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Six complete mitochondrial genomes from Early Bronze Age humans in the North Caucasus

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ABSTRACT

The North Caucasus region is rich in early Bronze Age sites, with burials yielding many artifacts, including those from the Chekon, Natukhaevskaya, Katusvina-Krivitsa kurgan groups (at Krasnodar Krai, Russia) and Klady kurgan (near Novosvobodnaya Village, Republic of Adygea, Russia). According to the mainstream archaeological hypothesis, these sites belong to the Maikop culture (3700–3000 years BC), with Novosvobodnaya communities representing an offshoot of Maikop ancestry. However, due to specific differences in Novosvobodnaya artifacts, the Maikop and Novosvobodnaya assemblages could represent two synchronous archaeological cultures living in almost sympatry but showing independent ancestry, from the Near East and Europe respectively. Here, we used target-enrichment together with high-throughput sequencing to characterize the complete mitochondrial sequence of three Maikop and three Novosvobodnaya individuals. We identified T2b, N1b1 and V7 haplogroups, all widely spread in Neolithic Europe. In addition, we identified the Paleolithic Eurasian U8b1a2 and M52 haplogroups, which are frequent in modern South Asia, particularly in modern India. Our data provide a deeper understanding of the diversity of Early Bronze Age North Caucasus communities and hypotheses of its origin. Analyzing non-human sequencing reads for microbial content, we found that one individual from the Klady kurgan was infected by the pathogen *Brucella abortus* that is responsible for zoonotic infections from cattle to humans. This finding is in agreement with Maikop/Novosvobodnaya livestock groups, mostly consisting of domestic pigs and cattle. This paper represents a first mitochondrial genome analysis of Maikop/Novosvobodnaya culture as well as the earliest brucellosis case in archaeological humans.

1. Introduction

The Neolithic period and the beginning of Bronze Age represent an essential transitional period in the history of Europe colonization. Based on the archaeological data, there are several controversial hypotheses that describe Europe colonization. However, there are only few genetic/genomics studies of ethnical characteristics of Neolithic and Bronze Age human populations that can provide an additional source of information on the migration routes of European ancestors (Bramanti et al., 2009; Brandt et al., 2013; Chikhi et al., 1998; Haak et al., 2005; Skoglund et al., 2012). Moreover, recent study attributed most of present-day Europeans to at least three highly differentiated populations: west European hunter-gatherers, ancient north Eurasians related to Upper Palaeolithic Siberians and early Middle East farmers who contributed to agriculture origin in Europe (Lazaridis et al., 2014).

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To date, several studies about Europeans from Bronze Age were published but data about genetic diversity and mitochondrial haplotype presence of Caucasus Bronze Age populations are unknown (Allentoft et al., 2015; Haak et al., 2015; Gamba et al., 2014). The Maikop culture was a main archaeological culture in the North Caucasus in the Early Bronze Age. It had several development stages and spanned the period of 3700–3000 years BC. First Maikop culture kurgans were excavated by Nikolay Veselovsky in 1897 near Maikop City (Republic of Adygea, Russia) (Rezepkin, 2012). Since then, abundant archaeological material was found near Maikop City and the Caucasus and Black Sea Region (Fig. 1).

The mainstream archaeological view suggests the presence of only one Maikop culture/community in this area, which had Near Eastern cultural ancestry (Iessen, 1950; Munchaev, 1975). At the same time, there is another hypothesis about the Western-European origin of the part of the Early Bronze Age Caucasus community based on the stratigraphic disposition of tombs in kurgans and artifacts found near Novosvobodnaya site (Republic of Adygea, Russia). Unlike the mainstream one, this hypothesis suggests the presence of the European cultural and potentially genetic flow to the North Caucasus at the turn of the Neolithic and Bronze Age. Moreover, the Novosvobodnaya tombs could be the eastern wing in the development of the north central European gallery graves, which include the Funnel Beaker (TRB) culture (Rezepkin, 2012).

In the past few years, the methods for ancient DNA extraction and analysis from archaeological material have been developed to allow retrieval of genomic information from a variety of sample types: hairs (Miller et al., 2008; Rasmussen et al., 2011), mummified tissue (Keller et al., 2012), calcified bones and teeth (Allentoft et al., 2012; Skoglund et al., 2012), and plant remains (Martin et al., 2013; Yoshida et al., 2013).

Moreover, millions or even billions of DNA sequences may be derived from ancient biological samples due to massive capacity of modern platforms for Next-Generation Sequencing (NGS) (Skryabin et al., 2009). Methods of modern genomics can be successfully applied to archaeological problems. The riddle of human migration in Europe at the turn of the Neolithic and Bronze Ages has become particularly attractive for archaeological genetics (Deguilloux et al., 2011; Haak et al., 2005; Izagirre and de la Rua, 1999; Skoglund et al., 2012; Sykes, 1999).

In the previous investigation, we applied the NGS approach to study ~5000-year-old human remains from the Klady kurgan grave (Novosvobodnaya site). Sequencing of the mitochondrial (mt) DNA with 13.4x coverage enabled us to determine the mtDNA haplogroup for that individual as V7 (Nedoluzhko et al., 2014). In the current study, we aimed at investigating the Early Bronze Age ancient communities from the North Caucasus and establish their origins and development by combining archaeological and genetic data. Here, we present the pilot ancient mitochondrial DNA analysis of 6 individuals, who lived in the piedmont area of the North Caucasus at approximately 3700 to 3000 years BC. Further investigation may require a power of hundreds ancient mt genomes as well as nuclear genomic data to support the archaeological hypotheses of Europe colonization.

Ancient remains of our ancestors provide information about the *Homo sapiens* origin, migration history and even diseases. For instance, deep sequencing of ancient strains of tuberculosis and plaque revealed important pieces of information about the origin of European epidemics throughout the centuries (Dabernat et al., 2014; Mutolo et al., 2012; Nguyen-Hieu et al., 2010; Wagner et al., 2014). However, molecular identification of historic pathogens can be complicated by limited information in public genetic databases and diversity of soil nonpathogenic contaminants that leads to false positive results in genetic analysis (Campana et al., 2014).

Brucellosis is an important livestock and human disease in many parts of the world. According to the WHO reports, brucellosis keeps patients from normal activity, thus, making it one of the major economic and medical problems in developing countries.

Based on several studies, *Bruccella*-induced diseases were common in Antiquity (Bendrey et al., 2008; Capasso, 1999; Kousoulis et al., 2012; Papagrigorakis et al., 2006; Shapiro et al., 2006) and the Middle Ages (Isidro, 2009; Mutolo et al., 2012). For the first time, we report a case of *Brucella abortus* in ancient human remains from the early Bronze Age North Caucasus using the ancient DNA analysis. This finding suggests that brucellosis affected early farmers in Europe, at least in the Caucasus.

2. Material and methods

2.1. Samples

Ten ancient human bones and teeth were collected for analysis. Human bones from the burials of the Maikop and Novosvobodnaya sites were used for ancient DNA (aDNA) analysis (which were only successful for five specimens, see below) (Supplementary 1).

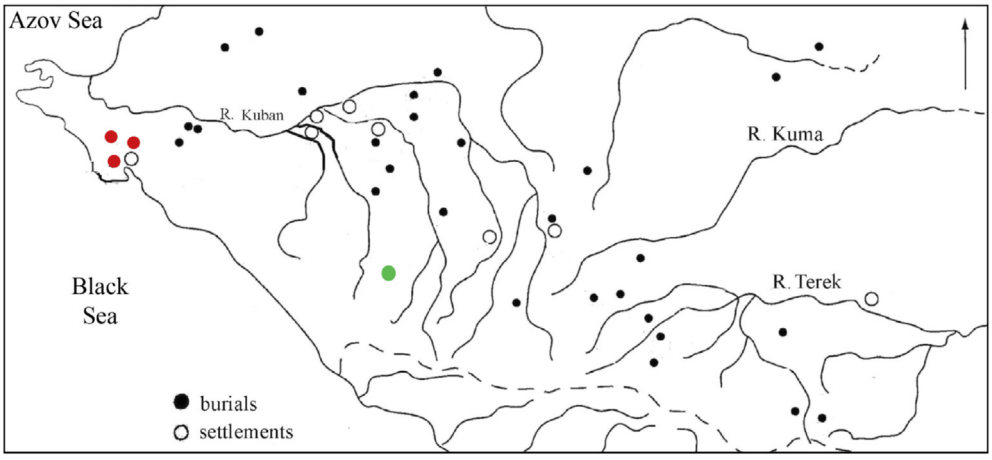

Fig. 1. The North Caucasus Early Bronze Age significant archaeological sites (modified after Rezepkin, 2012). Red circles – Maikop culture burials (Natukhayevskaya Village, Katusvina Krivitsa-2 and Chekon), specimens from those were used in this study, black circles – other Maikop culture burials, white circles – Maikop culture settlements, green circle – Klady burials (Novosvobodnaya settlement), specimens from those were used in this study. (https://www.google.com/maps/d/viewer?mid=zGhBH1rfe2eE.kfpObKz6NZr0).