

ORIGINAL ARTICLE

Ancient DNA from nomads in 2500-year-old archeological sites of Pengyang, China

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Six human remains (dating ~2500 years ago) were excavated from Pengyang, China, an area occupied by both ancient nomadic and farming people. The funerary objects found with these remains suggested they were nomads. To further confirm their ancestry, we analyzed both the maternal lineages and paternal lineages of the ancient DNA. From the mitochondrial DNA, six haplotypes were identified as three haplogroups: C, D4 and M10. The haplotype-sharing populations and phylogenetic analyses revealed that these individuals were closely associated with the ancient Xiongnu and modern northern Asians. Single-nucleotide polymorphism analysis of Y chromosomes from four male samples that were typed as haplogroup Q indicated that these people had originated in Siberia. These results show that these ancient people from Pengyang present a close genetic affinity to nomadic people, indicating that northern nomads had reached the Central Plain area of China nearly 2500 years ago.

Journal of Human Genetics (2010) 55, 215–218; doi:10.1038/jhg.2010.8; published online 26 February 2010

Keywords: ancient DNA; mitochondrial DNA; nomads; Pengyang; Y chromosome SNP

INTRODUCTION

The Ningxia area in China, where both ancient farming people and nomads lived 2000 years ago,¹ is in the southern inner Mongolia and northern Central Plain areas (Figure 1). As the farming people migrated and expanded in the Central Plain area, they became the ancestors of the modern Han Chinese; meanwhile, the nomads occupied Siberia and Mongolia. Owing to conflicts between these two ethnic groups in the frontier, the nomads assimilated gradually into the Han Chinese population or became the minorities in northern China.^{1,2} Therefore, the minorities in northern China and other northern Asians can trace their origins to the ancient nomads.¹ Archeological studies of the Zhongzhuang and Wangdahu sites, located in Pengyang in the southern Ningxia area in China (Figure 1), indicated that they were occupied from ~770 BC to 256 BC during the Dongzhou period. The funerary objects included many skulls of animals, bronze swords and daggers, but no farming tools. The ancient Pengyang people were identified anthropologically as northern Asians, implying that they were nomads. In this study, we analyzed the maternal and paternal lineages of the ancient DNA from the remains, confirming the evolution of the populations living in this area.

MATERIALS AND METHODS

Fragments (~2 cm long) of the unbroken femurs were sampled from six well-preserved ancient human remains at two sites, Zhongzhuang and Wangdahu (Table 1). All analyses were conducted in the professional ancient DNA

laboratory, following the criteria for ancient DNA studies.³ Ensuring authenticity, DNA extraction and polymerase chain reaction protocols were previously published.⁴ The mitochondrial hypervariable I segments (16050–16409) were amplified using three overlapping primer pairs. The mitochondrial haplogroups were identified using the amplified product-length polymorphism method.⁵ The sex of each sample was determined by the amelogenin gene polymerase chain reaction test. Y chromosome single-nucleotide polymorphisms M175 and M242 were determined by sequencing to type the haplogroups O and Q.⁶ In the Y chromosome single-nucleotide polymorphism procedure, any male researcher was excluded and the ancient female individuals were also amplified as negative controls. All primers in this study are listed in Supplementary Table S1. The sharing populations were searched from the published data of modern people across East Asia and Siberia, the source of which is provided in Supplementary Table S2. The mitochondrial hypervariable I sequence of every researcher was obtained to identify potential laboratory-based contamination (Supplementary Table S3).

RESULTS AND DISCUSSION

DNA samples from all individuals were successfully amplified, and the obtained mitochondrial hypervariable I sequences were inferred to be authentic. We found six haplotypes from haplogroups C, D4 and M10 (Table 1). Haplogroup D4 has a high distribution frequency (8–22.3%) in northern Asians^{7,8} and eastern Asians.⁹ Haplogroup C has a very high distribution frequency (15.9–67.2%) in Siberia,⁷ but a low frequency in China (3.5%).⁹ Haplogroup M10 is a new sub-haplogroup of M¹⁰ with a frequency range of 0–2.3% in modern Han Chinese,¹¹ while a few M10 individuals have also been found among Mongolians and Yakuts.^{8,12}

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Received 26 October 2009; revised 27 December 2009; accepted 21 January 2010; published online 26 February 2010

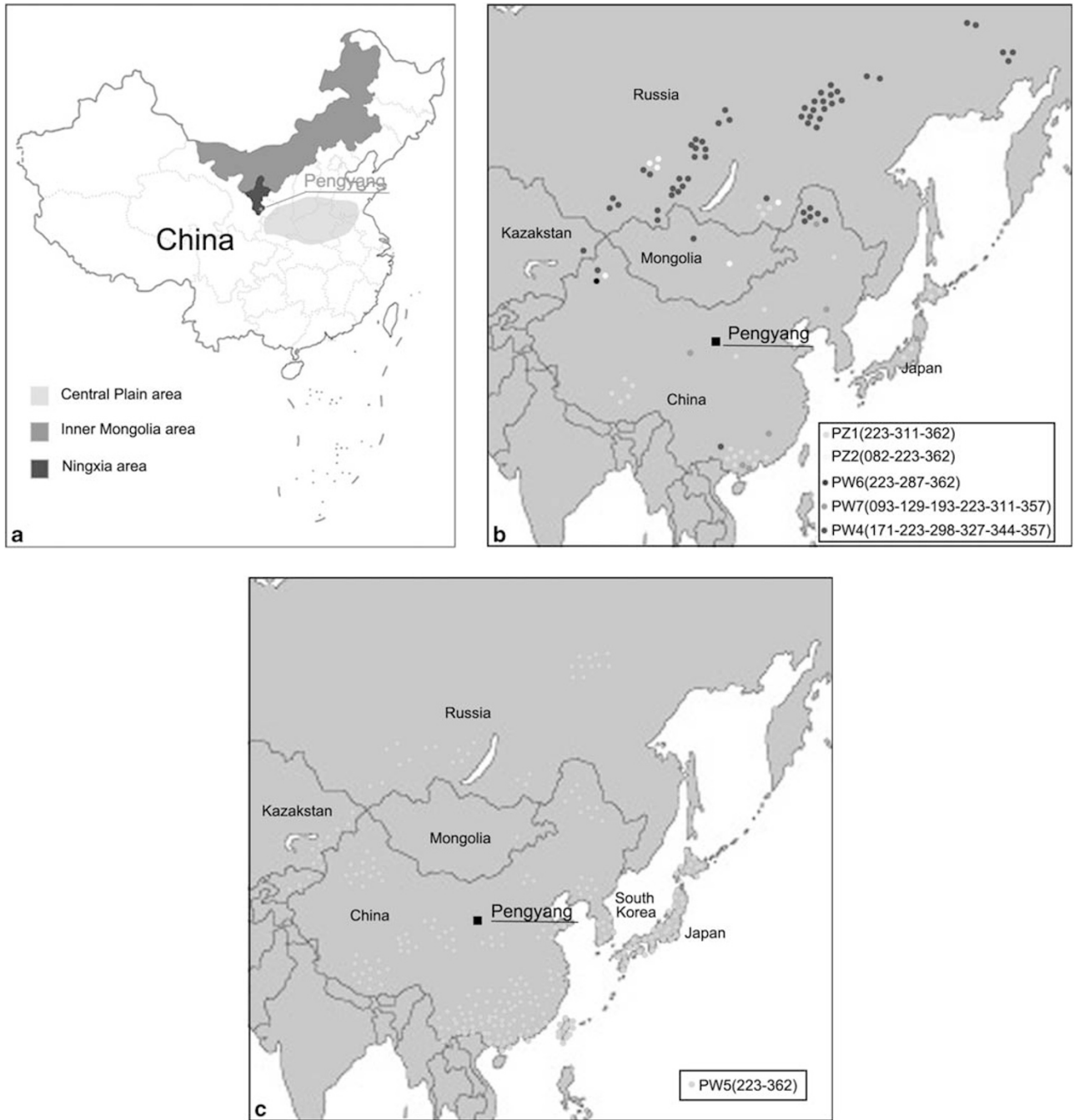


Figure 1 Geographic distribution of the ancient individuals from Pengyang and their sharing populations. (a) Geographic location of Pengyang. (b) Distribution of the populations shared by PZ1, PZ2, PW4, PW6 and PW7. (c) Distribution of the populations shared by PW5. Every dot represents an individual. Only the last three digits are given, for example, site 223 represents 16 223.

A phylogenetic tree was constructed to determine the genetic relationship between the ancient Pengyang people and related populations (Figure 2). In haplogroup C, the Han Chinese originating from the farming people^{1,9,10} are concentrated in one group. Meanwhile, PW4 is clustered into another group with minorities in northern China, northern Asians and ancient nomads,^{2,7,12–19} suggesting that PW4 is closely related to the nomads. In haplogroups D4 and M10, the ancient people from Pengyang consistently occupy the same nodes as northern Asians, northern minorities of China and ancient nomads, showing a close genetic relationship among them.

To further understand the genetic relationship among these populations, the six haplotypes from our study were used as queries to search for sharing populations in the published data of modern people across East Asia and Siberia, and we selected the matched sequences that belong to the same haplogroups for further comparisons (Figure 1; Supplementary Table S2). PW4 (16 171–16 223–16 298–16 327–16 344–16 357) shares a haplotype with 58 individuals. Among these, 49 individuals are from outer Mongolia and Siberia, 8 are from Uighur of Kazakstan and minorities of northern China who also originated from northern Asia,^{1,2} and only 1 is from the

Table 1 Genetic information of samples excavated from Pengyang

	MtDNA HVI polymorphic position													APLP analysis					
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	
	6	6	6	6	6	6	6	6	6	6	6	6	6	0	0	4	5	3	
	0	0	1	1	1	2	2	2	3	3	3	3	3	4	6	3	1	0	
	8	9	2	7	9	2	8	9	1	2	4	5	6	0	4	1	7	1	
Samples	2	3	9	1	3	3	7	8	1	7	4	7	2	0	6	8	8	0	

Site ^a	CRS	C	T	G	A	C	C	C	T	T	C	C	T	T	MtDNA haplogroups	C	G	T	C	G	Sex	Y-chromosome haplogroup
Zhongzhuang	PZ1	—	—	—	—	—	T	—	—	C	—	—	—	C	D4	T	—	—	A	A	♀	—
	PZ2	T	—	—	—	—	T	—	—	—	—	—	—	C	D4	T	—	—	A	A	♂	Q
	PW4	—	—	—	G	—	T	—	C	—	T	T	C	—	C	T	—	C	—	—	♂	Q
Wangdahu	PW5	—	—	—	—	—	T	—	—	—	—	—	—	C	D4	T	—	—	A	A	♂	Q
	PW6	—	—	—	—	—	T	T	—	—	—	—	—	C	D4	T	—	—	A	A	♂	Q
	PW7	—	C	A	—	T	T	—	—	C	—	—	C	—	M10	T	A	—	—	—	♀	—

Abbreviations: APLP, amplified product-length polymorphism; HVI, hypervariable I; mtDNA, mitochondrial DNA.
^aThe Zhongzhuang site contained two human remains, both of which were sampled. There were nine remains in the Wangdahu site, but only four remains were selected because the remains in the other burials were not intact.

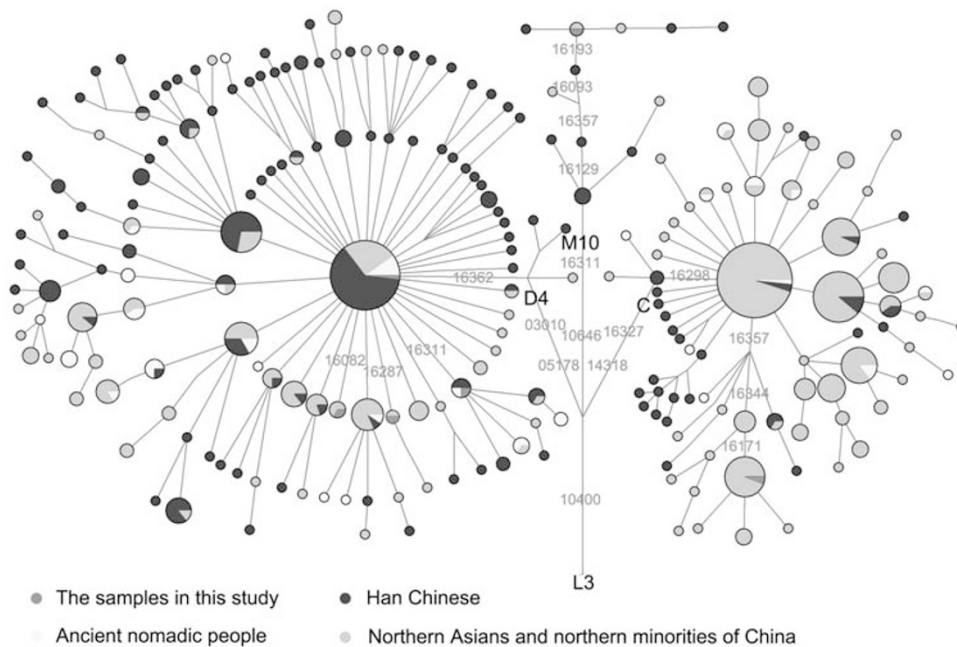


Figure 2 The maximum parsimony trees of median-joining networks of the mtDNA haplogroups of Pengyang ancient people in comparison with other populations. A phylogenetic tree was constructed by Network software ver. 4.5.1.0 (<http://www.fluxus-engineering.com>) using maximum parsimony methods and the mtDNA data of northern Han Chinese,^{9,10} northern Asians,^{7,12–14} minorities in northern China^{2,15} and the ancient nomadic people.^{16–19}

Daic population in southern China. PZ2 (16 082–16 223–16 362) shares a haplotype with four Siberians and two mongolians. PW6 (16 223–16 287–16 362) shares a haplotype with only one Kazak of China. Interestingly, all individuals sharing haplotypes with PZ2 and PW6 originated from nomads. PW7 (16 093–16 129–16 193–16 223–16 311–16 357) shares a haplotype with seven people, one of whom is an inner Mongolian, but the origins of the other six individuals do not trace back to the nomads. Haplotype 16 223–16 311–16 362 of PZ1 is observed in 25 people, including three Buryats, one Mongolian and five Tibetans, whose origin can be

traced back to the nomads.¹ Moreover, this haplotype is also found in ancient Xiongnu from northern Asia.¹⁶ Therefore, it is possible that PZ1 originated from the nomads. Haplotype 16 223–16 362 of PW5 is an ancient haplotype in haplogroup D and is found in various populations. The haplotype 16 223–16 362 is highly prevalent in eastern Asians, but its frequency is also high in northern Asians (Figure 1) and in ancient Xiongnu.¹⁶ Overall, every sample from Pengyang tested is related to northern Asians or northern minorities of China, and bears a high resemblance to northern nomads.

By surveying Y chromosome single-nucleotide polymorphisms, all four male individuals were typed as haplogroup Q, believed to have arisen in Siberia (Table 1). Generally, haplogroup Q is distributed at a very high frequency throughout Siberia, but at low frequencies outside of Siberia.²⁰ The frequency of haplogroup Q among Han Chinese was reported as 2.8%, whereas haplogroup O is predominant at 66.8%.⁹ These results demonstrate a close patrilineal genetic relationship between the ancient Pengyang people and the nomads.

Historical records documented that the nomadic culture was predominant in southern Inner Mongolia in the Dongzhou period, which was associated with historical events such as climate cooling, grassland degradation and farming people losing control of the border area.¹ Taken together, this study suggests that the northern nomads may have reached the southern Ningxia area in the Dongzhou period.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Key Laboratory for Evolution of Past Life and Environment in Northeast Asia, Ministry of Education, China (EPLNEA). We are grateful to the Institute of Cultural and Historical Relics and Archaeology in the Ningxia Hui Nationality Autonomous Region, China, for providing the samples. We also thank Wenxia Song from the Department of Cell Biology and Molecular Genetics of Maryland University for assistance with revision of the paper.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhg>)