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COVER Xinjiang was a frontier area for population admixture between eastern and western Eurasians. Although previous archaeological studies have indicated that Bronze Age Xinjiang residents exhibited predominantly western Eurasian features, our research reveals that population admixture between eastern and western Eurasians occurred at most sites in Xinjiang, including the Kingdom of Khotan in extreme southwestern Xinjiang. Eastern Eurasian genetic contributions were heavily represented in most Bronze Age populations in Xinjiang, with contributions as high as 79% in Khotan samples. In skulls with typical western features, we identified many craniometrical traits similar to those associated with eastern Eurasians. This result suggests that western forms of some observed cranial features might be genetically dominant to eastern forms, and that the genetic contributions can be more precisely predicted using craniometrical traits, which fit quantitative genetic models. Based on the results of this study, we argue that early Xinjiang populations were not solely from the west, but also received contributions from the east. Two male skulls from the same graveyard are shown in the cover figure: they exhibit features typical of either eastern Eurasians (right) or western Eurasians (left), especially with respect to orbit shape and nasion depression (see the invited article by TAN JingZe et al. on page 299).

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Volume 58 Number 3 January 2013

CONTENTS

Progress of Projects Supported by NSFC

ARTICLE

Acoustics

291 **Interaction between microbubble and elastic microvessel in low frequency ultrasound field using finite element method**
SHEN YuanYuan, WANG TianFu, CHIN ChienTing, DIAO XianFen & CHEN SiPing

INVITED ARTICLE

Geology

299 **Craniometrical evidence for population admixture between Eastern and Western Eurasians in Bronze Age southwest Xinjiang**
TAN JingZe, LI LiMing, ZHANG JianBo, FU WenQing, GUAN HaiJuan, AO Xue, WANG LingE, WU XinHua, HAN KangXin, JIN Li & LI Hui

REVIEW

Organic Chemistry

307 **Formation of silacycles via metal-mediated or catalyzed Si–C bond cleavage**
WANG LiLi & DUAN Zheng

ARTICLES

Acoustics

316 **Platform for dynamic virtual auditory environment real-time rendering system**
ZHANG ChengYun & XIE BoSun

Polymer Chemistry

328 **Structural variation of melt-crystallized PTT during the heating process revealed by FTIR and SAXS**
CHEN Zhen & YAN ShouKe

Plant Physiology

336 **ATHK1 acts downstream of hydrogen peroxide to mediate ABA signaling through regulation of calcium channel activity in *Arabidopsis* guard cells**
LÜ Dong, WANG Wei & MIAO Chen

Crop Germplasm Resources

344 **Genetic structure and eco-geographical differentiation of cultivated *Hsien* rice (*Oryza sativa* L. subsp. *indica*) in China revealed by microsatellites**
ZHANG DongLing, ZHANG HongLiang, QI YongWen, WANG MeiXing, SUN JunLi, DING Li & LI ZiChao

Geology

353 **The magnetic properties of Serbian loess and its environmental significance**
LIU XiuMing, LIU Zhi, LÜ Bin, MARKOVIĆ S B, CHEN JiaSheng, GUO Hui, MA MingMing, ZHAO GuoYong & FENG Hua

364 **Vegetation evolution and its response to climatic change during 3.15–0.67 Ma in deep-sea pollen record from northern South China Sea**
LUO YunLi & SUN XiangJun

Geochemistry

373 **The comparison of biomarkers released by hydropyrolysis and Soxhlet extraction from source rocks of different maturities**
WU LiangLiang, LIAO YuHong, FANG YunXin & GENG AnSong

Geography

384 **Magnetic records of heavy metal pollution in urban topsoil in Lanzhou, China**
WANG Bo, XIA DunSheng, YU Ye, JIA Jia & XU ShuJing

396 **Spatiotemporal variation in alpine grassland phenology in the Qinghai-Tibetan Plateau from 1999 to 2009**
DING MingJun, ZHANG YiLi, SUN XiaoMin, LIU LinShan, WANG ZhaoFeng & BAI WanQi

CONTENTS

Atmospheric Science

406 **Different types of La Niña events and different responses of the tropical atmosphere**
YUAN Yuan & YAN HongMing

Engineering Thermophysics

416 **A new model of selective photothermolysis to aid laser treatment of port wine stains**
LI Dong, HE YaLing, WANG GuoXiang, WANG YongXian & YING ZhaoXia

Optoelectronics

427 **Broad-band direct QPSK modulator/demodulator for wireless gigabit communication**
CAO YuXiong, WU DanYu, LIU XinYu & JIN Zhi

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Invited Article

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Craniometrical evidence for population admixture between Eastern and Western Eurasians in Bronze Age southwest Xinjiang

TAN JingZe[1,2], LI LiMing[1], ZHANG JianBo[1], FU WenQing[1], GUAN HaiJuan[2], AO Xue[2], WANG LingE[1], WU XinHua[3], HAN KangXin[3], JIN Li[1,2] & LI Hui[1,2*]

[1] *Key Laboratory of Contemporary Anthropology (Ministry of Education), School of Life Sciences, Fudan University, Shanghai 200433, China;*
[2] *Shanghai Society of Anthropology, Shanghai 200433, China;*
[3] *Institute of Archaeology, Chinese Academy of Social Sciences, Beijing 100710, China*

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Xinjiang, the most northwest provincial administrative area of China, was the area where the oriental people met the occidental. The populations in Xinjiang exhibit very high genetic diversity. Previous study revealed that the eastern Xinjiang populations of the Bronze Age were mixed by the Eastern and the Western Eurasians. However, few studies have been performed to reveal when the population admixture started and how far to the west it reached. In this paper, we studied 148 craniofacial traits of 18 skulls from the Bronze Age Liushui graveyard in Khotan (Keriya County) in the southwest of Xinjiang. Seventeen craniometrical parameters of the Khotan samples were then compared with those of other ancient samples from around Xinjiang using dendrogram cluster analysis, principal components analysis, and multidimensional scaling. The results indicated that population sample of Liushui graveyard was mixed by the Western and Eastern Eurasians with about 79% contribution from the east. Therefore, we demonstrated that population admixture between east and west Eurasia can be traced back to as early as 1000 BC in southwest Xinjiang.

craniometry, morphology, population admixture, Khotan Kingdom, Bronze Age

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Xinjiang is the most northwest provincial administrative area of China, locating between East Asia and Central Asia. The modern populations of Xinjiang were found to have both characters of Eastern and Western Eurasians since the very beginning of the relevant anthropological studies [1]. Both physical anthropology [2] and molecular anthropology [3–5] revealed that indigenous Xinjiang people exhibit intergradations between the oriental and occidental people. Most populations are more similar to the oriental people genetically. Even the Uyghur, a population with the lowest proportion of oriental genetic contribution, has at least 60% lineages from the orient [5]. The characters of intergradations might have come from either of two processes: (1) admixture between two different populations, or (2) ancestral population deriving into populations in both sides. According to the genomic analyses, the Xinjiang populations are not ancestor of the other Eurasian populations, but admixed populations [6]. Therefore, our question is how early the admixture happened, and where.

The early Xinjiang people were regarded as a different “race” from the East Asians by most central China ancient people [7]. Archaeological findings revealed that Bronze Age residents of Xinjiang were very similar to the western Eurasians [8]. Therefore, it was widely accepted that the East Asians did not influence the early Xinjiang people much during the Bronze Age. However, recent studies of the DNA from the Bronze Age Xinjiang Mummies led to very different conclusions. DNA analyses revealed pronounced eastern proportions in the genetic structures of the ancient mummies from several important archaeological

*Corresponding author (email: lhca@fudan.edu.cn)

sites in eastern Xinjiang, including Qumul [9], Kroran [10], Charchan [11], etc. These results proved that the East Asians had at least arrived in eastern Xinjiang at the Bronze Age. However, archaeological studies suggested that the ancient people of the western Xinjiang, the Saka, were different nation from the eastern Xinjiang Tocharians [8]. Therefore, the genetic admixture in western Xinjiang of the Bronze Age is necessary to investigate. Recently, we studied the cranial characters of a population sample of Bronze Age Khotan Kingdom from Keriya County in southwest Xinjiang, and found apparent population admixture between Eastern and Western Eurasians.

This population sample was found in 2002 from a Bronze Age graveyard near Liushui Villiage, Achan Township, Keriya County, Hotan Prefecture, Xinjiang Uygur Autonomous Region, and was in the Silk Road (Figure 1). The excavation was finished in 2005. Fifty-two tombs were found. The human remains from the tombs were dated by ^{14}C to 2950±50 year before present [12]. That is to say, this graveyard, the Liushui graveyard, is the oldest site found in southwest Xinjiang.

1 Materials and methods

1.1 Samples and craniometrical standards

We excavated 21 human skeletons from Liushui graveyard of Khotan in 2003. According to craniometrical standard, they were 12 males, eight females, and one minor. Eighteen adults had intact skulls. We analyzed 31 observational features and 88 metrical items on these skulls, and calculated 29 morphologic indices (Table S1). We used the international standard of craniometry [13]. To compare our sample to those from Central Asia and East Asia, we studied the craniometrical standards of Russia [14], China [15], and Japan [16], and did not find them different from the international standard.

1.2 Clustering analyses

The craniometrical results (17 indices) of Khotan were compared with those data from the Bronze Age samples from Northwestern Asia, Central Asia [17,18], Xinjiang [19–28], and Central China [29–32]. Multidimensional scaling analysis (MDS) and principal component analysis (PCA) were used in population comparison. The first eight principal components (94.2% of variance) were transformed by Pearson's r into genetic distances, and a dendrogram by Unweighted Pair Group Method with Arithmetic mean (UPGMA tree) was drawn based on these distances. As cranial features show much more variations among male samples than among female samples, previous studies usually only used data of males in clustering analyses. Our analyses also compared males only.

1.3 Admixture analysis

We developed a method to explore the population structures among our metric data. The method is to reveal the contribution rates of two presumed ancestral populations from Eastern and Western Eurasians to the Xinjiang populations based on the mean values of 17 traits of each population.

Eastern ancestral population is represented by '$A1$' and Western '$A2$'. Let C_{it} denote the mean values of the group i and the trait t in dataset, and ω_i represent the contribution rate of $A1$ to group i as well as $1-\omega_i$ to $A2$. The function of residual error can be built

$$\text{Residual error} = \sum_{i=1}^{30}\sum_{t=1}^{17}[C_{it} - \omega_i \times A1_t - (1-\omega_i) \times A2_t]^2, \quad (1)$$

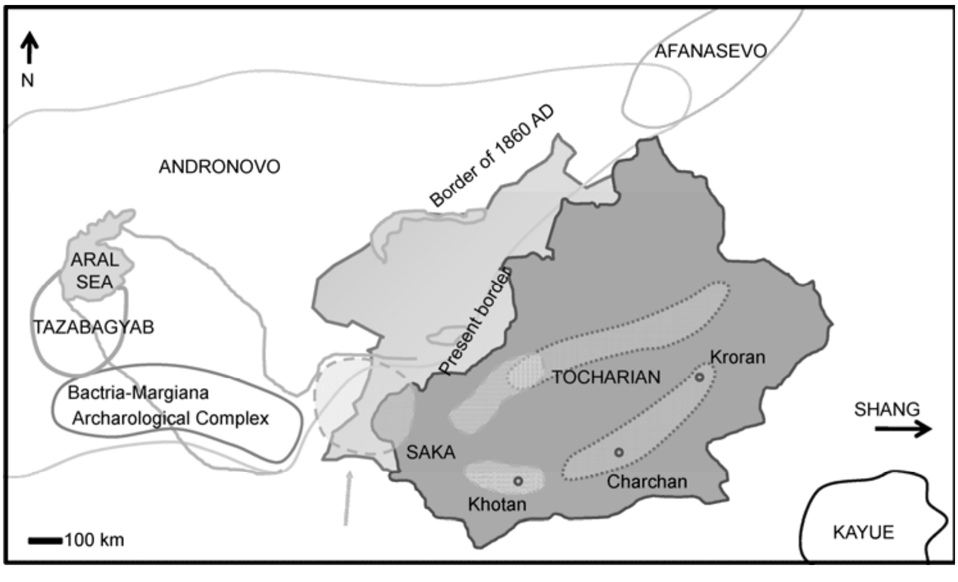

Figure 1 Distribution of Khotan in Xinjiang and the archaeological cultures around.

where $A1_t$ and $A2_t$ are the mean values of trait t of presumed ancestral populations $A1$ and $A2$, respectively. Note that both $A1_t$ and $A2_t$ are unknown and two group sets point to Eastern and Western Eurasians are distinct in our dataset, thus we let the mean of these two sets be the estimates of $A1_t$ and $A2_t$, respectively.

Our aim is that find the most possible set of ω_i which minimize the function of residual error. Note that ω_i is independent to ω_j for $i \neq j$, the set of ω_i has sole solution

$$\omega_i = \frac{\sum_t [(A2_t - C_{it}) \times (A2_t - A1_t)]}{\sum_t (A2_t - A1_t)^2}. \tag{2}$$

The solution of the function above may be negative or greater than 1, and according to the property of the function, it should be corrected by letting negative value to be 0 and the value greater than 1 to be 1.

The data standardized by original data was used in this analysis.

2 Results

2.1 Craniofacial features and ancestry identification for individuals

In our samples, 11 males, seven females, and a child have intact skulls. We collected craniometrical data of these 19 skulls (Table S1). As features of children are not fully developed, we excluded the data of the child skull from our analyses hereafter.

Populations from different geographic regions of the world have apparently different skull features [7,17,18], especially the shapes of orbital cavity and nasion. We observed these features on our samples, and found that some samples were similar either to the Eastern Eurasians or to the Western Eurasians, while some had characters of both sides, which suggested admixture between Eastern and Western Eurasians (Figure 2). We calculated the allele frequencies of 32 features in our samples (Table S2), and compared 12 features with ancestry identification characters with the reference western and eastern populations. Among the 12 features, seven features of most Khotan samples were western types, two features were eastern types, and three features were different in ancestry identification between males and females (Table 1). Therefore, the observation features of the Khotan samples were mostly western types, which made this population sample more like Western Eurasians.

Some metrical data can be transformed into morphological indices of skulls. We also calculated these indices in our samples and classified the indices of the samples into grades (Table S3). Among these indices, four items are different between Eastern and Western Eurasians. Most Khotan samples had western types for these four items, which was consistent with the observational features. Totally, the Khotan samples were more like Western Eurasians for the observational features of individual samples.

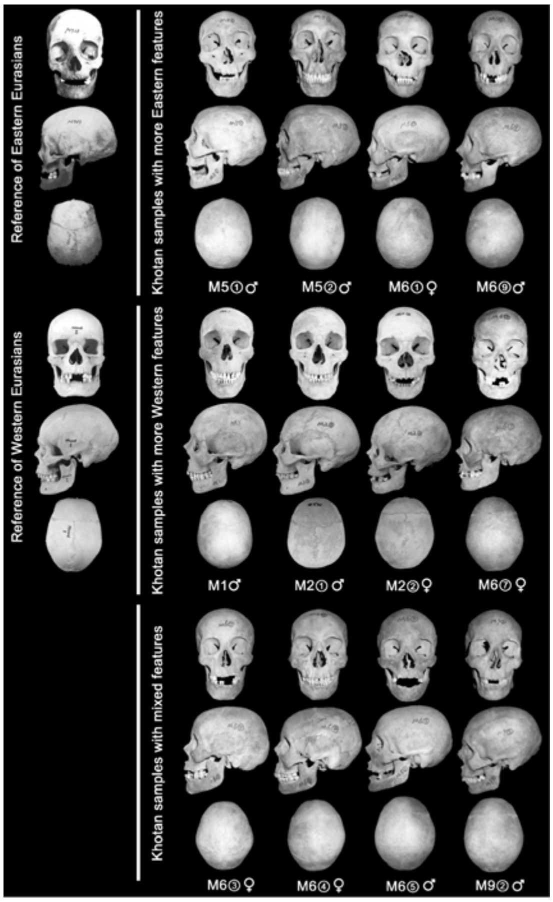

Figure 2 Some of the Khotan skulls compared to the referential samples of Eastern and Western Eurasians. Sex information is marked by ♀ or ♂ under each skull.

2.2 Population comparison and ethnic classification

It is imprecise to judge the origin of a population only by observing individual samples. Instead, many software programs can analyze the population data to assess the distances among populations. Here, we performed multiple methods (MDS and PCA) to estimate the distances between Khotan and other neighboring populations and influences from the Eastern and Western Eurasians.

We included 17 metrical items in the analyses (Table S4). These items are key items normally used in the craniometrical analyses, including sizes and angles of cranium, face, orbita, nose, zygoma, etc. Thirty populations from Xinjiang, East China, Central Asia, Siberia, and East Europe were included in the analyses, and were classified into three groups: 1, populations in Xinjiang (within the border of Xinjiang before 1860 AD); 2, populations to the west of Xinjiang; and 3, populations to the east of Xinjiang. Distances among the populations were plotted in the maps (Figure 3).

In the MDS map (Figure 3(a)), the populations from the

Table 1 Craniofacial features of Khotan samples compared to those of the typical Eastern and Western Eurasians

Item	Western	Eastern	Khotan	
			Most individuals	More like
Superciliary arch	Distinct	Weak	Distinct ♂ /Weak ♀	Western ♂ / Eastern ♀
Glabella projection	Strong	Weak	Strong ♂ /Light ♀	Western ♂ / Eastern ♀
Orbit shapes	Rectangle/Orthorhombic	Circular/Ellipsoid	Orthorhombic	Western
Orbital inclination	Forward oblique	Backward oblique	Backward oblique	Eastern
Nasion depression	Deep	Shallow/None	Deep	Western
Types of nasal roof	Straight/Convex-concave	Straight/Concave	Convex-concave	Western
Piriform aperture	Pear-shaped	Heart-shaped	Pear-shaped ♂ /Heart-shaped ♀	Western ♂ / Eastern ♀
Canine fossa	Deep	Shallow	Shallow	Eastern
Zygomatic shape	Oblique	Blunt	Oblique	Western
Malar jnt	Distinct	Light	Distinct	Western
Palate shape	C shape	Parabola	C shape	Western
Shovel incisor	None/Weak	Significant/Moderate	None	Western
Total profile angle	Orthognathous	Prognathous	Orthognathous	Western
Nasal index	Leptorrhiny	Mesorrhiny/Chamaerrhiny	Leptorrhiny ♂ /Mesorrhiny ♀	Western ♂ /Eastern ♀
Orbital index	Chamaeconchy	Mesoconchy/Hypsiconchy	Hypsiconchy ♂ /Mesoconchy ♀	Eastern
Simotic index	Protrudent	Weak	Protrudent	Western

same geographic region were clustered closely, i.e., the Eastern Eurasians were in the left, the Western Eurasians were in the right, and the Xinjiang populations, including the Huns, Wusun, and Saka from western Xinjiang, were in the middle. This distribution indicated that the craniometrical data can well predict the fact of Xinjiang populations to be the admixture between the east and the west. Among the Xinjiang populations, the Yanbulaq M in the east was in the Eastern Eurasian cluster, while Yanbulaq C from another graveyard in Yanbulaq was in the Xinjiang cluster, showing that the populations with different ancestries might have migrated into the same place. The Khotan was also very close to the Eastern cluster, indicating that Khotan might have more eastern components than most of the other Xinjiang populations.

The PCA map (Figure 3(b)) was similar to the MDS map, but displayed much clearer clusters. The East Asians were in the upper-left side of the map. The East European and Siberian populations were in the upper-right of the map. Two Central Asian populations were in the lower-right of the map. Therefore, the Western Eurasians were all in the right of the map. The Xinjiang populations were scattered between the Eastern and Western clusters. The Khotan was closest to the East Asians among the Xinjiang populations, especially in the first component.

We calculated the Pearson's r distances using the former eight principal components (94.2% of the variances), and transformed into a UPGMA (unweighted pair-group method with arithmetic means) tree (Figure 4). The tree was divided into two major clades, Eastern Eurasians and Western Eurasians. The Xinjiang populations were mostly in the Eastern Eurasians. Khotan, Yanbulaq, and Alwighul were even closer to the East Asians. However, the earliest Xinjiang sample, Qawrighul (1800 BC), was in the Western Eurasian cluster, indicating a western origin of this population.

2.3 Admixture analysis

To assess the contributions of the Eastern and Western Eurasians to the Xinjiang populations, we developed a statistic method to calculate the admixture proportions. The current most widely used admixture estimation program, STRUCTURE [33], are based on the individual data of the populations. However, almost all of the published ancient craniometrical data are average data of the populations, not the individual data. Therefore, STRUCTURE cannot be applied to these data. Although it is not perfect enough yet, our new method is based on the population average data that can be used to estimate the admixture. In the analysis, we assumed that there were two parental populations for the ancient Xinjiang people, i.e., Eastern Eurasians and Western Eurasians. We used the Anyang (Yinxu) sample as the Eastern reference and the Volga Pit Tomb sample as the Western reference. The results (Figure 5) were credible. The East Asian populations all exhibited high Eastern proportions (92.6%–100%), while the East European, Siberian, and Central Asian populations all exhibited very low Eastern proportions (0–23.3%), which was consist with the common sense. The average Eastern proportion in the Xinjiang samples was 60.8% (34.4%–87.3%). Only three out of the 15 Xinjiang samples had less than 50% of the Eastern proportions. The Eastern proportion in the Khotan sample (87.3%) was the highest among the Xinjiang samples, which was unpredictable. To verify this result, we changed different population samples as the parental references. The Eastern proportion in the Khotan varied from 78.7% to 91.2%. This result indicated that our Khotan sample was more similar to the Eastern Eurasians. The high Eastern proportion in the Khotan might have resulted from the high Eastern contribution to the original Khotan population, or the subsequent genetic drift.

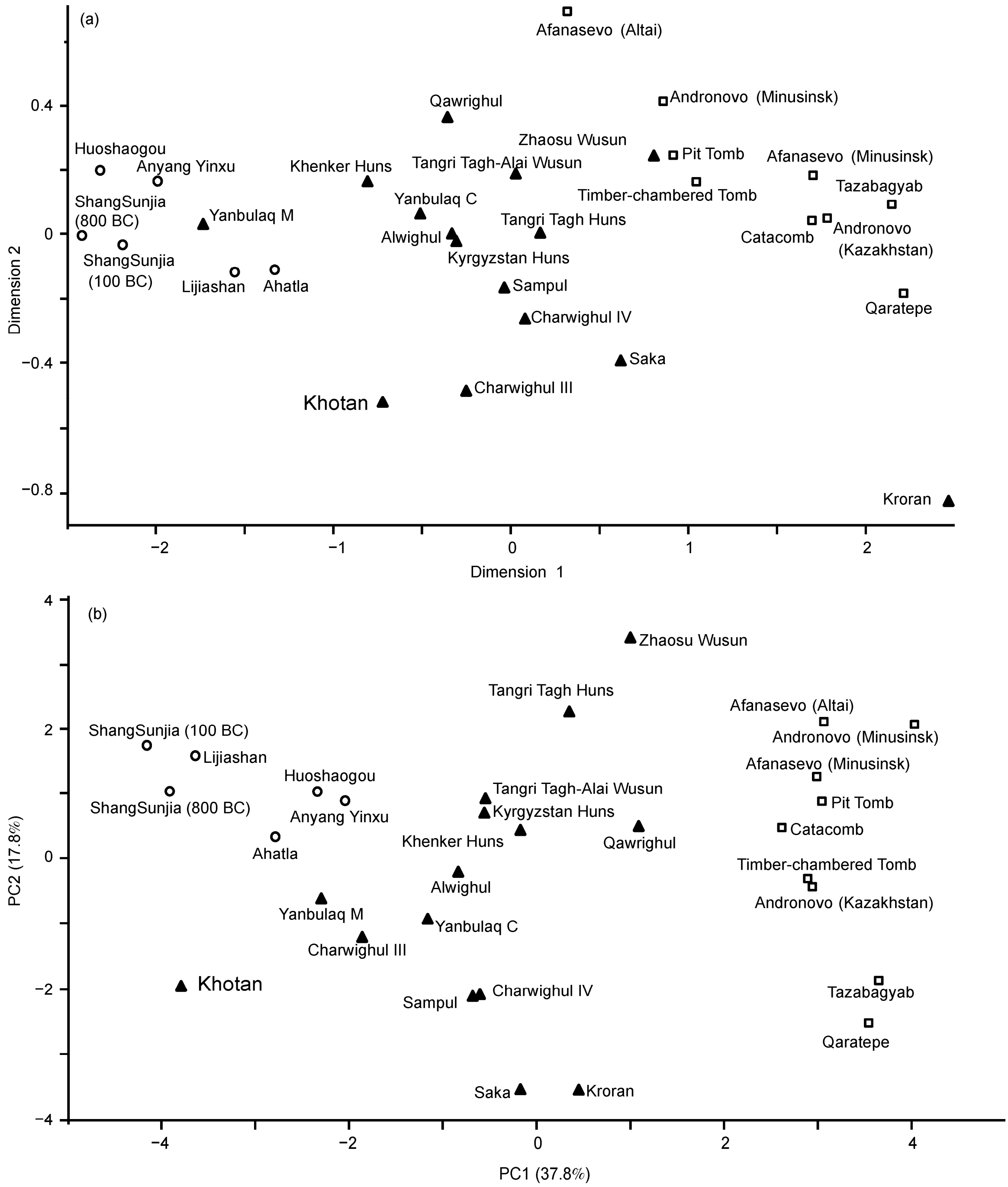

Figure 3 Multidimensional scaling (a) and principal component analysis (b) of craniometrical data of Khotan and surrounding ancient samples (males).

3 Discussion

3.1 Inconsistency of ancestry identification by observational features and by metrical traits

The ancestry identification of the Khotan sample by observational features seemed inconsistent from that by metrical traits. Judged from the observational features, the Khotan samples were more like Western Eurasians, while they were more similar to the Eastern Eurasians in the metrical traits. This phenomenon indicated that different data might issue different results during population relationship analyses. Some result may even be incompatible. This kind of incompatibility may have resulted from various reasons, including different genetic modes of the traits. However, very few genetic modes for the craniometrical traits have been decided, therefore, we cannot determine how different genetic modes influenced our results. One possible explanation can be that most metrical traits may be quantitative traits influenced by many genes, while observational features may be related to very few genes. For example, nasion depression followed a monogenic inheritance mode in pedigrees, and the western high nasion is dominant [34]. If most western features are dominant to the eastern features, the admixed population will be more like the western people, resulting in apparent bias. Differently, this bias will not appear when judged from quantitative traits. If the craniometrical traits are quantitative, the relevant results of population genetic structure will be more reliable. However, this hypothesis requires more genetic evidence.

3.2 Khotan population was more similar to Eastern Eurasians

Judged from the metrical data, especially from the PCA (Figures 3 and 4) and admixture analysis (Figure 5), the Khotan samples were obviously close to the populations from the Eastern and Central China. Among the Xinjiang populations, the Khotan was close to the Yanbulaq M and Charwighul III, and the later two populations had Eastern Eurasian features with cranial characters of northeastern Tibetans [18,25,27]. Therefore, there were additional anthropological clues to the eastern ancestry of Khotan people.

Furthermore, archaeological evidence also supported an eastern influence of the Khotan, as well as influences from central Asia and Siberia. The styles of the pottery in Khotan were very similar to the Chust Culture found in Fergana Valley of Uzbekistan. However, geometric carving patterns on the Khotan pottery were most similar to those in Tibet and Qinghai. A single-eared gallipot found in Khotan can find an almost exact copy in the Qosgong graveyard, Lhasa, Tibet. There were also bronze wares excavated from Khotan. Those bronze wares had the same styles with those from Charwighul and Yanbulaq. The bronze or golden bell-shaped eardrops from Khotan were most similar to those of the early Scythians from Siberia. Bronze harnesses and arrows with tailhooks were most similar to those of Arzan in Siberia [12]. Therefore, the population admixture in early Khotan had both physical anthropological and archaeological evidences.

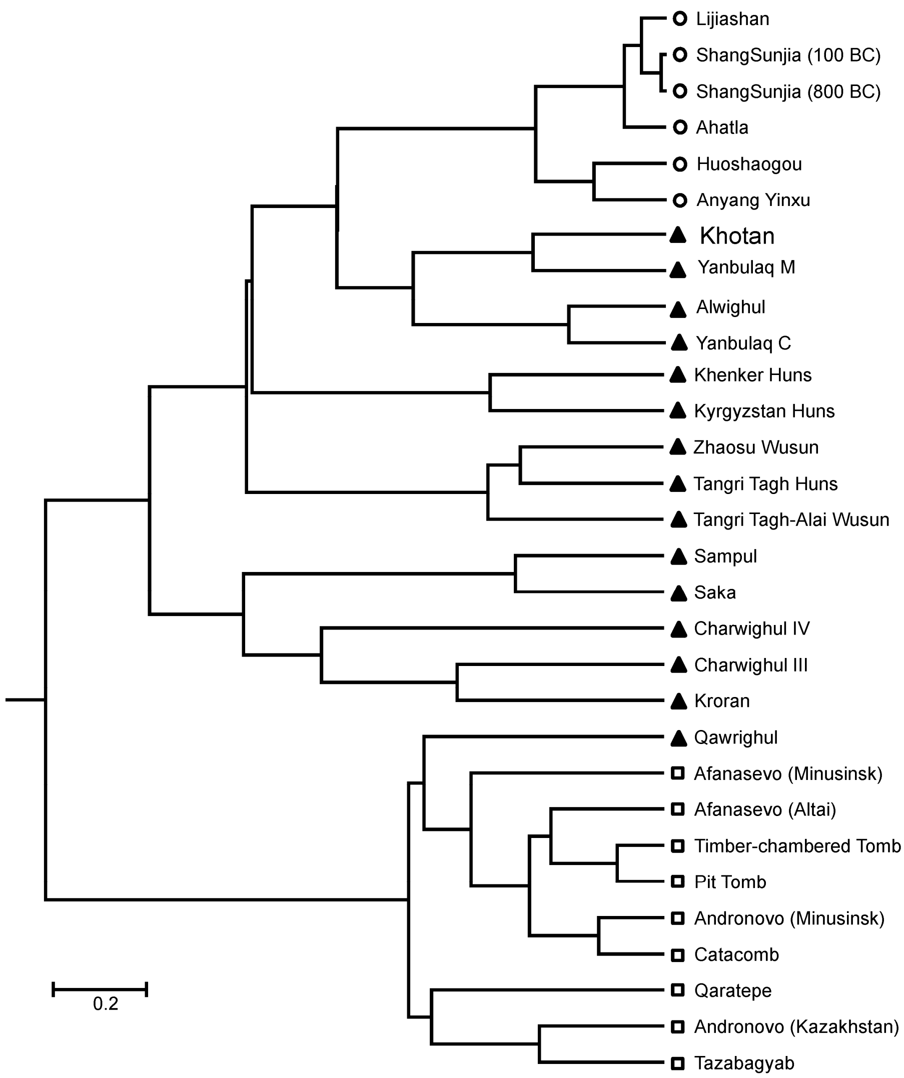

Figure 4 UPGMA cluster analysis of Pearson's *r* distances among 30 male population samples.

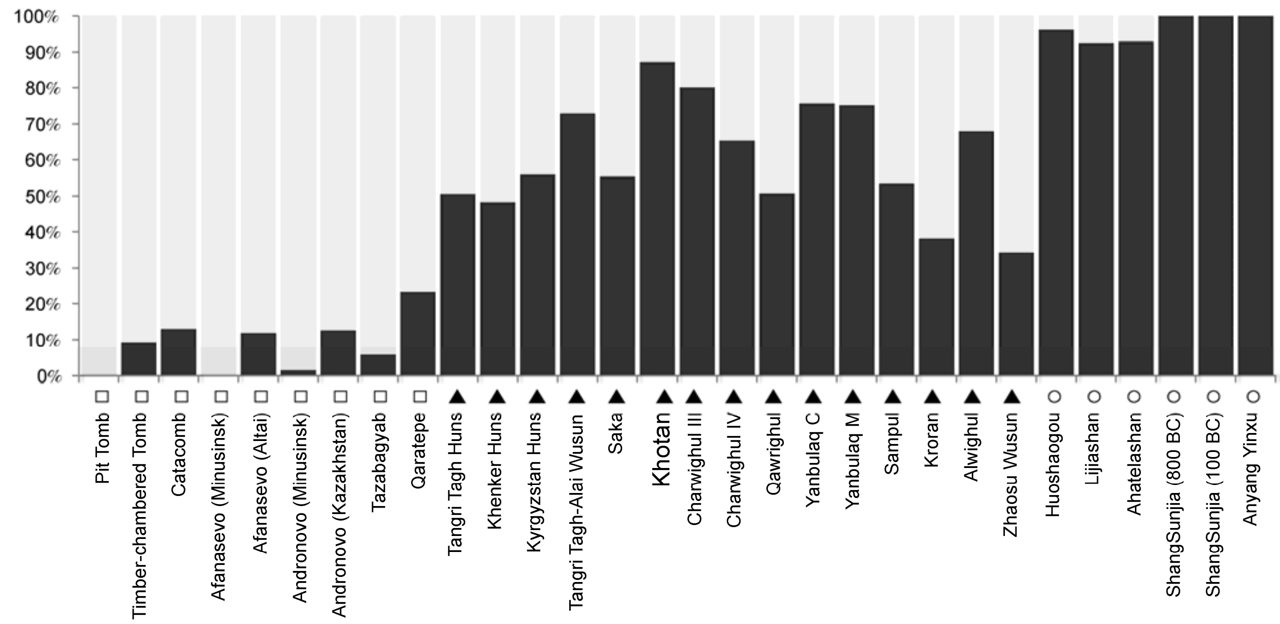

Figure 5 Population admixture analyses with Anyang as eastern parental reference and Volga (Pit Tomb) as western parental reference.

3.3 Population admixture in ancient Xinjiang might be much wider

Population admixture was widely found in the Bronze Age Xinjiang populations. The contributions were from East Asia, Central Asia, Siberia, etc. There were many Bronze Age population samples from East Asia which is close to Xinjiang, including Anyang Yinxu (1300–1027 BC), Huoshaogou (1600 BC), ShangSunjia (800–100 BC), Lijiashan (1550–690 BC), Ahatla (1550–710 BC). These Bronze Age populations were all similar to each other in cranial features and also similar to the modern northern Chinese and northeastern Tibetans [29–32]. The Siberian populations, including Afanasievo (3500–2500 BC) and Andronovo (2100–900 BC), and the Volga populations, including Timber-chambered Tomb (1500–800 BC), Catacombs (2000–1500 BC, Pit Tomb (3600–2200 BC), were also variants of the Proto-Europeans. The Xinjiang Qawrighul (1800 BC) people were most similar to these populations [19,30]. The Central Asian populations, including Qaratepe (4000–3000 BC), Tazabagyab (2000–1000 BC), and Pamir Saka (600–400 BC), were called Mediterranean European, or Indo-Afghan [18]. Some Xinjiang populations were also similar to the Indo-Afghan type, including the Kroran (100 BC–100 AD), Sampul (100 BC–300 AD), and Shambabay (900–500 BC) [18,20,22,23]. However, those population samples all exhibited admixed features. For example, the one of the six Kroran samples showed very pronounced East Asian features, and very similar to the Kayue people in central China [18,20]. Moreover, the DNA analysis on the Kroran samples also revealed Eastern contribution [10].

Beside the Indo-Afghan type, there were also Pamir-Ferghana type people in Central Asia. This type was mostly found in western Xinjiang. The Xinjiang Tangri Tagh-Alai Wusun (600–100 BC) showed most features of Pamir-Ferghana type, but also some features of the Proto-Europeans. The Huns (Xiongnu) in western Xinjiang showed apparent admixture between eastern and western Eurasians [18,21,26]. Most of the Zhaosu Wusun people were Pamir-Ferghana type, while two females among them showed obvious East Asian features [18,21,26].

Sometimes, it seemed that two totally different populations were using the same graveyard. The most typical example was Yanbulaq (1300–550 BC) in eastern Xinjiang. Individual samples were divided into M group (East Asians) and C group (Proto-Europeans) [18,25]. In central Xinjiang, the admixture pattern was even complicated. A population sample in Toksun, Turpan, the Alwighul (600–200 BC), comprised Indo-Afghan, Pamir-Ferghana, East Asian, Proto-European, and intergraded types [18]. In southern Xinjiang, the early Charwighul IV (1000–500 BC) were most similar to the Proto-Europeans. The later Charwighul III (200 BC–220 AD) were more like East Asians, with three skulls showing characters of the Huns [18,27].

Totally, as was shown in the MDS and PCA of this study, all of the Bronze Age Xinjiang populations exhibited admixed features with more eastern contributions than western contributions. In the previous studies, very few samples from southwestern Xinjiang were studied. By this study, we know that the population admixture happened not only in eastern and northern Xinjiang, but also in the most southwestern part of Xinjiang. The eastern contributions to the Khotan samples might have come from Tibet. Furthermore, we suppose the populations to the west of Khotan might also be admixed, although relevant studies are yet to be done.

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Supporting Information

Table S1 Individual craniometric data of Liushui graveyard in Khotan
Table S2 Frequencies of craniofacial observational features among the human remains of Khotan Liushui graveyard
Table S3 Hierarchical classification of craniofacial indices of Khotan Liushui graveyard
Table S4 Population comparison of the mean values of 17 craniofacial metric variables of males

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