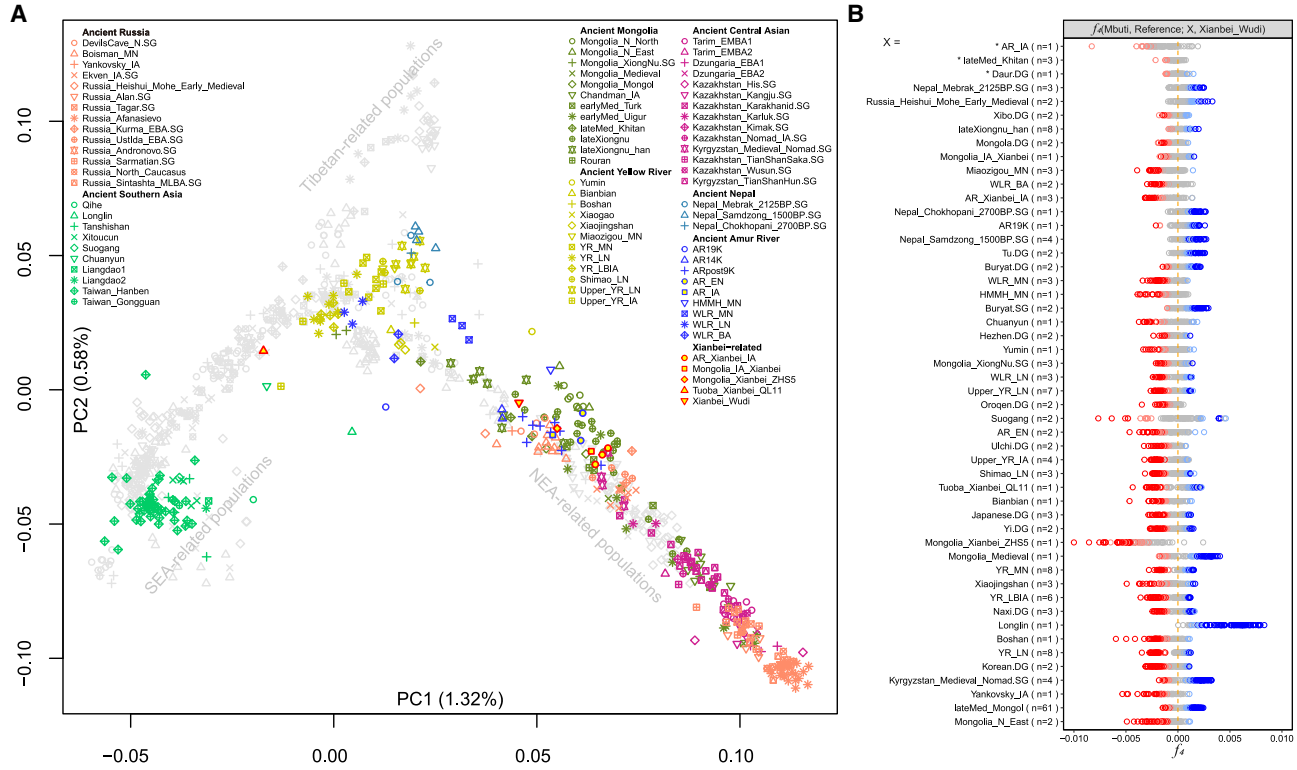

Figure 2. Genetic structure of Xianbei_Wudi

(A) Principal component analysis (PCA) plot of Xianbei_Wudi and other ancient East Asians. Modern populations were used for calculating PCs and are plotted by gray dots. The detailed result of modern populations can be found in Figure S2A.

(B) Top 50 results of f_4-statistics test in the form of f_4(Mbuti, Reference; X, Xianbei_Wudi) indicating the close genetic profiles between Xianbei_Wudi with ancient NEA individuals and modern Mongolic-speaking populations. $|Z| \geq 3$ was colored by deep red or deep blue, $3 > |Z| \geq 2$ was colored by light red or light blue.

See also Figures S1 and S2, Table S1, and Data S1.

with the Xianbei_Wudi, represented by the Z scores of f_4(Mbuti, Reference; AR_IA/lateMed_Khitan, Xianbei_Wudi) in the order: $-4.19 < Z < 1.28$. Next, we compared the genetic profiles of Xianbei_Wudi with other Xianbei-related individuals. We observed that AR_Xianbei_IA individuals have similar genetic profiles with AR_EN (Figure 2A and Data S1C) and show similar patterns with AR_EN in symmetric f_4-statistics. Ancient Xianbei-related individuals from farther west in Mongolia showed more genetic affinity with ancient individuals from the central Mongolian Plateau, represented by f_4(Mbuti, earlyMed_Turk; Mongolia_Xianbei_ZHS5, Xianbei_Wudi) with Z scores of -8.79, and f_4(Mbuti, Mongolia_LBA_3_MongunTaiga; Mongolia_IA_Xianbei, Xianbei_Wudi) with Z scores of -4.27, respectively.[19,24,26]

Next, we used *qpAdm* to estimate the ancestry proportions of Xianbei_Wudi. The Donghu (东胡) was a tribal alliance that roamed the northeast of China from the early Shang dynasty (~1600 BCE) to the Western Han dynasty (~206 BCE). Additionally, the Wuhuan (乌桓), Xianbei, Rouran (柔然), and Khitan tribes were widely believed to have originated from the Donghu (referred to as Donghu-related populations hereafter).[27,28] We modeled the Xianbei_Wudi individual along with previously published Donghu-related populations using four groups: ANA, Ancient North Eurasian, western Eurasian steppe (WES), and YR groups (Figure 3A, Data S1D and S1E). The earliest Xianbei-related individual AR_IA_Xianbei dated to 50–250 AD and could be modeled as ~85% derived from the ANA group, ~13% from the YR group, and the rest from the WES group. In contrast, the Tuoba_Xianbei_QL11 individual about 200 years after AR_IA_Xianbei could be modeled using the YR group as a single source.[18,25] Moreover, four contemporaneous Xianbei-related populations dated to ~550 AD also revealed a different ancestral makeup. Rouran_TL1 and Mongolia_Xianbei_ZHS5 could be modeled as deriving ~96%–100% from the ANA group, with the remainder from the WES group. Meanwhile, we observed a genetic influence from the YR group in Mongolia_IA_Xianbei and Xianbei_Wudi, which could be modeled as three-way admixtures deriving ~61%–77% from the ANA group, ~14%–32% from the YR group, and the rest from the WES group.[19,26] The lateMed_Khitan individual, who could be dated to ~1020 AD,[24] about 460 years after Xianbei_Wudi, had almost the same genetic compositions as Xianbei_Wudi.

DISCUSSION

Previous studies proposed that, from a patrilineal perspective, high reproductive success is related to both wealth and socio-political power.[30,31] Therefore, ancient royal families were

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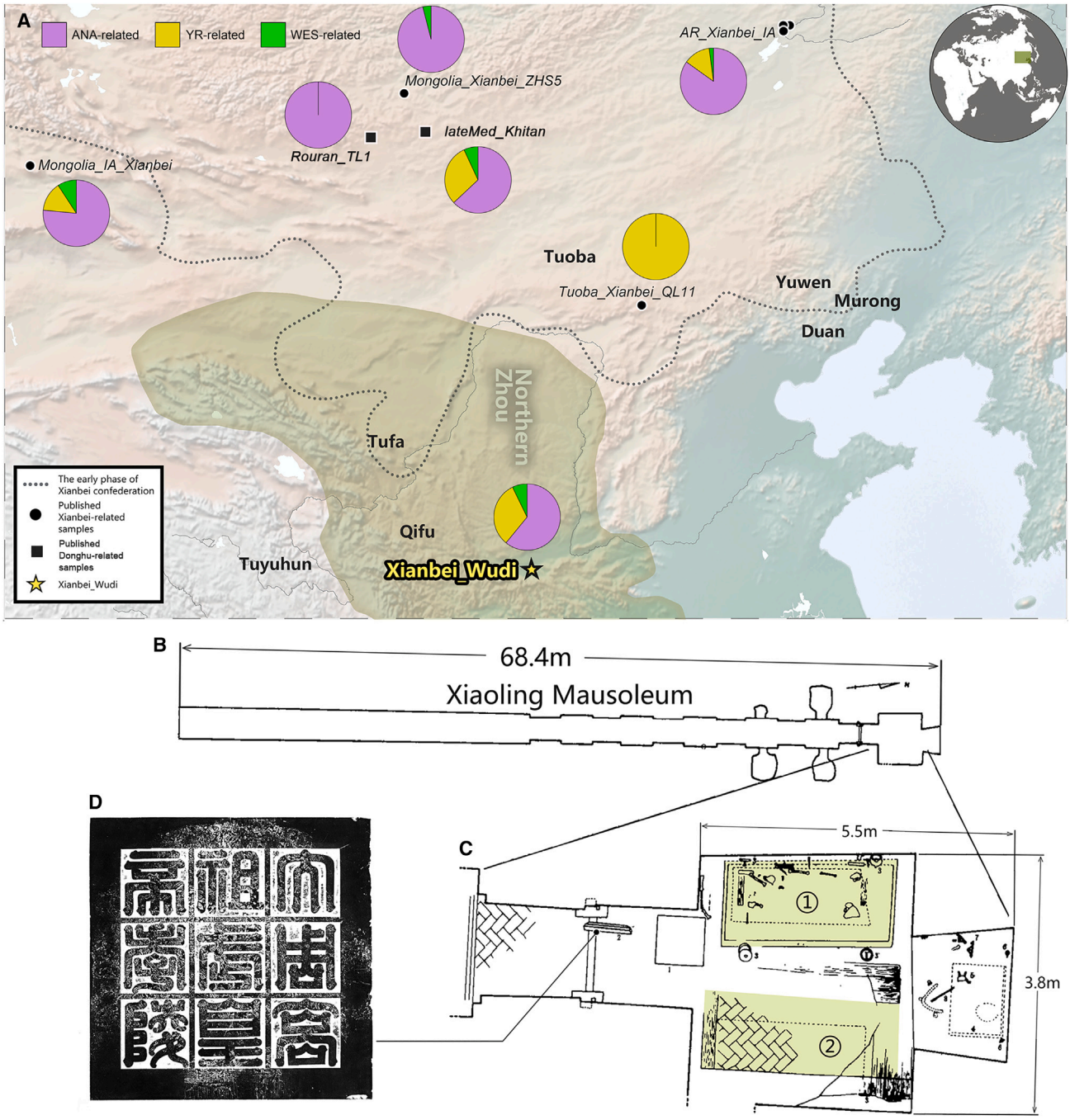

Figure 3. Background information and admixture compositions of Wudi and other Donghu-related populations

(A) Historical geography background of this study and the ancestry proportions obtained from *qpAdm*. Xianbei clans and sphere of influences presented in this map are Duan (281 CE), Murong (281 CE), Yuwen (281 CE), Tuoba (281 CE), Tuyuhun (382 CE), Qifu (395 CE), Tufa (409 CE), and Northern Zhou (572 CE). Historical records split the Xianbei into early and later phases (Kradin[11]; Miller[10]). In the early phase, from the 2nd to 3rd centuries CE, a loose Xianbei confederation ruled a vast steppe region stretching from Inner Mongolia to Central Asia. During the late phase, from the 4th to 6th centuries, Xianbei states under the Murong Yan (慕容燕) and Tuoba Wei (拓跋魏) rose and fell in northern China. Xianbei gradually proliferated and fragmented into three groups: east, center, and west (Holcombe[29]). The eastern group contains the Murong clan, the Duan clan (段), and the Yuwen clan (宇文) in northeastern China. The central group was mostly formed of Tuoba clan (拓跋), ranging from Mongolia to the Yin Mountains atop the Ordos Loop of the Yellow River. The western group includes the Qifu clan (乞伏), the Tufa clan (秃发) in present-day Gansu Province, and the Tuyuhun clan (吐谷浑) in Qinghai; information in this map is mainly sourced from *The Historical Atlas of China*. (Notes: The areas under clan names designate rough spheres of influence. The darker green zone designates the territory of Northern Zhou in 572 CE.); ancestry proportion estimated for Xianbei_Wudi and previously reported Donghu-related ancient individuals via *qpAdm*.

AR_EN, YR_LBIA, and Russia_Sintashta_MLBA represent ANA-related, YR-related, and WES-related populations, respectively.

(legend continued on next page)

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believed to have a profound impact on the genetic pool of populations. Genetic study of the patrilineal lineage in imperial families is crucial for testing this hypothesis. However, the patrilineal lineage of Emperor Wu is only found at a low frequency (<5%) among populations in East Asia and Northeast Asia.[32] Further research on the influence of cultural selection on the patrilineal genetic pool may require the observation of more families.

For Wudi himself, some questions fascinated physical anthropologists, historians, archeologists, and the public. First, what does Wudi look like? Using the most up-to-date facial reconstruction software, our study has also attempted to paint a picture of the former Emperor of the Northern Zhou based on Yuwen Yong's genomic data. The only surviving full portrait of Wudi is included in the renowned *Thirteen Emperors Scroll* (历代帝王图) (Figure 1E), attributed to Yan Liben (阎立本, 601–673 CE). Our facial reconstruction provides further details based on cranial CT data and SNP-predicted results. We learned that the Emperor would have had brown eyes, dark black hair, and dark to intermediate (between dark and intermediate) skin, in line with the phenotypes of present-day East or Northeast Asians. In addition, Xianbei's appearance remains controversial in historical records. Some have described the Xianbei people as having some exotic characteristics with thick beards, yellow hair, and protuberant "high" noses[33–35]; others believe that most Xianbei were not visibly dramatically different from the general population of northeastern Asia.[29] The latter view is in line with our genetic prediction. Second, our analysis has also allowed a paleopathological inference of potential significance. The sudden death of Wudi made great differences in the trajectory of subsequent Chinese history. There are two main hypotheses about the cause of Emperor Wudi's death: illness due to poisonous ulceration, as stated in official dynastic histories such as the *Book of Zhou* (周书), or the non-court historical cause of death by poisoning. The latter exploits the timing of the Emperor's illness on the dawn of a campaign against Turkic peoples and seems largely a conspiracy theory, given the lack of further information. Alternatively, the *Book of Zhou* adds that Yuwen Yong presented several severe symptoms: aphasia, drooping of the eyelid (ptosis), blindness, and an affected gait through lameness in one leg. These may be symptoms of a stroke. Interestingly, although the sequencing depth is low, one of the six risk loci reported by Promethease is related to stroke.

In seeking to understand Xianbei's genetic history, the genome of Wudi is important in expanding our knowledge of this question since Wudi belonged to the royal clan of the Northern Zhou dynasty. First, we discovered that Wudi showed the closest relationship with ancient Khitan and Heishui Mohe populations as well as modern Daur and Mongolian ones. From the genetic aspect, we were able to deduce the likely admixture events between individuals from Northeast Asia and those in the YR Valley. In the reconstructed genealogy of Wudi (Figure S3), his grandmother, with the family name Wang (王), is regarded as northern Han or Koguryo.[1] Therefore, nearly one-third of YR group-related ancestry in the Emperor might be explained by consecutive intermarriage between Xianbei royal families and local Han aristocrats. In contrast, Wudi's wife—Empress Ashina—derived her ancestry mainly from ANA, without the influence from YR-related Han Chinese, showing the limited genetic admixture of the ancient Turkic royal family with Han.[36] Second, in our population analyses, aside from one controversial Xianbei Tuoba individual from Qilangshan, genomic data revealed the dominant genetic ancestry (at least 61%) of Xianbei derived from the ANA group (Figure 3A). Third, regarding the genetic diversity among the Xianbei groups, we observed a genetic heterogeneity among them, tied closely to geographic regions. This could be evidenced by both *f*-statistics and *qpAdm* analyses (Figure 3A and Data S1F). Specifically, the southernmost Xianbei_Wudi possessed the highest genetic ancestry (~32%) out of the YR group. The westernmost Mongolia_IA_Xianbei, located near the Northern Xinjiang and Altai Mountain region, had the most WES group genetic component, up to ~7%. The Mongolia_Xianbei_ZHS5 and AR_Xianbei_IA individuals from eastern Mongolia to the Amur River Basin derived the most genetic ancestry (up to 96%) from the ANA group. The observed genetic landscape likely mirrored the large-scale population migration from Northeast Asia and the subsequent admixture between the immigrant ANA group and the local populations. In summary, the genetic origins of Wudi provided direct evidence for the admixture process between the Han and non-Han aristocracy in the formation of the Han Chinese, and the Xianbei group likely primarily consists of a consistent, dominant genetic ancestry.

STAR★METHODS

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  - Data merging

(B) Plan view of Xiaoling Mausoleum.
(C) Relics distribution map of passage for tomb and tomb chamber. (①, relics of the coffin and bones for Wudi; ②, relics of the coffin for Empress Ashina).
(D) Epitaph of Xiaoling.
See also Figure S3 and Data S1.

- Phenotypic analysis
- Principal components analysis
- ADMIXTURE analysis
- *f*-statistics
- Admixture modeling

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2024.02.059.

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AUTHOR CONTRIBUTIONS

S.W., C.-C.W., P.W. and L.J. conducted the project and conceived the idea. P.D., J.Z., H.M., J.X., B.Z., X.C., X.R., and Y.X. collected the samples and conducted the experiments. K.Z., H.Q., Y.Y., P.W., and C.-C.W analyzed the data. P.D., K.Z., Z.H., S.X., E.A., Q.Z., S.H., C.-C.W., and S.W. wrote the paper. P.D., K.Z., and Z.H. wrote and edited the supplementary text. All the authors revised the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Ancient skeletal element	this study	H12501
Chemicals, peptides, and recombinant proteins		
Ethanol	Sinopharm	100092008
NaClO	Sinopharm	80010428
0.5 M EDTA, pH 8.0	Thermo Fisher	AM9262
5 M NaCl	Invitrogen	AM9760G
1 M Tris-HCl, pH 8.0	Sangon Biotech	B548127
20× SSC buffer	Thermo Fisher	AM9770
20% (wt/vol) SDS solution	Thermo Fisher	AM9820
Proteinase K	Merck	539480
Silica magnetic beads	G-Biosciences	786–915
Guanidine hydrochloride	Sigma Aldrich	50933
Isopropanol	Sigma Aldrich	67-63-0
Sodium acetate	Sigma Aldrich	S2889
Tween 20	Sigma Aldrich	P9416
ATP solution (100 mM)	Thermo Fisher	R0441
dNTP mix (25 mM each)	Thermo Fisher	R1121
T4 polynucleotide kinase (10U/μL)	Thermo Fisher	EK0031
T4 DNA polymerase (5U/μL)	Thermo Fisher	EP0062
Bst enzyme	New England Biolabs	M0275S
Q5 High-Fidelity DNA polymerase	New England Biolabs	M0491S
BSA 20 mg/mL	New England Biolabs	B9000S
T4 RNA ligase reaction buffer	New England Biolabs	B0216L
Klenow fragment	Thermo Fisher	EP0052
FastAP thermosensitive alkaline phosphatase	Thermo Fisher	EF0651
T4 DNA ligase (30U/μL)	Thermo Fisher	EL0013
T4 DNA ligase (5U/μL)	Thermo Fisher	EL0012
AccuPrime Pfx DNA polymerase	Thermo Fisher	12344–024
Dynabeads MyOne Streptavidin C1	Life Technologies	65001
Agencourt AMPure XP beads	Beckman Coulter	A63881
Critical commercial assays		
MinElute PCR Purification Kit	QIAGEN	28006
Quick Ligation Kit	New England Biolabs	M2200
MGI Easy Universal Library Conversion Kit (App-A)	MGI	1000004155
DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM APP-C PE150)	MGI	940-000413-00
Twist Ancient Human DNA Panel	Twist	106658
Twist Mitochondrial Panel	Twist	102039
Twist Diversity SNP Panel	Twist	104376
Twist Universal Blockers	Twist	100767
Twist Binding and Purification Beads Kit	Twist	100984
Twist Hybridization and Wash Kit	Twist	101026
Twist Wash Buffers	Twist	100846

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
BAM files and genotype calls reported in this paper have been deposited in the GSA-Human (https://ngdc.cncb.ac.cn/gsa-human/)	This paper	HRA003871 HRA003872
Software and algorithms		
AdapterRemoval v2.3.1	Schubert et al.[37]	https://github.com/MikkelSchubert/adapterremoval; RRID:SCR_011834
BWA v0.7.17	Li and Durbin[38]	https://bio-bwa.sourceforge.net/; RRID:SCR_010910
bamUtil v1.0.14	https://github.com/statgen/bamUtil	https://github.com/statgen/bamUtil
DeDup v0.12.3	Peltzer et al.[39]	https://github.com/apeltzer/DeDup
pileupCaller	https://github.com/stschiff/sequenceTools	https://github.com/stschiff/sequenceTools
pmdtools	Skoglund et al.[40]	https://github.com/pontussk/PMDtools
ANGSD v0.931	Korneliussen et al.[13]	http://www.popgen.dk/angsd/index.php/ANGSD; RRID:SCR_021865
Schmutzi v1.5.5.5	Renaud et al.[12]	https://github.com/grenaud/schmutzi
HaploGrep2	Weissensteiner et al.[41]	https://haplogrep.i-med.ac.at/
Yleaf	Ralf et al.[42]	https://github.com/genid/Yleaf
IGV	Thorvaldsdottir et al.[43]	https://igv.org/; RRID:SCR_011793
EIGENSOFT v7.2.1	Patterson et al.[44]	https://github.com/DReichLab/EIG; RRID:SCR_004965
ADMIXTOOLS v7.0.2	Patterson et al.[20]	https://github.com/DReichLab/AdmixTools/; RRID:SCR_018495
ADMIXTURE v1.3.0	Alexander et al.[45]	http://dalexander.github.io/admixture/index.html; RRID:SCR_001263

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Shaoqing Wen (wenshaoqing@fudan.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human, accession number: HRA006585 and HRA003872), which is publicly accessible at GSA-Human: https://ngdc.cncb.ac.cn/gsa-human.
- Haploid genotype data of ancient individuals in this study on the 1240K panel are available in the EIGENSTRAT format from the following link: https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data.
- This paper does not report the original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Archaeological and anthropological information

Yuwen Yong was buried at the Xiaoling Mausoleum with his wife, Empress Ashina. The archaeologically excavated mausoleum is located in modern-day Dizhang Town, Xianyang City, Shaanxi (Figures 3A–3C).[46] Unearthed cultural relics at this site, such as

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Xiaolingzhi (Epitaph of Xiaoling, 孝陵志) (Figure 3D), consistent with historical records contained in the *Book of Zhou*. The osteological evidence consists of some scattered bones and a relatively intact skull.[47] Tooth wear analysis confirmed the male's age as around 30–40,[47] which is also in accordance with the age when the Emperor passed away. Human remains in this high-rank cemetery were poorly preserved.

We collected this ancient sample from the Xiaoling Mausoleum. Approval for its use was curated by co-authors and obtained with permission from the respective provincial archaeology institutes or universities that managed the samples. The permission and oversight were also provided by the institutional review board at the Ethics Committee for Biological Research at Fudan University.

Genealogical information

Tracing the origins of the Northern Zhou Dynasty royal family Yuwen is one of the significant issues in mediaeval history because it is closely related to ethnic integration, the historical process, and the political system at that time. Based on primary sources from the *History of the Northern Dynasties*, *Book of Wei* and *Book of Zhou*, we reconstructed the simplified genealogy of Wudi. The genealogy could be split into two parts: For the first part, from Gewutu (葛乌菟) to Houdougui (侯豆归), there are several doubts about generations' orders. For example, some described Gewutu as a descendant of the Sothern Xiongnu Chanyu (南匈奴单于)[48]; others believed this was a means of fabricating a family genealogy with the claim of distinguished ancestry.[49] Moreover, there have been some doubts whether Houdougui (侯豆归) is a progeny of Qidegui(乞得龟) from the *History of the Northern Dynasties*. The genealogy is relatively precise for the second part, from Houdougui to Yuwen Yun (宇文赟). Notably, the mother of Wudi is from a prominent Xianbei noble named Chinu (叱奴), and his grandmother is regarded as Koguryo,[1] or most likely, a northern Han[2] aristocratic family named Wang from Lelang (乐浪).

METHOD DETAILS

Skull scanning and facial reconstruction

The skull of sample Xianbei_Wudi was surface scanned with an Artecs 3D non-contact three-dimensional scanner Artecs space spider. The resolution of the surface scanner is up to 0.1 mm. Surface scans were processed using Artec Studio 13 Professional software, followed by the export of the final 3D model (.stl format) to the Blender software (https://www.blender.org). Within an open-source 3D environment of Blender, it is possible to perform standard skull-based virtual facial reconstruction of Xianbei_Wudi. Since the Emperor's remains did not preserve the mandible, the reconstruction of his jaw was referred to as an illustration from the *Thirteen Emperors Scroll* attributed to Yan Liben in the Tang Dynasty (Figure 1E).[50]

After the preliminary operations, the skull models were placed on the Frankfurt plane. As the forensic facial reconstruction is aimed at a final portrait, no facial muscles have been sculpted. The soft tissue depth average of modern Chinese (30–40 years old, male) was used for facial reconstruction of Xianbei_Wudi, with the respective values of sex (male), ancestry (Mongoloid) and age (36 years old). According to the chosen reference data, twenty-nine markers were selected for the depth measurement (Data S2C). Twelve bilateral landmarks and five landmarks (1, 3, 5, 6, 7) located on the midline were added to the skull landmarks. Every landmark was measured three times. After that, we used the facial-morphology-related SNPs to predict the skin, eye, and hair color, relying on the IrisPlex, HIrisPlex and HIrisPlex-S systems (https://hirisplex.erasmusmc.nl/).

Ancient DNA extraction and library preparation

We extracted DNA from one limb bone in a dedicated aDNA facility at Fudan University, following established precautions for working with ancient human DNA.[51,52] Human remains were surface-cleaned and ground to a fine powder. We used 50 mg of bone powder to extract DNA.[53] The lysis step included the addition of 1 mL extraction buffer to each 50 mg sample, containing 0.45 M EDTA (PH 8.0), 0.25 mg/mL Proteinase K (Merck, Germany), 0.05% (v/v) Tween 20 (Sigma Aldrich, Germany). After suspending the sample powder by vortexing, the sample was incubated overnight (15–24 h) at 37°C. After centrifugation, we transfer the lysate supernatant to a fresh tube. We mixed 17.5 μL magnetic beads (G-Biosciences, USA) with 2.5 mL binding buffer containing 5 M GuHCl, 40% (v/v) Isopropanol, 0.12 M sodium acetate, 0.05% (v/v) Tween 20 (Sigma Aldrich, Germany). Then, we transferred the supernatant (500μL) to a binding buffer/bead mixture, followed by an extraction using a robot (Enlighten Biotech, China) procedure.[51] Finally, the DNA was eluted with 50 μL TET buffer (QIAGEN, Germany). We prepared initial double-stranded and double-indexing libraries following Meyer's protocols[54] but with minor modifications[55,56] outlined below. The end-repair step was performed in 25 μL reactions using 20 μL DNA extract. This was incubated for 20 min at 12°C and 15 min at 37°C, purified using a standard MinElute (Qiagen, Germany) purification step and eluted in 15 μL TET (Qiagen, Germany). Next, Illumina-specific adapters were ligated to the end-repaired DNA in 25 μL reactions. The reaction was incubated for 15 min at 20°C and purified with another MinElute purification step before being eluted in 20 μL EB Buffer (Qiagen, Germany). The adapter fill-in reaction was performed in a final volume of 25 μL and incubated for 20 min at 37°C followed by 20 min at 80°C to inactivate the Bst enzyme (NEB, USA). Libraries were amplified with indexing primers in two parallel PCRs using Q5 High-Fidelity DNA Polymerase (NEB, USA). Single-stranded libraries using 30μL of purified extract were additionally prepared to improve endogenous DNA content.[57,58] We purified indexed products using the MinElute PCR Purification Kit (Qiagen, Germany). We qualified the clean-up libraries using Qubit 2.0 (Thermo Fisher, USA).

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Nuclear SNPs capture and sequencing

The double-stranded library prepared from bone was firstly captured using the Twist Bioscience Twist Alliance Diversity SNP Panel + Twist Target Enrichment Standard Hybridization v1 Protocol (short for twist 1.4M). This panel targeted a set of about 1.4M nuclear SNPs. 2-plex hybridization capture was carried out using 120-bp biotinylated probes. Following the DNA input requirements of the standard protocol, 1000 ng of input from the libraries was used. After the washing steps to remove nonspecific targets, the remaining material was eluted in 23 μL of water without keeping the backup slurry. Fourteen amplification cycles were performed rather than the seven cycles recommended by the protocol. The final PCR clean-up was carried out using a 1.5 × ratio of Twist Bioscience Beads. The enriched library was validated using an Agilent Bioanalyzer High Sensitivity DNA Kit and a Thermo Fisher Scientific Qubit dsDNA High Sensitivity Quantitation Assay. We then sequenced a half volume of the libraries (~10 μL) on an Illumina NovaSeq 6000 instrument at the Annoroad Company, China, in the 150-bp paired-end sequencing design. In the meantime, we converted the rest of the libraries (~10 μL) into circular single-strand libraries adapted to the DNBSEQ-G99 instrument using the MGI Easy Universal Library Conversion Kit (App-A, Cat. No.: 1000004155).[51] Finally, we made DNBs and sequenced the libraries with the DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM APP-C PE150, Cat. No.: 940-000413-00).

In addition, we also enriched the double and single-stranded shotgun libraries using the Twist Ancient DNA panel, following the protocol developed by David Reich's lab (short for twist 1240K).[59] Finally, the sequencing of the double-stranded library was performed using the Illumina Nova6000 platform at the Mingma Technologies Company, China, in the 150-bp paired-end sequencing design. The single-stranded library was sequenced using the Element AVITI platform at the Mingma Technologies Company in the 75-bp paired-end sequencing design.

Y chromosome targeted amplification

Given the characteristics of highly degraded DNA in this study, we designed a more sensitive short amplifier primer system[60] and conducted tests on samples in this study. The panel comprises 485 Y-SNPs, covering common lineages in East Asia. SNP details, PCR amplification, sequencing, and data analysis can be found in our previous studies.[61] The sequencing was performed using a NovaSeq 6000 platform at Mingma Technologies Company (Shanghai, China). 150 bp paired-end reads were generated according to the manufacturer's instructions.

QUANTIFICATION AND STATISTICAL ANALYSIS

Sequence data processing

We trimmed the adapters and merged the paired-end reads into one sequence using AdapterRemoval v2.3.1.[37] Merged reads were then mapped onto the human reference genome (hs37d5; GRCh37 with decoy sequences) using BWA v0.7.17[38] samse and parameters -l 1024 and -n 0.01. We then used Dedup v0.12.3[39] to remove the PCR duplicates. Eight bases from both ends of merged reads were clipped to avoid excess C- > T and G- > A transitions at the ends of the sequences using trimBam module implemented in BamUtil v1.0.14 (https://github.com/statgen/bamUtil). We filtered the alignment quality using mpileup module implemented in SAMtools using parameters -q30, -Q30, then generated pseudo-haploid calls using pileupCaller (https://github.com/stschiff/sequenceTools) and parameterRandomHaploid. In this study, three different SNPs panel were used, Affymetrix "Human Origins" panel, which includes 597,573 SNPs[20] and Illumina "1240k" panel, which includes 1,233,013 SNPs.[21–23] As a result, 285,469 and 568,608 SNPs were called on each panel for our sample, respectively.

Authentication of ancient DNA

Multiple methods were used to assess the quality of our sample. First, pmdtools[62] (https://github.com/pontussk/PMDtools) was used to detect the postmortem pattern of ancient DNA and to estimate 5′ C>T misincorporation rates. Second, we used Schmutzi v1.5.5.5 to estimate mitochondrial contamination rates and call the consensus sequences of the mitochondrial genome.[12] We then estimated the nuclear genome contamination rate in our sample using ANGSD v0.910.[13]

Genetic sexing and uniparental haplogroup assignment

Two methods were used to assign the genetic sex to our sample.[40,63] To assign the haplogroup for our sample, the log2fasta program implemented in Schmutzi was first used to call the mtDNA consensus sequences, and then mitochondrial haplogroups were assigned using Haplogrep2.[41] Mutations that appeared when checked against rCRS were also re-checked in BAM (Binary Alignment Map) files through visual inspection using the IGV software.[43] Y chromosome haplogroups were assigned by aligning a set of positions in the ISOGG (International Society of Genetic Genealogy, http://isogg.org/) and Y-full (https://www.yfull.com/tree/) databases, in which only base and mapping quality higher than 30 were used for analysis. Haplogroup determination was performed with the script Yleaf.py in Yleaf software,[42] which provides outputs for allele counts of ancestral and derived SNPs along a path of branches of the Y-chromosome tree. Finally, we re-checked the SNPs by visual inspection with IGV software.[43]

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Data merging

We merged our data with previously published datasets[64] using mergeit implemented in EIGENSOFT.[44] The "Human Origin" dataset was used in analyses that involved various modern populations, including smartpca and ADMIXTURE. The "1240k" dataset includes a small number of representative modern populations and a large number of ancient populations, making it suitable for *f*-statistics analysis.

Phenotypic analysis

We used the gatk HalotypeCaller[65] to generate the variant call format file (VCF file) for the Xianbei_Wudi individual, then uploaded the VCF file to the web service Promethease for phenotypic prediction.

Principal components analysis

PCA analysis was performed on the Human Origin dataset using smartpca v16000 with the default parameters and set lsqproject to YES.[44] The modern populations were used to calculate the principal components (PCs), and then the ancient samples were projected onto the top two PCs. In total, 471,995 SNPs were used to calculate the PCs.

ADMIXTURE analysis

The unsupervised admixture analysis was performed using ADMIXTURE v1.3.0.[45] After pruning for linkage disequilibrium in plink v1.90[66] using parameters --indep-pairwise 200 25 0.4,[67,68] program was run with 5-fold cross-validation and 100 bootstrap replicates and varies of ancestral populations K ranging from 2 to 10. After pruning for linkage disequilibrium, 258,896 SNPs were used to perform the admixture analysis.

f-statistics

We used *qpDstat* v980 to calculate the f_4-statistics using parameter f_4-mode: YES,[20,67] and *qp3Pop* v651 was used to calculate the outgroup-f_3 using parameter inbreed: YES. Both software are implemented in ADMIXTOOLS.[20] In f_4-statistics test, we used various populations from East Asia as Reference to find any Reference population that could distinguish population X from Xianbei_Wudi. If all f_4 results of a population X show non-significant Z score, it means that population X form a clade to Xianbei_Wudi from all the References, in such case, we conclude that population X has close genetic relationship with Xianbei_Wudi given our current resolution.

Admixture modeling

Q*pAdm* implemented in ADMIXTOOLS was used to estimate the ancestries proportion of our sample as the combination of the source populations.[20] We set the parameters allsnps: YES and inbreed: YES, and used the rotation strategy described in Harney et al.[69] Specifically, we used a set of distantly related outgroup as fixed outgroup to model our sample as the combination of one to three potential sources, and a population were added to the outgroup whenever it was not chosen as a potential source.