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Ethnic affinities of the ancient human Jety-Asar population by mitochondrial DNA analysis

An anthropological study of the remains has indicated uniformity of the ancient human Jety-Asar population (Central Asia) and suggests a mixed Euro-Mongoloid genesis. DNA was extracted from teeth from three Caucasoid skulls recovered from a burial site dated at approximately 2000 years ago. Ancient mitochondrial DNA (mtDNA) was analysed by restriction fragment length polymorphism (RFLP) analysis for the A, B, C and D haplogroups and the sequencing of hypervariable region I of the mtDNA control region. The full set of mtDNA control region variants determined for Jety-Asar specimens was only found among a modern Mongolian population (Mongoloid people), indicating some discordance of molecular and morphological data.

Keywords: Ancient mitochondrial DNA / Nomads / Jety-Asar population EL 3482

The eastern Aral region in Central Asia is important for human evolution and the study of history because the area was regularly inhabited from the lower Paleolithic and was the crossing point of migration routes of ancient nomad tribes and of permanent contacts between the nomads of the Eurasian steppe and the agriculturists of Middle Asia. Archaeological artefacts and ancient texts (Pompejus Trogus) lead us to believe that the ancient people who lived in the low valley of the Syr-Darya river in southern Kazakhstan were known as the Saka. The Jety-Asar archaeological culture which existed in the eastern Aral region (Fig. 1) from the 5th century before Christ (B.C.) until the 8th century *anno Domini* (A.D.) had a mixed cattle-breeding, hunting, agricultural and fishing economy without visible traces of social differentiation. This civilization played an important role in the formation of the ancient and early medieval Eurasian nomads [1]. The people of the Jety-Asar culture left a great number of fortified settlements with towers and a sophisticated irrigation system. The Jety-Asar burial sites were excavated by the Khoresm expedition from the Institute of Ethnology and Anthropology (Russian Academy of Sciences) in 1988 and 1990. Most of the burials had taken place between the 3rd century B.C. and the first century A.D.

Migrations of nomad tribes from Asia to Europe in the steppe belt were common during this period. Because of its geographic location and long survival, the Jety-Asar culture is of particular significance as a focus of ethnic, trade and cultural contacts between nomadic tribes and agriculturists. Morphological analysis of the remains of

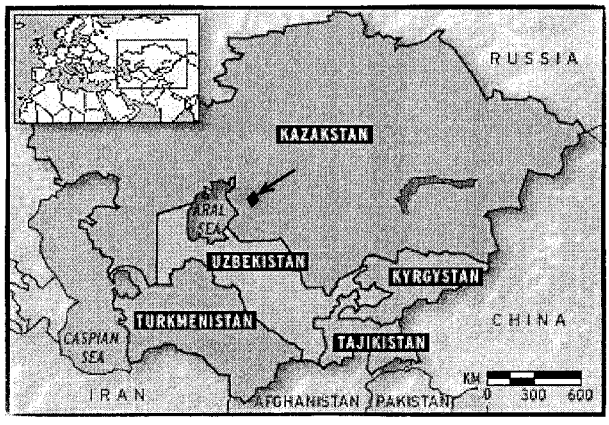

Figure 1. Map showing the location of the Jety-Asar archaeological culture in southern Kazakhstan (◆ and arrow).

the Jety-Asar people, primarily of their skulls and teeth, reveals a number of traits characteristic of the ancient peoples of Southern Europe, Central Asia and Ural [1]. The implication is that the Jety-Asar are of mixed Euro-Mongoloid origin. The most specific feature of the Jety-Asar culture is the widespread artificial deformation of the head. The comparative cranial analysis with other Eurasian steppe populations has shown some resemblance, but has not allowed any ethnic relationships to be clearly detected [2]. Little accurate data exist on the numerous nomad tribes of the Eurasian steppe belt and their interrelationships. Thus, ethnic identification of the ancient Jety-Asar population, which existed approximately thirteen centuries in the central part of the migration corridor, has become one of the most important problems of Central Asian archaeology.

The aim of this study was to determine the ethnic origin of one of the ancient nomad populations within the Central Asian region and steppe belt of Eurasia using mitochon-

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drial DNA (mtDNA) analysis. Three Caucasoid skulls from a burial site dated at approximately 2000 years old, at which time several migration events took place in this region [2], were available and sufficiently well-preserved to undertake molecular genetic analysis. DNA was extracted from well-preserved molars without caries and cracks from each of these skulls to minimize the possible modern DNA contamination. Teeth were first soaked in 10% bleach for 20 min, rinsed with ethanol and distilled water, and then UV-irradiated for 30 min on each side. Each tooth was powdered in a coffee grinder. We used a DNA extraction method excluding the silica-based stage for two of the samples [3]. All manipulations for protection from modern DNA contamination were as described previously for routine ancient DNA work [4].

Ancient mtDNA was analysed by restriction fragment length polymorphism (RFLP) analysis for the A, B, C, and D haplogroups [5, 6] and the sequencing of hypervariable region I (HVRI) of the mtDNA control region between L16,208 and H16,401. PCRs were successful and all analyses were duplicated for all three samples. The primers used were designed according to Dr. D. A. Merriwether (personal communication), Handt *et al.* [7], and Vigilant *et al.* [8] to amplify specific segments of mtDNA less than 230 bp. Forty cycles of PCR were carried out with *Taq* polymerase (Promega, Madison, WI, USA). The blank DNA extraction and PCR controls consistently gave negative results. Restriction digestions were carried out following the manufacturer's recommendations (Gibco-BRL, Grand Island, NJ, USA). Magnetic beads (Dynal) were used for the separation and purification of the biotinylated strand of the amplified DNA, followed by direct sequencing with T7 Sequenase Version 2.0 DNA sequencing kit (Amersham, Oakville, ON, Canada) in accordance with the manufacturer's manual.

The DNA typing results of this study were compared with data from the following populations: various European samples [9–13], Finnish and Saami [14], Bulgarian [15], Turkish [12, 15], Druze and Adygei [16], Mongolian [17], Uyghurs [18], lowland and highland Kirghiz [18], Kazakhs [18], Taiwanese Han [19], Koreans [19], Ainu [19], Ryukyuans [19], modern non-Ainu Japanese [20], 700-year-old Oneota [4], Native American [6], Indian [21], Yanomami [22], Native American and European belonging to putative haplogroup X [23], ancient desiccated corpses from Xinjiang [24], 2000-year-old Japanese [20], and 2000-year-old Chinese [25].

The results of RFLP and sequencing are shown in Table 1. The well-established four mtDNA haplogroups (A, B, C, and D) were not observed among the samples typed. Nevertheless, the *Alu*I 5176 site loss that is characteristic of haplogroup D was found in tooth 2, but in an unusual combination with the *Dde*I 10394 and *Alu*I 10397 site loss. The same RFLP variant is found in 1 of 51 Turkmen (I. Ovchinnikov, unpublished data) and in 5 of 103 Mongols [17]. MtDNA from this sample contains 16233 C→T transition. This is one of three characteristic control region polymorphisms associated with haplogroup D. Previously it was shown that in the Mongolian population none of the haplotypes carried all three control region polymorphisms for haplogroup D and this RFLP variant was considered to be haplotype D2 found in the Old and New World [17].

Three sequences of HVRI of the mtDNA control region were different without any ambiguities. Individual 1 had one substitution: a T to C change at 16362. Individual 2 had three substitutions: a C to T change at 16223, a C to A change at 16257, and a C to T change at 16261. Individual 3 with the *Alu*I 5176 site loss had three substitutions: a C to T change at 16223, a T to C change at 16311, and a T to C change at 16357. These haplotypes are shared with some modern human populations (Table 2).

We did not find any mtDNA haplotypes specific for the Europeans [16] despite the obvious Caucasoid characteristics of three ancient individuals. One of the typed haplo-

Table 1. Restriction sites and polymorphic nucleotides, numbered according to [26], identified for the Jety-Asar samples

Sample	*Hae*III	*Alu*I	9 bp	*Dde*I	*Alu*I	*Hin*cII	*Hae*III	16223C	16257C	16261C	16311T	16357T	16362C
	663	5176	8272/89	10394	10397	13259	16517						
1	0	1	2	0	0	1	1	•	•	•	•	•	C
2	0	1	2	0	0	1	0	T	A	T	•	•	•
3	0	0	2	0	0	1	1	T	•	•	C	C	•

Presence and absence of a restriction site are indicated by 1 or 0, respectively. Two copies of the 9 bp repeat are indicated by 2. A dot (•) indicates identity with the reference sequence [26].

Table 2. Distribution of Jety-Asar haplotypes among modern human populations

Ancient individual	Sharing populations (No. of individuals shared from common number of analyzed samples)
1	Caucasian (2 of 100), German (3 of 200), Danish (1 of 33), Cornwall (2 of 69), Alava and Viscaya (3 of 61), Western Turkey (1 of 22), Turkish (1 of 29), Druze (2 of 45), Mongolian (2 of 103), Uyghurs (2 of 55), lowland Kirghiz (1 of 48), ancient Japanese (3 of 14), modern non-Ainu Japanese (8 of 26)
2	Mongolian (2 of 103), lowland Kirghiz (2 of 48), highland Kirghiz (1 of 47)
3	German (1 of 200), Mongolian (2 of 103)

types from the Jety-Asar samples is only distributed among the Mongoloid populations of Central Asia, based on HVRI motifs [17, 18]. Two samples have a 16223 C→T substitution, which occurs in a small number of Europeans (7%) [12] and a significant proportion of Mongoloid populations including the Mongolian (65%) [17], Kazakh (50.9%), highland Kirghiz (48.9%), lowland Kirghiz (68.8%), and Uyghur (41.8%) [18] haplotypes. Also, the 16362 T→C substitution, which was found in individual 1, occurs with highest frequency in Central Asian populations (40% in Mongols [17], 32.7% in Kazakhs, 29.8% in highland Kirghiz, 43.8% in lowland Kirghiz, and 32.7% in Uyghurs [18]) in comparison with the low frequency in Europe and the Middle East, from 0 to 5.1% in different studies [9–13, 15].

The discovery of West and East Eurasian haplotypes in the Jety-Asar population emphasizes their affinities with several modern populations that live in neighbouring areas of Mongolia [17], Kirghizstan, and Kazakhstan [18]. These data are particularly notable given the pronounced Caucasoid morphological traits of the ancient skulls.

We conclude tentatively that the east-west genetic exchange in this human population must date back many thousands of years B.C. Our preliminary data imply an ancient mingling of Euro-Asian populations, and detailed analyses of a wide range of ancient nomads will undoubtedly provide a more complete picture of the history of human migrations in this area.

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