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E-mail: Eurasia@archaeology.nsc.ru

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ANTHROPOLOGY AND PALEOGENETICS

A.S. Pilipenko[1, 2], R.O. Trapezov[2], and N.V. Polosmak[1]
[1]Institute of Archaeology and Ethnography, Siberian Branch, Russian Academy of Sciences, Pr. Akademika Lavrentieva 17, Novosibirsk, 630090, Russia
E-mail: polosmaknatalia@gmail.com
[2]Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Pr. Akademika Lavrentieva 10, Novosibirsk, 630090, Russia
E-mail: alexpil@bionet.nsc.ru; rtrapezov@yandex.ru

A GENETIC ANALYSIS OF HUMAN REMAINS FROM AK-ALAKHA-3 BURIAL MOUND 1, GORNY ALTAI*

A genetic analysis of human remains from burial 1 in mound 1 at Ak-Alakha-3, Gorny Altai, focused on mitochondrial DNA, sex markers, and autosomal hypervariable STR markers. Variants of mtDNA extracted from the remains of an adult individual and a child fall into Eastern Eurasian haplogroups A4 and C, respectively, which are common in modern and prehistoric populations of Gorny Altai and the adjacent regions of southern Siberia and Central Asia. These variants must be considered autochthonous in the gene pool of the Early Iron Age Altai, and were shared by otherwise dissimilar populations of that region in the Scythian Age. The adult individual was shown to be male, and the child was a girl. The results corroborate the efficiency of aDNA testing using the well preserved cancellous bone samples.

Keywords: *Paleogenetics, ancient DNA, mitochondrial DNA, sex markers, Gorny Altai, Pazyryk culture, Kara-Koba burials.*

Introduction

Ak-Alakha-3 mound 1, studied at the Ukok Plateau, Gorny Altai under the leadership of N.V. Polosmak in 1993 (Polosmak, 2001: 62–67; 2013), contained two burials. Mummified female remains, and an abundant accompanying inventory nicely preserved under the permafrost conditions, were discovered in intact "frozen" (lower) burial 2. The burial was performed in accordance with the Pazyryk culture canons. (Upper) burial 1, located directly on the roof of the Pazyryk burial chamber, contained remains of two individuals: a 25–30 year-old adult man and a 9–10 year-old child. The burial was performed in accordance with the so called Kara-Koba funeral traditions. A detailed description and anthropological characteristics of the buried individuals have been published (Chikisheva, Polosmak, Zubova, 2015). The data obtained in the course of the excavations are indicative of simultaneous construction of both burials and their interrelationship. Burial 1 was looted in ancient times, with the result that the remains of buried individuals were disturbed. A nearly complete skeleton of an adult male has become available to the

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researchers, though its lower part was found beyond the burial chamber boundaries. Considering that only fragments of parietal and temporal bones of the skull, one lower molar, and several small fragments of the postcranial skeleton (the latter were transferred for paleogenetic research) have been identified in the course of excavations and subsequent office control of paleoanthropological materials, the remains of the child had presumably been withdrawn from the burial by looters. In view of the close attention given to the burial complex of Ak-Alakha-3 mound 1 by specialists of various research areas, who previously focused on studying the materials of "frozen" burial 2 (among the latest publications, see (Letyagin, Savelov, Polosmak, 2014)), the comprehensive analysis of the remains of two individuals found in burial 1 of this mound seems to be topical. The present article sets out the results of their genetic testing.

Materials and methods

Paleoanthropological samples. A humerus of the adult male (skeleton 1), and small fragments of the postcranial skeleton bones of the child (skeleton 2) which contained mainly cancellous bone tissue partially covered by thin plates of the compact bone tissue, were used as material for DNA extraction.

Pre-treatment of paleoanthropological material and DNA extraction were performed as described previously (Pilipenko et al., 2010; Pilipenko, Molodin, Romaschenko, 2011a; Molodin et al., 2012). The surface of the adult male's humerus was treated with bleach (5 % solution) to destroy possible contamination by modern DNA. Then the external layer, approximately 1–2 mm thick, was mechanically removed, and the sample was irradiated with UV light for at least one hour. Fine powder was drilled from the internal layer of compact bone tissue.

Surface layers (a thin plate of compact bone tissue and underlying layers of cancellous bone tissue) were removed from the fragments of child's cancellous bones. The obtained fragments containing deep layers of cancellous bone tissue were kept in 5 % solution of bleach for approximately 5 minutes, following which they were thoroughly washed with a large amount of purified water, put into 96 % ethyl alcohol, dried, and ground to a fine powder using a mill.

DNA was extracted by incubating the bone powder with a 5M guanidinium thiocyanate buffer at 65 °C and continuously stirring with the use of a thermal shaker for 36–48 hours (Pilipenko et al., 2010), followed by phenol-chloroform extraction. The DNA was precipitated from the aqueous phase with isopropanol.

Mitochondrial DNA sequence analysis. The amplification of hypervariable region (HVR) I of the mitochondrial (mt) DNA was performed using two different techniques: amplification of four short overlapping fragments in one round of PCR (Haak et al., 2005) and amplification of one long fragment by nested PCR (consisted of two reaction rounds) (Pilipenko et al., 2008). DNA sequencing was performed with an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA). Sequencing reaction products were analyzed with an ABI Prism 3100XL Genetic Analyzer automatic capillary sequencer (Applied Biosystems, USA) at the Center for Collective Use of Automatic DNA Sequencing of the SB RAS (Novosibirsk).

The obtained sequences were compared with the revised Cambridge Reference Sequence (rCRS) for human mitochondrial DNA (Andrews et al., 1999). Phylogenetic interpretation of sequences was performed on the basis of the current classification of mitochondrial DNA variability (van Oven, Kayser, 2009) (http://www.phylotree.org). The obtained results were additionally verified using the HaploGrep software tool (Kloss-Brandstatter et al., 2011) (http://haplogrep.uibk.ac.at/). Phylogeographic analysis of the obtained results was carried out using literature data on mtDNA HVR I variability in modern populations of Eurasia, which consist of more than 25 thousand samples.

Profiling of STR loci and sex identification of the remains being studied were conducted using an AmpFlSTR® Profiler® Plus PCR Amplification Kit (Applied Biosystems, USA) according to the manufacturer's instructions.

Precautions against contamination and verification of results. All stages of work with ancient materials were conducted in laboratory rooms specially equipped for paleogenetic research. All laboratory staff used special workwear suits for the clean-rooms. All work surfaces and equipment were routinely cleaned with a 5 % solution of bleach and irradiated by UV light. Ancient samples and tubes containing blank controls (without adding the paleomaterials) underwent DNA extraction and amplification procedures simultaneously in order to identify possible contamination of reagents and equipment used. Three independent DNA extractions were conducted for each individual. Amplification was conducted several times for each extract. The mtDNA HVR I was sequenced for all paleogenetic laboratory staff who had access to the clean rooms, in order to identify possible contamination of ancient materials.