



# Ancient DNA from Tubo Kingdom-related tombs in northeastern Tibetan Plateau revealed their genetic affinity to both Tibeto-Burman and Altaic populations

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Received: 9 December 2021 / Accepted: 27 August 2022

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## Abstract

The rise of the Tubo Kingdom is considered as the key period for the formation of modern groups on the Tibetan Plateau. The ethnic origin of the residents of the Tubo Kingdom is quite complex, and their genetic structure remains unclear. The tombs of the Tubo Kingdom period in Dulan County, Qinghai Province, dating back to the seventh century, are considered to be the remains left by Tubo conquerors or the Tuyuhun people dominated by the Tubo Kingdom. The human remains of these tombs are ideal materials for studying the population dynamics in the Tubo Kingdom. In this paper, we analyzed the genome-wide data of eight remains from these tombs by shotgun sequencing and multiplex PCR panels and compared the results with data of available ancient and modern populations across East Asia. Genetic continuity between ancient Dulan people with ancient Xianbei tribes in Northeast Asia, ancient settlers on the Tibetan Plateau, and modern Tibeto-Burman populations was found. Surprisingly, one out of eight individuals showed typical genetic features of populations from Central Asia. In summary, the genetic diversity of ancient Dulan people and their affiliations with other populations provide an example of the complex origin of the residents in the Tubo Kingdom and their long-distance connection with populations in a vast geographic region across ancient Asia.

**Keywords** Ancient DNA · Dulan County · Tubo Kingdom · Modern Tibetan populations

## Introduction

In its heyday, the Tubo Kingdom (633–842 AD) covered most of Northwest China and parts of neighboring South and Central Asia. In the northeastern Tibetan Plateau (NETP), including Qinghai Province and its adjacent areas, Tubo

Kingdom defeated the Tuyuhun Kingdom and incorporated this area into its territory in the seventh century (Zhou 1985). Tuyuhun Kingdom (313–663 AD) was established by the Tuyuhun people, descendants of Xianbei people who originated in the northeast part of China (Zhou 1985). After migrating to NETP, Tuyuhun people established Tuyuhun Kingdom and chose Dulan as the capital. Under the regime of the Tubo Kingdom, the Tuyuhun people likely integrated with those from the Tubo Kingdom and later gave rise to present-day Ando Tibetans (Li and Li 1992; Janhunen 2006). The earliest known ancient Tibetan documents were found in the Tuyuhun territory, and ancient Tibetan documents found in Dulan were mostly written in the Ando dialect (Lin 2007). In addition, the southern branch of the Silk Road brought more chances of cultural exchanges and human interactions to Dulan (Mu and Wang 1994; Zhu 2020). In conclusion, the high diversity and complex composition of modern Tibetans in NETP were shaped during the Tubo Kingdom period, with great contribution from Tuyuhun residents.

High diversity and intense admixture were observed in modern populations on the Tibetan Plateau in previous

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Communicated by Shuhua Xu.

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literature. Neolithic farmers from Yellow River Basin represented by Y-chromosome haplogroups O $\alpha$ 1c1b-CTS5308 and O $\alpha$ 1c1a-Z25929 migrated to the plateau hinterland through NETP and mixed with local Paleolithic hunter-gatherers represented by D1a1-M15 and D1a2-P47 (Shi et al. 2005, 2008; Qi et al. 2013; Wang et al. 2018). From the maternal perspective, plateau groups share some mitochondrial DNA haplotypes with populations in northern China, northwestern China, and northern Asia (Kang et al. 2013, 2016; Qi et al. 2013; Li et al. 2019). Previous whole-genome studies have shown that Tibeto-Burman populations share most components with Sinitic populations, with admixture from Mongolic-Tungusic groups, Central Asians and South Asians, and hold a small amount of archaic ancestry (HUGO Pan-Asian SNP Consortium et al. 2009; Lu et al. 2016; He et al. 2021; Wang et al. 2021; Yang et al. 2021).

Previous studies of ancient DNA provide some clues for the genetic structure of ancient people on the Tibetan Plateau. Neolithic, historical and modern highlanders derived most of their ancestry from Neolithic Northern East Asians (He et al. 2021). In NETP, Neolithic and Iron Age populations were genetically similar to farmers in the Yellow River Basin (Ning et al. 2020). On the southwestern edge of the plateau, ancient Himalayan individuals harbored a major ancestry from groups related to Late Neolithic populations in NETP and displayed long-term genetic stability (Jeong et al. 2016; Liu et al. 2022). However, the genetic scenario of the Tubo Kingdom period in NETP still remains poorly investigated. Although Cui et al. obtained partial mitochondrial sequences from three of four Tubo individuals in Dulan, which showed a close relationship with modern Tibetans

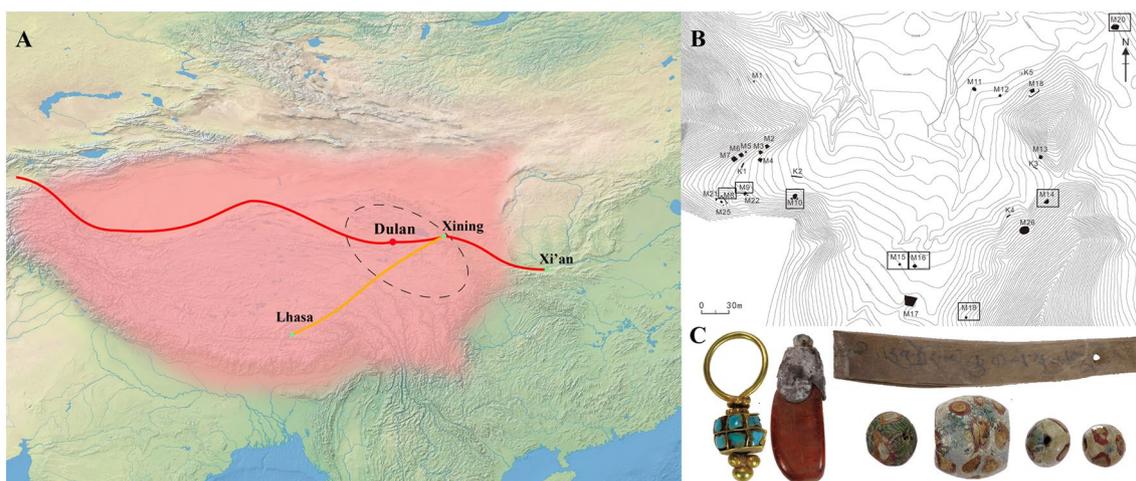
(Cui et al. 2003), more thorough and precise genome-wide data is lack of reporting.

In this paper, we analyzed the ancient DNA from the remains of Dulan tombs excavated in 2014. Our objectives are to (1) determine the paternal, maternal and autosomal genetic structure of this ancient NETP population and (2) analyze the relationship of the studied population with ancient people in eastern Eurasia and modern Tibeto-Burman populations. Overall, we generated a new set of ancient DNA data which provided important clues for the genetic structure and population dynamics during the Tubo Kingdom period in NETP.

## Materials and methods

### Site and samples

The studied cemetery is located in Wayan reservoir, Reshui Town, Dulan County, Qinghai Province (Fig. 1). In 2014, salvage archaeology for the Wayan reservoir construction project in Dulan was conducted by Qinghai Provincial Institute of Cultural Relics and Archaeology and Shaanxi Provincial Institute of Archaeology jointly (Qinghai Provincial Institute of Cultural Relics and Archaeology, Shaanxi Provincial Institute of Archaeology 2018). Burial customs and unearthed artifacts showed characteristics of Tubo, Tuyuhun, and Tang cultural remains. Thus, the ethnicity of tomb occupants in this research remains controversial. In total, 25 tombs and 5 sacrifice pits were excavated, and the human remains of eight male tomb occupants are collected from 8 of 25 tombs (Table 1, Fig. 1).



**Fig. 1** Geographic location of Dulan tombs in this study. **A** Map showing the location of the Dulan site. Red line represents the southern Silk Road and yellow line represents Sino-Tibetan Road. Light red shape represents Tubo territory in the ninth century and

dashed ellipse represents Tuyuhun territory under the regime of the Tubo Kingdom. **B** Map showing the distribution of tombs and sacrifice pits. Occupants of boxed tombs were analyzed in this study. **C** Archaeological relics excavated from the Dulan site in 2014

**Table 1** Dulan site sampling information

Sample ID	Burial numbers	Skeletal element	Sex (morphology)	Sex (molecular)	Date (Cal. BP)	mtDNA haplogroups	mtDNA coverage	Y-chromosome haplogroups	Y-SNP
DL1	2014QDRM14	Radius	Male	Male	1295–1174	D4j1b	16.291	O2a2b*	–
DL2	2014QDRM16	Tibia	Male	Male	1270–1060	M13a2	2.226	–	196
DL3	2014QDRM15	Radius	Male	Male	1276–1066	D4g	1.174	O2a2b1a1a1	285
DL4	2014QDRM10	Fibula	Male	Male	1290–1121	C4d	9.125	O2	312
DL5	2014QDRM9	Tibia	Male	Male	1295–1174	D4g2a1	27.207	O2a2b1a1a1	480
DL6	2014QDRM19	Radius	Male	Male	1294–1130	A21	13.2462	N1b2	478
DL7	2014QDRM8	Femur	Male	Male	1352–1280	M9a1b1	60.1738	R1a1a1b2a	479
DL8	2014QDRM20	Tibia	Male	Male	1289–1077	C4b1a	16.7218	R1a1a1b2a	479

### DNA extraction, library preparation, and sequencing

Tooth and bone samples were handled with care in the dedicated ancient DNA laboratory at the Ministry of Education Key Laboratory of Contemporary Anthropology, Fudan University, following the standard procedure (Knapp et al. 2012). Samples were first polished 1–2 mm in depth to remove potential contamination on the surface. Then, samples were soaked in 5% sodium hypochlorite solution for 15 min and rinsed with 50% ethanol twice, and then UV-irradiated for 30 min. Dried samples were powdered under liquid nitrogen in a 6750 Freezer Mill (SPEX, USA).

DNA was then extracted from 200 mg powder of each sample. The powder was mixed with 4.5 mL 0.5M EDTA (pH8.0) and 0.5 mL 2.5 mg/mL proteinase K and then placed in the shaker at 150 rpm/min for 24 h at 37 °C. The clear supernatant was obtained by centrifugation (2 min at 15,000 g) and then processed with 25 mL binding buffer (5M guanidine hydrochloride, 40% isopropanol, 1M sodium acetate, and 0.05% Tween-20, pH5.2) on the MinElute silica spin column (QIAGEN, Germany), followed by washes with PE buffer (QIAGEN, Germany) twice. DNA was eluted in 50 µL EB buffer (QIAGEN, Germany).

The double-stranded DNA libraries were prepared according to Meyer's protocol (Meyer and Kircher 2010). The libraries were purified with the MinElute PCR Purification Kit (QIAGEN, Germany) instead of AMPure XP Beads (Beckman, Germany). The PCR products were verified amplification success via Qubit (Thermo Fisher, USA).

Sequencing was conducted by Novogene, China on the Illumina Platform HiSeq-PE150. We clipped the Illumina sequencing adapters and merged reads by Adapter Removal v2.3.1 (Schubert et al. 2016). We then mapped merged reads to the human reference genome (hs37d5) by BWA 0.7.17 (Li and Durbin 2009). We removed PCR duplicates by DeDup v0.12.8 (Peltzer et al. 2016). We generated sequencing metrics using Qualimap 2.2.1 (Okonechnikov

et al. 2016). Before SNP calling, we clipped 10 bp from both ends of each read to reduce wrong calls due to postmortem DNA damage by the trimBam module of bamUtil (Jun et al. 2015). We then called haploid genotypes for the SNPs in the 1240k-Illumina panel (“1240k”; 1,233,013 SNPs) (Mathieson et al. 2015) using pileupCaller (<https://github.com/stschiff/sequenceTools>) with the default ‘–randomHaploid’ mode by randomly choosing one base with Phred base quality score  $\geq 30$ . For mitogenomes, we mapped merged reads to rCRS (Andrews et al. 1999). The final consensus sequences were generated by Schmutzi 1.5.146 (Renaud et al. 2015) or bcftools (Li 2011). The mtDNA haplogroups were assigned by HaploGrep2 (Weissensteiner et al. 2016) according to PhyloTree mtDNA tree Build 17 (Van Oven and Kayser 2009).

For Y-chromosome haplogroup determination, we performed multiplex PCR targeting enrichment with short amplicons comprising 485 Y-SNPs based on the Next Generation Sequencing platform described in Xiong et al. 2022 other than merely shotgun sequencing. The result is also validated by calling informative SNPs from whole-genome sequencing data.

We used the aforementioned software to clip adapters, map reads to reference genome, and then call SNPs with SAMtools (Li et al. 2009). We assigned the Y-chromosome haplogroups by yHaplo (Poznik 2016) or Yleaf (Ralf et al. 2018) according to ISOGG 2019 (Genealogy ISOGG 2019) and checked manually.

### Measures taken to ensure authenticity

#### Laboratory environment

We prepared all the samples and reagents in the clean, dedicated ancient DNA laboratory and each step was conducted in isolated rooms. We strictly followed protocols to avoid contamination caused by wrong operations. We used negative controls to ensure no contamination from reagents or

containers and no cross-contamination during PCR amplification or other steps.

### Ancient DNA authentication

We used several software programs to verify the sequencing data is ancient DNA. We demonstrated patterns of postmortem chemical modifications by mapDamage 2.0.8 (Jónsson et al. 2013). We estimated the mitochondrial contamination rate by Schmutzi (Renaud et al. 2015). Finally, we measured the nuclear genome contamination rate based on X chromosome data as implemented in ANGSD 0.91047 (Korneliusson et al. 2014).

### Population analysis

For mitogenome analysis, genetic distance  $F_{ST}$  was calculated using Arlequin 3.5 (Excoffier and Lischer 2010). Nonmetric Multidimensional Scaling (NMDS) plot was generated by R package metaMDS based on  $F_{ST}$  matrix. For Y-chromosome haplogroups, we constructed principal component analysis (PCA) based on 83 worldwide linguistic populations and projected the Dulan group onto it.

For whole-genome analysis, we performed PCA using 2217 present-day Eurasians or 1212 ancient and present-day East Asian individuals (detailed populations listed in Table S1) based on the Affymetrix HumanOrigins array (“Human Origins”; “HO”; 597,573 SNPs) (Patterson et al. 2012) and the 1240k panel from the AADR dataset v44.3 (AADR 2021) and other published results with the smartpca module of Eigensoft (Patterson et al. 2006; Price et al. 2006). We removed SNPs with minor allele frequency smaller than 1% and pruned for linkage disequilibrium with parameters “-maf 0.01 -indep-pairwise 200 25 0.2” using PLINK v1.90 (Chang et al. 2015). Then, we performed unsupervised admixture analysis using ADMIXTURE (Alexander et al. 2009). We computed  $f_3$ -statistics and  $f_4$ -statistics to measure genetic relationships between populations using both ancient and present-day individuals aforementioned by the qp3Pop and qpDstat module of AdmixTools (Patterson et al. 2012). We used qpAdm (Patterson et al. 2012) to investigate the ancestral sources and estimate mixture proportions in the Dulan individuals.

Visualization of all results was produced by R-4.0.2 and python 3.7.3.

## Results

### Mitochondrial DNA analysis

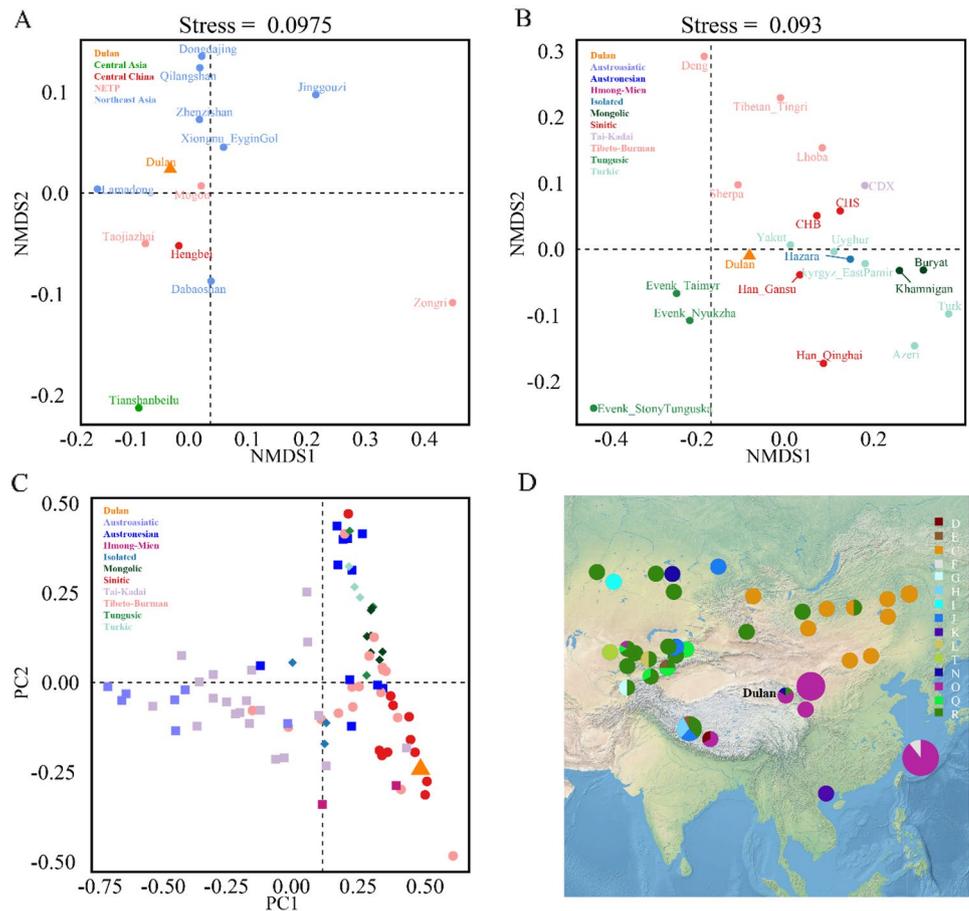
Complete mitogenome sequences were obtained from all eight individuals and assigned to haplogroups A21, C4b1a,

C4d, D4g, D4g2a1, D4j1b, M9a1b1, and M13a2, respectively. DL7 is excluded in NMDS analysis due to high contamination (23%) and his haplogroup is M9a1b1. The high diversity of maternal origins revealed intense admixture for the formation of Tibetans. The occurrence of haplogroup A21, D4j1b, and M13a2 shows maternal continuity on the plateau, while the presence of haplogroup C4b1a, C4d, D4g, and D4g2a1 reveals wide human interactions across Asia. Haplogroup A21 and M13a2 are found at high frequency and high diversity in modern Tibetans (Wang et al. 2014; Kang et al. 2016), Sherpas (Kang et al. 2013), and Han Chinese (Li et al. 2019). Haplogroup D4j1b has existed in Tibetan Plateau since at least Neolithic Time (Ding et al. 2020). Haplogroup C4b1a occurs frequently in present-day North Asian populations (Volodko et al. 2008; Derenko et al. 2010, 2018) and northern East Asian groups (Kong et al. 2006). Haplogroup C4d is commonly seen in Tibetans (Qin et al. 2010; Kang et al. 2016), Han Chinese (Li et al. 2019), modern Southeast Asians (1000 Genomes Project Consortium et al. 2015; Kutanan et al. 2017), and ancient steppe nomads (Damgaard et al. 2018; Mary et al. 2019). Haplogroup D4g2a1 distributes widely in Han Chinese (Li et al. 2019) and Thailanders (Kutanan et al. 2017). In the NMDS plot (Fig. 2A, B, Table S2, Table S3), Dulan group locates between ancient and modern plateau groups and northern East Asian groups.  $F_{ST}$  based on HVS1 region reveals that Dulan group is surrounded by ancient North and Northeast Asia groups, and is closest to local Mogou people (~4000BP), but far from local Taojiazhai (family cemetery) and Zongri (~5000BP) people. Dulan group does not cluster with specific present-day linguistic populations in the NMDS plot of  $F_{ST}$  constructed from whole mitogenomes, but does show genetic affinity with northeastern Asian groups like Yakut, Han\_Gansu, and Evenk\_Taimyr (Slatkin  $F_{ST} = 0$ ).

### Y-chromosome analysis

Eight individuals were identified as male and seven were successfully assigned to Y-chromosome haplogroups, namely N1b2-F830, O2, O2a2b1a1a1-F8, O2a2b-CTS11109\*, and R1a1a1b2a-Z94. DL7 is excluded in the analysis due to high contamination as well, which is assigned to R1a1a1b2a-Z94. Compared to diversified maternal lineages observed in Dulan individuals, their paternal origins shrink to three haplogroups N, O, and R. N and O indicate ancestry from Neolithic NETP farmers, while R indicates ancestry from steppe-related nomads. Half of eight Dulan individuals are assigned to haplogroup O2. O2a2b-P164\* occurs at a higher frequency in Southeast Asia and is usually associated with Austronesian populations (Trejaut et al. 2014; Wei et al. 2017). Two

**Fig. 2** Uniparental analysis for Dulan people. **A** NMDS plot based on slatkin  $F_{ST}$  of mitochondrial HVS-I sequences between Dulan and 12 ancient groups. **B** NMDS plot based on slatkin  $F_{ST}$  of whole mitogenomes between Dulan and 20 modern populations. **C** Projection of Dulan onto PCA plot based on Y-chromosome haplogroup frequencies for 83 worldwide modern linguistic populations. **D** Y-chromosome haplogroup distribution during the first millennium AD



individuals are assigned to O2a2b1a1a1-F8, a sub-lineage immediately under O2a2b1a1a-F5 ( $O\alpha$ ).  $O\alpha$ , together with O2a2b1a2a-F46 ( $O\beta$ ) and O2a1b1a1a1a1-F325 ( $O\gamma$ ), contributes to 40% of modern Han Chinese (Yan et al. 2014) and is also predominant in Tibeto-Burman populations (Wang et al. 2018). Previous studies showed N lineages in Tibeto-Burman and Tai-Kadai groups in southern China mainly belong to the N1b-F2930 haplogroup (Hu et al. 2015). N1b also occurred at a high frequency in Shandong, eastern China during Early Neolithic (Yang et al. 2020). N1b2-F830 lineages share the most recent common ancestor 6900BP and distribute mainly in southern East Asia and Southeast Asia (1000 Genomes Project Consortium et al. 2015; Mallick et al. 2016; McColl et al. 2018). Intriguingly, DL8 was assigned to apparent west Eurasian haplogroups R1a1a1b2a-Z94. R1a1a1b2a-Z94 originated in the Eurasian Steppe and is widely distributed in present-day South Asians, Central Asians, and West Asians (Allentoft et al. 2015; Underhill et al. 2015). In the PCA plot (Fig. 2C, Table S4), Dulan clusters with Sino-Tibetan groups, as high frequency (50%) of O2 and its sublineages. The Y-chromosome haplogroup distribution among

ancient East Asia reveals admixtures of Sino-Tibetan populations and northern nomads in Dulan people (Fig. 2D, Table S5).

### Genomic analysis

Three individuals (DL5, DL6, and DL8) covered more than 10,000 target SNPs and were used to perform genomic analysis (Table 2, Figs. S1–S8) while the others were excluded due to high contamination (DL7) or insufficient data (DL1–DL4). Although DL5, DL6, and DL8 failed to perform pairwise missing rate (PMR) (Kennett et al. 2017) calculation to test genetic relatedness as no individual pairs shared more than 8000 overlap SNPs, we considered these individuals unrelated to each other. DL5, DL6, and DL8 belonged to distinct mtDNA haplotypes or Y-chromosome haplogroups. Moreover, the long distance between the three tombs and different burial customs provided the evidence of irrelevance as well. We firstly performed principle component analysis to test the genetic affinities between Dulan individuals and ancient and modern Eurasian populations. PCA plot (Fig. 3, Fig. S9) showed that DL5 and DL6 mainly clustered with present-day Tibetans and ancient plateau individuals,

**Table 2** Sequencing metrics for the eight Dulan individuals

Sample ID	Total reads	Mapped reads	Human DNA (%)	Mean length (bp)	Mean coverage	X-chromosome contamination	mtDNA contamination	1240k SNPs hit	HO SNPs hit
DL1	3,152,682	132,946	4.22	139.05	0.0041 ± 0.0666	–	–	3888	2035
DL2	11,102,693	343,401	3.09	147.16	0.0105 ± 0.1689	–	–	6284	2910
DL3	19,792,804	150,950	0.76	130.76	0.0056 ± 0.078	–	–	5366	2848
DL4	3,565,695	181,065	5.08	130.65	0.0049 ± 0.0767	–	–	4548	2342
DL5	15,733,331	2,234,222	14.20	119.9	0.0773 ± 0.5241	0.075	–	84,527	47,202
DL6	9,159,847	1,458,992	15.93	105.73	0.0464 ± 0.3223	–	0.03	50,211	26,599
DL7	8,551,840	2,502,879	29.27	113.4	0.078 ± 0.5134	0.233	–	78,675	40,689
DL8	9,297,707	1,845,942	19.85	121.19	0.0586 ± 0.406	6.611e–05 ± 0.095	0.04	57,971	29,071

however, DL8 clustered with modern Turkic populations and ancient Steppe nomads. Unsupervised admixture analysis produced similar results at best  $K=5$  where the lowest cross-validation error was observed (Fig. 3D, Fig. S10–S12), as DL5 and DL6 harbored similar genetic components to modern Tibeto-Burman populations and DL8 shared similar composition with modern Turkic populations like Kazakh\_China and Kyrgyz\_China. We then performed  $f_3$ -statistics and  $f_4$ -statistics tests for DL5, DL6, and DL8, respectively, and observed the same trends from the PCA and the ADMIXTURE analysis (Figs. 4, S13, S14, Table S6). While all showing genetic drift with Steppe-related populations, DL5 and DL6 were indistinguishable from plateau settlers, and DL8 differed from plateau settlers significantly. We further modeled the genetic components and ancestry sources by qpAdm (Table S7). DL5, DL6, and DL8 can be modeled as a two-way admixture of Russia\_Afanasievo and China\_Upper\_YR\_LN. DL5 and DL6 showed near exclusive China\_Upper\_YR\_LN-related ancestry (~95%–99%), while DL8 was admixture of China\_Upper\_YR\_LN-related ancestry (~53%) and Steppe-related ancestry (~47%).

## Discussion

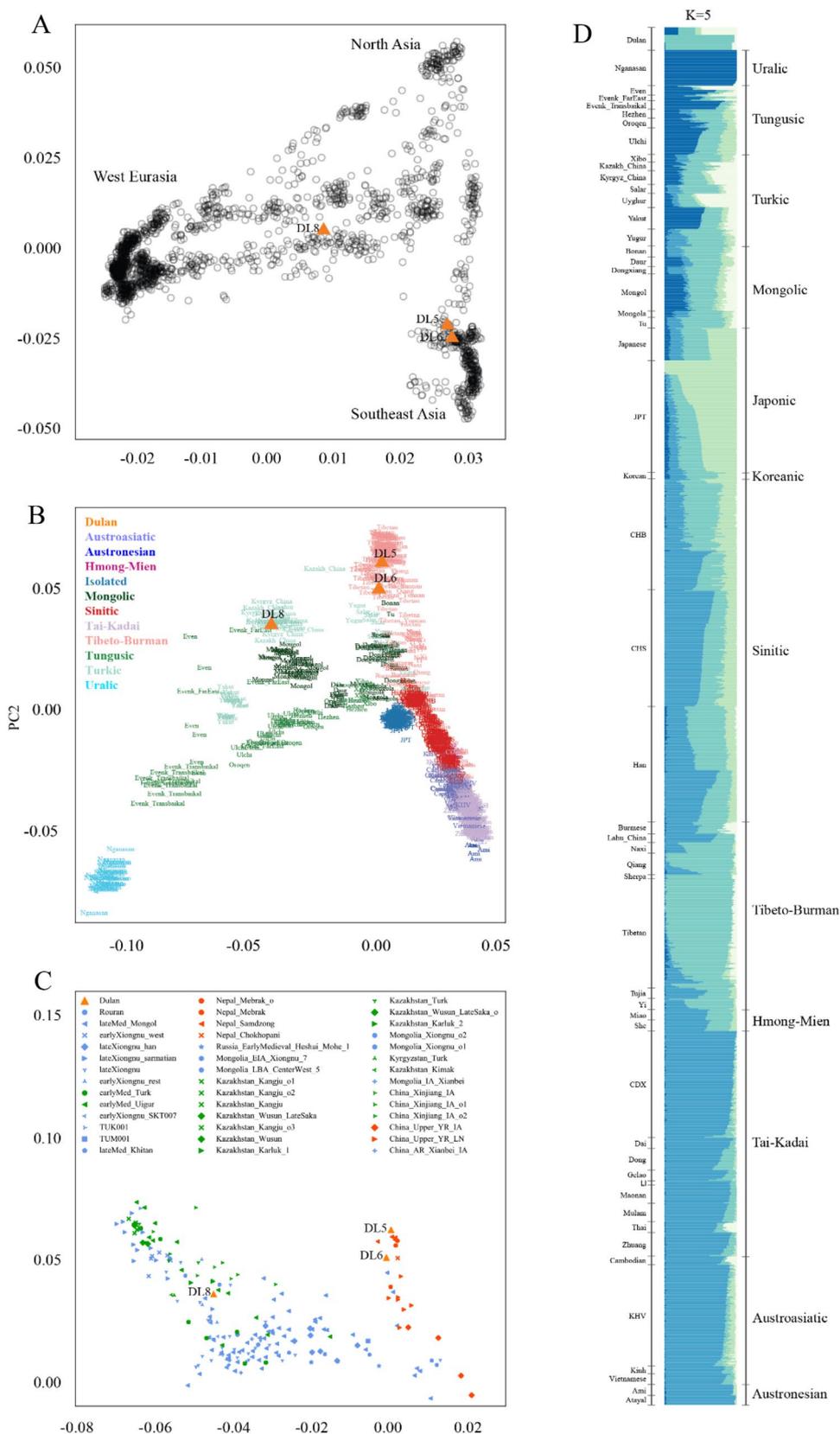
In this paper, we sequenced eight individuals from Tubo tombs and gave the genomic look into residents in the north-eastern part of the Tubo Kingdom. By comparing the genetic composition differences between residents of the Tubo Kingdom and other ancient or modern East Asians, our results showed that these ancient Tibetans owned complex origins.

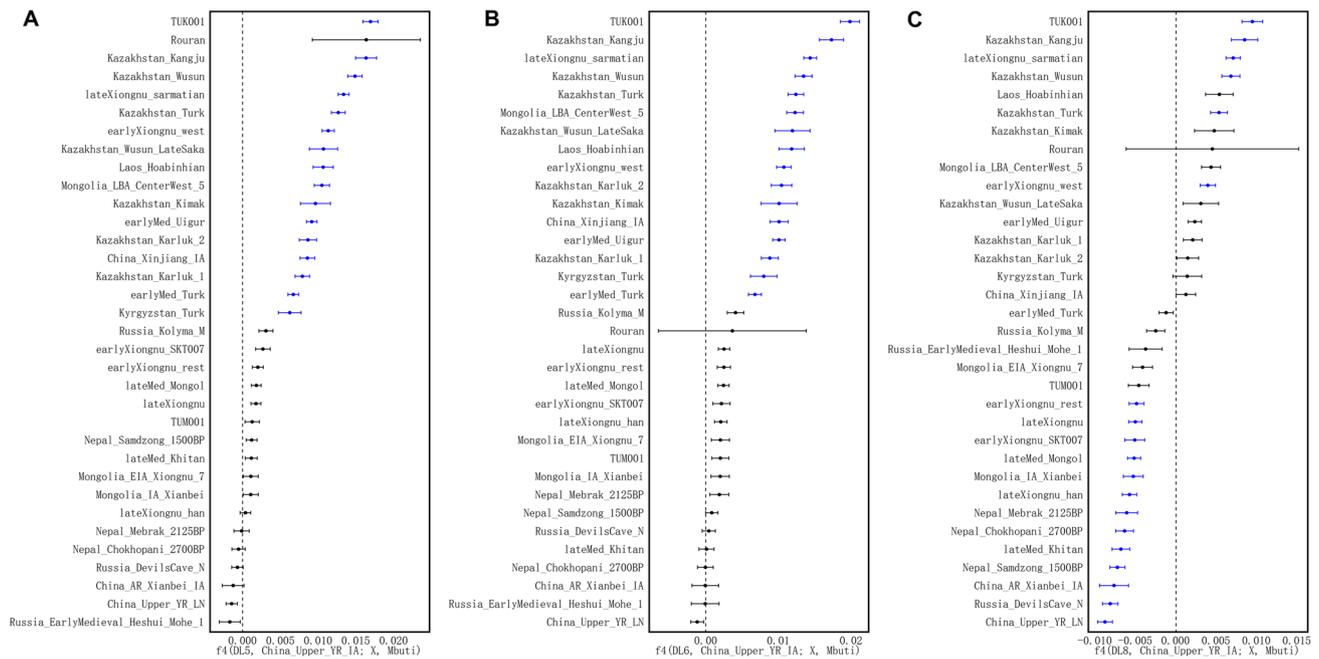
Uniparental markers showed that Dulan individuals owned highly diverse ancestries. Genomic analysis revealed similar but more precise results to uniparental researches since a small sample size led to biased population analyses in uniparental data. Dulan people are an admixture of East Asians and West Eurasians, while the local Tibeto-Burman

component constituted a major part. We did not find representative Y-chromosome C lineages of ancient Xianbei tribes (Li et al. 2018; Zhang et al. 2018) in Dulan samples, but the rank of shared genetic drift between Dulan people and Xianbei tribes (Figs. S13, S14) partially evidenced connections between Xianbei, Tuyuhun, and Tubo.

The complexity of Dulan people may arise from population movement through the southern route of the Silk Road. Compared to the famous northern route of the Silk Road through Gansu and Xinjiang, the southern route of the Silk Road through Qinghai is much lesser known. However, the Qinghai Road was an important trade route during the fifth to eighth Century when the Tuyuhun Kingdom and the Tubo Kingdom thrived. Our genetic results of studied tomb occupants together with their burial structures, burial customs, and burial goods confirm that people across Eurasia converged here and lived until their death. DL2 from 2014QDRM16 was mummified, buried in the wooden-chambered tomb with ancient Tibetan written on the wood, and buried with turquoise jewelry, amber pendant, and shell ornament. The wooden chamber of 2014QDRM16 and 2014QDRM23 was made up of nine layers and thirteen layers of cypress respectively, which also corresponded to the Tubo funerary rituals and hierarchy. Consistently, DL2 showed close maternal proximity to modern Tibetans for his mtDNA haplogroup M13a2. DL6 from 2014QDRM19 was buried in the only one brick-chambered tomb (which is a typical cultural characteristic of Han Chinese from Tang Empire) in this excavation and also showed affinity to modern Sino-Tibetan populations for his paternal and maternal lineages. DL8 from 2014QDRM20 was buried in the vertical pit tomb covered with round stone piles. Scattered horse remains on the raw-soil secondary platform and ironware were also excavated in 2014QDRM20. The burial structure of 2014QDRM20 showed typical steppe style, and DL8 showed abundant steppe ancestry from all markers analyzed in our research. Overall, our genetic evidence

**Fig. 3** Genetic patterns of Dulan people and present-day East Asians, data collected from Allen Ancient DNA Resource version 44.3 (AADR 2021). **A** Projection of Dulan onto PCA for Eurasians. PC1 and PC2 were calculated using 2217 present-day Eurasian individuals and Dulan individuals were projected onto PC1 and PC2. **B** Projection of Dulan onto PCA for 1212 East Asians. **C** PCA for Dulan and 206 ancient Asian individuals, principal components constructed from present-day East Asians. **D** ADMIXTURE results at best  $K=5$  for Dulan individuals and present-day East Asian populations sorted by linguistic families





**Fig. 4**  $f_4$ -statistics between Dulan individuals and ancient Eurasian populations of the form  $f_4(\text{DL5/DL6/DL8}, \text{China\_Upper\_YR\_IA}; \text{X}, \text{Mbuti})$ . The positive values show the greater affinity of the Dulan

individual and X, and the negative values show the greater affinity of China\_Upper\_YR\_IA and X compared to the Dulan individual and X.  $F_4$ -statistics with  $|Z$  score  $> 4$  are marked in blue

and archaeological findings demonstrate that residents in Dulan were highly diverse and the importance of the southern route of the Silk Road through Qinghai is massively underestimated.

One individual (DL7) is excluded in all analyses due to high contamination. However, his maternal lineage is assigned to M9a1b, which reaches the highest frequency in the southern part of Tibet around the Himalayas (Peng et al. 2011). Considering his haplogroup is distinct from other samples and the experimenter, and his tomb is robbed severely, this sample needs further investigation. For the left four samples excluded due to insufficient data (DL1-DL4), the low coverage is potentially due to the skeletal elements and shotgun sequencing, while performing capture on petrous could efficiently enrich endogenous DNA and thus generate more convincing data.

In conclusion, we provide essential genomic insights into the formation of populations on the Tibetan Plateau through eight ancient genomes of the Tubo Kingdom period in Dulan County, Qinghai Province. We demonstrate genetic continuity between Dulan people and ancient and modern Tibetan highlanders together with long-distance connections with populations in a vast geographic region across ancient Asia. Our findings support the complex origins but a major high-altitude ancestry and intense admixture for Tubo residents in NETP. Further analysis of ancient genomes from the hinterland of the Tibetan Plateau and contemporary samples from adjacent areas are needed to study the cultural and demic

diffusion of the Tubo Kingdom, which is the key part of the demographic history of Tibetans.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00438-022-01955-6>.

**Acknowledgements** This work was supported by the National Key R&D Program of China (2020YFE0201600), B&R Joint Laboratory of Eurasian Anthropology (18490750300), and the National Natural Science Foundation of China (91731303 and 31671297 to Hui Li). The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

**Author contributions** XEY carried out the molecular genetic studies and drafted the manuscript. JYL and XYR collected the samples. XEY and YTZ participated in the experiments. XEY and CS performed the statistical analysis. HL designed the study and revised the manuscript. All authors read and approved the final manuscript.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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