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RESEARCH ARTICLE

Molecular genetic analysis of Dongzhou-period ancient human of Helingeer in Inner Mongolia, China

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Abstract The mtDNA hypervariable region I (HVR-I) of 10 ancient individuals from Dongzhou-period ancient human populations in Helingeer county of Inner Mongolia were amplified and sequenced to investigate the genetic structure. The relationships between the ancient population and related extant populations, as well as its possible origin at the molecular level, were also studied. Moreover, phylogenetic analysis and multi-dimensional scaling analysis were also performed based on the mtDNA data of the ancient population in Helingeer and the related Eurasian population. The results showed that the ancient population in Helingeer were closer to the northern Asian populations than to the other compared populations in matrilineal lineage. Combining the research results of archaeology and anthropology as well as molecular biology, we inferred that they were nomads who migrated from Mongolia plateau and cis-Baikal region to Helingeer in Inner Mongolia, China.

Keywords ancient DNA, mtDNA, genetic structure, phylogenetic analysis

1 Introduction

Based on the study of ancient DNA, we can recover the genetic information of ancient populations, reconstruct ancestor-descendant relationships of populations from different periods, cultures and tribes, and provide additional evidence for human origins, evolution, migration, and culture exchanges (Kings et al., 1997; Shinoda and Kanai, 1999; Ricaut et al., 2005; Xie et al., 2005).

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The archaeological site of Xindianzi cemetery is located in Xindianzi, Helingeer county of Inner Mongolia, China. It is the largest Dongzhou-period cemetery that has been excavated in Hunhe River. The burial style and cultural relic of this cemetery appear to have obvious northern nomadic characteristics. Both the geographical location and the historic culture belong to the middle of the Great Wall zone in Northern China. Broadly, the Great Wall zone of Inner Mongolia locates in the east of Eurasian steppes, where humans have procreated and lived in the past. The Great Wall zone in Northern China is an agricultural zone established by the East Asian Mongoloid in the late Neolithic period. The nomadic lifestyle became more popular due to the dry and cold climate 4000 years ago. Before the Spring and Autumn period (770 B.C.–476 B.C.), the ancient tribes such as the Rong and the Di mentioned in Chinese historical documents, still lived in half agricultural and half pastoral ways. However, the populations in the Great Wall zone were completely changed to the nomadic custom in the late Warring States period (475 B.C.–221B.C.) (Lin, 2003). Whether the change resulted only from culture transmission or population migrations has attracted great interest from archeologists and anthropologists. The ancient Helingeer population was one of these groups living in the mid-late Dongzhou-period, which was an intergrade between the nomadic and agricultural culture. Therefore, the ancient DNA analysis of this population is very important to determine the possible origin of ancient humans and the forming process of the nomadic culture of the Great Wall zone in Northern China.

We investigated the genetic structure of the ancient Helingeer population and the relationships between the ancient tribe and extant populations by analyzing mtDNA polymorphisms of these ancient remains.

2 Materials and methods

2.1 Materials

In this study, intact femur samples from ten ancient individuals were chosen for mitochondrial DNA analysis. Each side of these bone samples was exposed to ultraviolet (UV) light for 45 minutes and a fragment of about 5–6 cm was cut from the bone. Subsequently, 3–4 mm of the outer surface of the bone fragment was removed to eliminate possible surface contamination. The bone fragments were ground under liquid nitrogen with a Planetary Mill Pulverisette 6 (Fritsch, Germany). All fine powders were stored at −20°C. DNA was carefully extracted from the powders using GENECLEAN® Kit for ancient DNA (BIO101, USA).

The mtDNA hypervariable region I (HVR-I) with 364 bp (nucleotide positions; np 16035–16398) was amplified with the use of four overlapping primer pairs (L16035-H16142, L16131-H16281, L16185-H16307, L16281-H16398). The PCR mixture of 12.5 μL contained 2μL ancient DNA extracts, 1 U Taq DNA polymerase (Promega, USA), 50 mM KCl, 2.5 mM $MgCl_2$ (Promega, USA), 0.2 mM dNTP (Promega, USA), 2 mg/mL BSA (Takara, Japan) and 0.2 mM each primer. The reaction was as follows: an initial denaturation at 94°C for 4 min, 36 cycles of 1 min at 94°C, 55 s at 55°C and 1 min at 72°C, followed by a final extension at 72°C for 10 min. PCR products were characterized on 2% agarose gels stained with ethidium bromide and then purified with the use of the QIAEX II Gel Extraction Kit (QIAGEN, German). Cycle sequencing that was carried out with the ABI Prism BigDye Terminator Cycle Sequencing kit 3.1 (Applied Biosystems, USA) including 25 cycles denaturation at 92°C for 30 s, annealing at 55°C for 15 s and elongation at 72°C for 2.5 min. Sequencing reaction was performed using the same primers. The sequences were analyzed by ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, USA), according to the manufacturer's instructions.

2.2 Prevention from exogenous DNA contamination

To eliminate exogenous DNA contamination with modern human DNA, a series of strict procedures were performed during the study of the ancient DNA. The sample preparations, DNA extractions and PCR amplifications were performed in an isolated pre-PCR area that was exclusively dedicated to ancient DNA, with air filter, positive air pressure, overnight UV-light (254 nm) exposure and frequent bench cleaning with DNA-Off™ (Q·BIO gene, USA). To prevent the laboratory from being contaminated, sterile reagents, sterile gloves, face masks, filter pipette tips and other standard precautions of ancient DNA study were adopted. Extraction and amplification controls were incorporated throughout the analysis. To assess the reproducibility and authentication of the results, all samples were extracted at least two times and each extract was amplified more than two times. The mtDNA HVR-I of all lab personnel and archaeologists involved in the project was genetically typed and kept on record for a final comparison with the results to eliminate contamination of the analyzer.

2.3 Genetic analysis

Genetic distance (D_A) values among populations was calculated with the use of the software ARLEQUIN 2.0, based on Tamura/Nei distance matrix, a gamma-parameter value of 0.26 and transition/transversion weight value of 10/1. Neighbor Joining tree (NJ tree) and Multidimensional Scaling (MDS) were performed based on D_A values by using PHYLIP 3.63 (Zhang et al., 2005). Analysis of molecular variance (AMOVA) was performed by using the software ARLEQUIN 2.0.

2.4 Comparing populations

To compare the relationships between the ancient population and extant populations, 14 extant populations were included in the genetic analysis based on the geographical distribution, including East Asian (Northern Han of China, Japanese, Korean), Central Asia (Uighurs, Kazakhs, Uzbek, Turkmen, Kirghiz), North Asian (Buryats, Yakuts, Evenks, Mongols), Turk, and European (Finlander, Basque, Swiss, Britisher).

3 Results

3.1 Mitochondrial DNA polymorphisms

Fragments of mtDNA HVR-I, 364 bp in length (corresponding to nucleotide positions 16035–16398 of the Cambridge reference sequence, CRS) (Anderson et al., 1981), were obtained from a total of 10 individuals from the ancient Helingeer population (Table 1).

Of the 10 successful samples, 10 sequence types, 12 polymorphism sites and 29 substitutions were found. All substitutions were transitions and there were no transversion or insertion/deletion. The majority of the transitions were concerned with pyrimidine, and the percentage of C→T transitions was obvious higher than that of T→C transitions.

3.2 Phylogenetic analysis

To analyze the relationships between the Dongzhou-period ancient population of Helingeer in Inner Mongolian and extant populations, the NJ tree was

Table 1 mtDNA HVR-I sequence variation of 10 ancient individuals

CRS	1	1	1	1	1	1	1	1	1	1	1	1
	6	6	6	6	6	6	6	6	6	6	6	6
	0	1	1	2	2	2	2	2	2	3	3	3
	9	8	9	2	3	5	6	6	9	1	2	6
	3	5	2	3	4	6	0	1	8	9	7	2
	T	C	C	C	C	C	C	C	T	G	C	T
2	.	.	.	T	.	.	.	.	C	.	T	.
3	.	.	.	T	.	.	.	T	.	A	.	C
4	.	T	.	T	.	.	T	.	.	.	.	.
5	C	.	.	T	.	.	.	.	.	.	T	.
7	.	.	.	T	.	T		.	.	.	T	.
9	.	.	.	T	.	.	T	.	.	.	.	.
10	C	.	.	T	.	.	.	.	.	.	.	.
11	.	.	.	T	.	.	.	.	.	.	T	.
12	.	.	.	T	T	.	.	.	.	A	.	C
13	.	.	T	T	.	T	.	.	.	.	.	.

constructed based on the sequences of mtDNA HVR-I region of 14 relative extant populations. The NJ tree (Fig. 1) showed that the 15 populations distinctly belong to three branches according to their geographic distributions, which were North Asian branch (Yakuts, Evenks, ancient Helingeer), East Asian branch (Northern Han of China, Japanese and Korean), Central Asian and European branch (Uighurs, Kirghiz, Kazakhs, Buryats, Turkmen, Uzbeks, Turk and European). The ancient Helingeer population was closer to modern Yakuts, Mongols and Evenks.

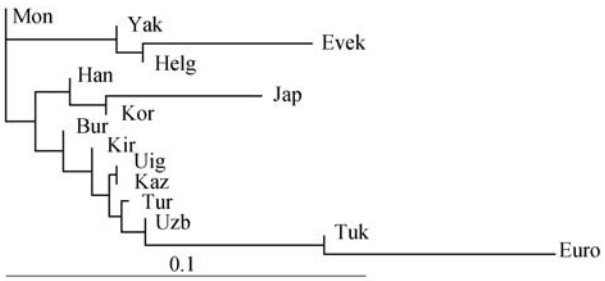

Fig. 1 NJ tree based on genetic distance between 15 populations. Note: Mon (Mongols), Bur (Buryats), Yak (Yakuts), Helg (Helingeer), Evek (Evenks), Han (Northern Han of China), Jap (Japanese), Kor (Korean), Kir (Kirghiz), Kaz (Kazakhs), Uig (Uighurs), Tur (Turkmen; Uzb (Uzbek); Tuk (Turk), Euro (European).

3.3 Multidimensional scaling analysis

MDS can reflect the genetic relationship among the populations by two-dimensional or multi-dimensional plot, based on the D_A values among populations. The two-dimensional MDS plot between the ancient Helingeer population and the 14 related modern populations is shown in Fig. 2, and the distribution of these

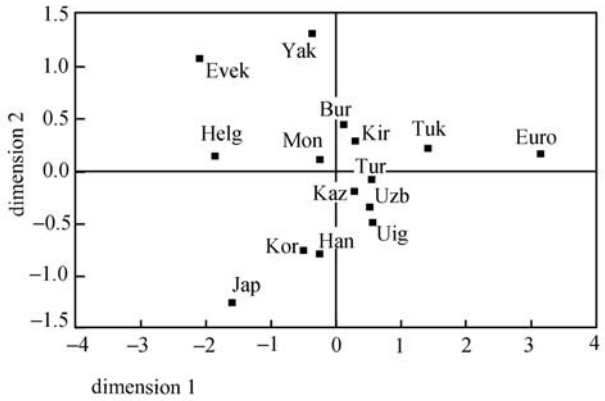

Fig. 2 MDS plot based on genetic distance between 15 populations

populations in MDS were similar to that of the NJ tree. Compared with other modern populations, the ancient Helingeer population was closer to Northern Asian populations.

3.4 Analysis of molecular variance

To analyze the genetic structure of the ancient Helingeer population and the variance between the ancient population and its relative modern populations, 15 modern populations were divided into five groups according to geographical distribution: East Asian group (Northern Han of China, Japanese and Korean), Central Asian group (Uighurs, Kazakhs, Uzbek, Turkmen and Kirghiz), North Asian group (Buryats, Yakuts, Evenks and Mongols), European group (Turk and European) and the ancient Helingeer population. The F_{CT} values (an index to scale the genetic distance among populations) between the ancient population and East Asian, North Asian, Central Asian, European were 0.078, 0.049, 0.108 and 0.291, respectively. A high F_{CT} value indicates long genetic distance, while low F_{CT} value means closer relationship. The result of F_{CT} value showed that the ancient Helingeer population had close relationship with the North Asian group.

4 Discussion

Geneticists, archaeologists and anthropologists pay attention to the applications of ancient DNA, including the study of human origins, evolution, migration and admixture etc. Meanwhile, the authentication of the results is more important. The elimination of extraneous DNA is of paramount importance in the study of ancient DNA, where a series of strict procedures are performed. In our study, the negative result of the extraction and amplification control, and the consensus sequences of two independent extracts from the same sample indicated

authentic results. In addition, the DNA of all laboratory personnel and archaeologists involved in the project was genetically typed and found to be absent from all sequence results.

No identical sequences are found from the ten Helingeer individuals, which showed that these samples have no familial genetic relationship. The oldest Asian C→T mutation at np 16223 are found in all Helingeer samples (Kivisild et al., 2002). Among these samples, four individuals have the C→T mutations at np 16327, which were the characteristic motif of the mtDNA haplogroup C. Haplogroup C is especially concentrated in North Asian populations at about 15%–38% frequency (Pakendorf et al., 2003; Schurr et al., 1999). This indicates that the ancient Helingeer population perhaps has maternal genetic relationship with the North Asian populations.

Here, to analyze the genetic relationship between the ancient Helingeer population and other modern populations, 14 related modern populations were selected for phylogenetic analysis, multidimensional scaling analysis and analysis of molecular variance. All results showed that the Dongzhou-period ancient Helingeer population had close maternal genetic relationship with North Asian populations.

In addition, it was found that the Buryats was located near the Central Asian in the NJ tree and the MDS plot, although the archaeological site of the Buryats was located in the North Asian. The modern Buryats population was mixed by Mongolian-speaking tribes and Turki-speaking people (Pakendorf et al., 2003). According to the historical records, the Central Asian Uighurs, Kazakhs, Uzbek and Kirghiz all belong to the Turkic-speaking tribes of the Altaic linguistic family, and the Uighurs and Uzbek are the descendant of different Turkic-speaking tribes (Yang and Ding, 2003). All these Central Asian tribes had been affected by the Mongolian group in history, meanwhile, the Mongols was also affected by the Turki-speaking peoples. In our study, the result indicated that the Buryats was close to the Mongols and the Central Asian, which was consistent with the historical records. This provides additional evidence for the close relationship between the Helingeer population and the North Asian populations.

The anthropology analysis has shown that the appearance of the skulls of the Dongzhou-period ancient Heligeer people is similar to that of the North Asian type of the modern Mongoloid. The isotopic composition of dietary and funerary objects, etc., indicate that the Dongzhou-period ancient Helingeer people have developed a pastoral economy and nomadic culture.

In conclusion, our analysis showed that the ancient Helingeer population was nomadic and immigrated to the modern Mongolia from the ancient Mongolia plateau and the Baikal Lake region, based on molecular biology, anthropology and archaeology studies. The southward migration of the nomads from the ancient Mongolia plateau and the Baikal Lake region perhaps was caused by the degeneration of the meadow and the cold weather of the Dongzhou-period. This southward migration brought the nomadic culture to the farming border zone, and played an important role in the formation of the nomadic culture of the Great Wall Zone in Northern China. Furthermore, the result provided a molecular biology evidence for the formation of the nomadic culture of the Great Wall in Northern China. However, there is a demand for a study based on more ancient samples to confirm the migration of the populations in the north Great Wall zone of China.

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