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Mitochondrial and Y-chromosome diversity of the prehistoric Koban culture of the North Caucasus

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ABSTRACT

The Koban archaeological culture is a well-known Northern and Central Caucasus culture that has been widely distributed throughout this region during the end of Bronze Age, and the beginning of the Iron Age. Named after the Koban cemetery (Republic of North Ossetia, Russia), it had highly developed agriculture and metallurgy. The Koban culture had been dramatically transformed under the influence of Scythian invasions and left a significant cultural legacy, including a number of historical puzzles. One of them is related to the origin, development, and ancestry of Koban culture due to significantly different opinions on the matter.

Here, we characterize, using Sanger and high-throughput sequencing, the mitochondrial and Y-chromosomal diversity of Koban culture individuals, whose remains were excavated at the Zayukovo-3 and Klin-Yar 3 cemeteries in the North Caucasus.

In this study we provide a new data for better understanding of the origin and genetic diversity of the North Caucasus communities during the Bronze and Iron Ages and show that the Koban archaeological culture has genetic continuity with other ancient cultures of the Caucasus.

1. Introduction

DNA analysis of individuals of ancient archaeological cultures becomes a routine investigation that helps to describe human ancestry and cultural origins in Eurasia at the turn of the Neolithic, Bronze, and Iron Ages (Nedoluzhko et al., 2014; Sokolov et al., 2016; Juras et al., 2017; Nikitin et al., 2017; Pilipenko et al., 2018).

In recent years, a significant amount of new information has been generated using genomic analysis of various ethnic groups, both modern and ancient (Nielsen et al., 2017; Triska et al., 2017). From this point of view, it seems very relevant to compile ancient and modern genetic portraits of the North Caucasus population, that known as one of the most ethnically diverse in the world. Recently, significant success has been achieved in ancient population-wide studies of the foothills and steppes of the Caucasus in the Early and Middle Bronze Age (Wang et al., 2019); the first information on the Caucasian population of the Iron and the Middle Ages against a broad Eurasian background (Damgaard et al., 2018) was also obtained. However, numerous palaeoanthropological materials of vivid archaeological cultures that have had a significant impact on the formation of the modern ethnic composition of the North Caucasus population remain unexplored.

The Koban culture of the Late Bronze and the Early Iron Ages (the

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end of the 2nd - the middle of the 1st millennium BC) is of significant interest, since it created a “bridge” between the ancient and modern times and, according to many experts, served as the basis for the formation of modern autochthonous peoples of the Caucasus (Kozenkova, 1989). Named after the Koban cemetery in the North Ossetia Republic (Russia), the Koban culture was widespread on both sides of the Great Caucasus Range during the 13th/12th – 4th centuries BC. It is widely known because of its advanced metallurgy as well as developed terraced agriculture (Korobov and Borisov, 2013). However, the genetic origin and diversity of the Koban population have not been explored to date.

Mitochondrial and Y-chromosomal DNA sequencing enabled analysis of the maternal and paternal haplogroups in the present study, aimed at investigating the origin and development of the Koban culture by combining archaeological and genetic data. Here, we present the preliminary ancient DNA analysis (mitochondrial and Y-chromosome) of human remains from the Klin-Yar 3 and Zayukovo-3 cemeteries providing new paleogenetic data of Koban culture representatives in the North Caucasus. Sanger and next-generation sequencing approaches were applied to study human remains from the Koban archaeological culture.

The people of the Koban culture had a mtDNA pool that included haplogroups and subgroups previously described in the European Paleolithic and Neolithic archaeological cultures. The same statement applies to the majority of described Y-chromosomal haplogroups; however, one of them (D1a2a1) was common in Eastern Asian ancient communities (Gayden et al., 2007; Wang et al., 2014). This specimen origin will be studied in the future by whole nuclear genome sequencing.

We suppose that further investigations, including additional archaeological fieldwork and new studies of ancient mitochondrial/nuclear genomes for the Koban, Sarmatian-Alanic, and other ancient cultures of the Caucasus compared with modern ones, will shed light on their ancestry, history, and diversity.

2. Materials and methods

2.1. Samples

The materials used in the study were obtained during archaeological excavations of Koban culture burials, found at two cemeteries (Fig. 1). Fourteen Koban culture ancient human remains were collected and then used for ancient DNA (aDNA) analysis.

Five burials of the Klin-Yar 3 burial ground in the vicinity of modern Kislovodsk in the Stavropol Krai were excavated in 1994–1996 by Andrey Belinskij (“Nasledie” Cultural Heritage Unit, Stavropol, Russia) and Heinrich Härke (then University of Reading, Reading, UK) and included in the recently published monograph (Belinskij and Härke, 2018).

The complex of archaeological sites near the village of Zayukovo, Baksan district of the Kabardino-Balkar Republic (Russia) includes the burial ground of Zayukovo-3 (8th – 5th centuries BC) which has been systematically studied since 2014 by the joint expedition of the State Historical Museum (Anna Kadieva) and the Institute of Archaeology, Russian Academy of Sciences (Sergey Demidenko). To date, more than 80 burials have been excavated, with at least 50 of them belonging to the Koban archaeological culture (Kadieva and Demidenko, 2017). The dating of the burials was based on the typological analysis of cultural artifacts in graves. Detailed information about the studied specimens and their origin is presented in Table 1.

2.2. DNA extraction and sequencing

Genetic material was extracted from the bones and teeth in the aDNA facilities of the National Research Center “Kurchatov Institute” (Moscow, Russia), as previously described (Orlando et al., 2013).

This method includes several steps:

- Bones and teeth drilling for powder;
- DNA extraction in buffer containing EDTA and proteinase K;
- DNA enrichment on silica beads in binding buffer, which contains guanidine thiocyanate, tris(hydroxymethyl)aminomethane (TRIS), sodium acetate and sodium chloride;
- DNA washing in ethanol;
- DNA elution using low-salt buffer.

D-loop region sequencing was used for mitochondrial DNA (mtDNA) haplogroup identification. We used several primers pairs to amplify the parts of D-loop region that were previously designed by Sampietro with colleagues (Sampietro et al., 2005). Mitochondrial hypervariable region

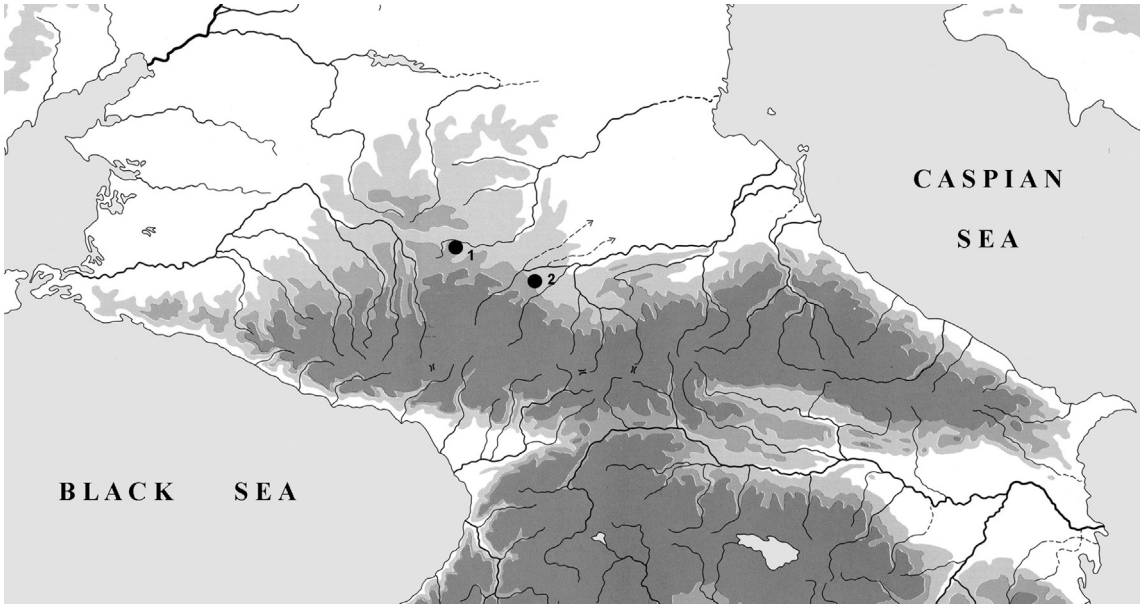

Fig. 1. Geographical map of the Caucasus showing the archaeological sites (black circles) where human samples were collected for the ancient DNA analysis: 1 – Klin-Yar-3, and 2 – Zayukovo-3.

Table 1
Detailed information on Koban/Sarmatian culture samples used in ancient DNA analysis. The human remains had been deposited in the collections of (IA) – Institute of Archaeology, Russian Academy of Sciences (Moscow, Russia), and (RM) – Rostov Regional Museum of Local History (Rostov-on-Don, Russia).

#	Burial site	Excavation ID	Sex	Age	Culture	Century (BC/AD)	Excavations by	Collection
1	Klin-Yar 3	353	F	25–29	Koban	IX-VII BC	Andrey Belinskij/Heinrich Härke	IA
2	Klin-Yar 3	355	M	40–49	Koban	IX-VII BC	Andrey Belinskij/Heinrich Härke	IA
3	Klin-Yar 3	375	N/A	8–10	Koban	IX-VII BC	Andrey Belinskij/Heinrich Härke	IA
4	Klin-Yar 3	376	N/A	15–19	Koban	IX-VII BC	Andrey Belinskij/Heinrich Härke	IA
5	Klin-Yar 3	377	N/A	9–10	Koban	IX-VII BC	Andrey Belinskij/Heinrich Härke	IA
6	Zayukovo-3	71	N/A		Koban	VIII-VII BC	Anna Kadieva/Sergej Demidenko	RM
7	Zayukovo-3	72	N/A		Koban	V BC	Anna Kadieva/Sergej Demidenko	RM
8	Zayukovo-3	79	N/A		Koban	VIII-VII BC	Anna Kadieva/Sergej Demidenko	RM
9	Zayukovo-3	80	N/A		Koban	VIII-VII BC	Anna Kadieva/Sergej Demidenko	RM
10	Zayukovo-3	81	N/A		Koban	VIII-VII BC	Anna Kadieva/Sergej Demidenko	RM
11	Zayukovo-3	82/1	N/A		Koban	VI-V BC	Anna Kadieva/Sergej Demidenko	RM
12	Zayukovo-3	82	N/A		Koban	VI-V BC	Anna Kadieva/Sergej Demidenko	RM
13	Zayukovo-3	91	N/A		Sarmatian	II-III AD	Anna Kadieva/Sergej Demidenko	RM
14	Zayukovo-3	95	N/A		Koban	VIII-VI BC	Anna Kadieva/Sergej Demidenko	RM
15	Zayukovo-3	105	N/A		Koban	VI-V BC	Anna Kadieva/Sergej Demidenko	RM

1 (HVR1) primer pairs and their sequences are presented in Supplementary Table S1. PCR fragments (138–210 base pairs (bp) in length) covering the mtDNA D-loop were sequenced using the ABI 3730xl platform (Thermo Fisher Scientific, USA).

Ovation® Ultralow Library System V2 (NuGEN, USA) kit was used for DNA-libraries preparation. These libraries were quantified using a high-sensitivity chip on a 2100 Bioanalyser instrument (Agilent, USA) and sequenced on the Illumina Novaseq6000 system (Illumina, USA) using paired-end reads (150 bp length). The Illumina sequencing data was combined with Sanger sequencing results for validation (mtDNA-haplogroups) and for Y-chromosomal haplogroup analysis.

2.3. Ancient DNA analysis

After Sanger sequencing, DNA sequences were aligned to the human mitochondrial Cambridge Reference Sequence (NC_012920.1, rCRS) using Bioedit 5.0.9. mtDNAprofiler package (Yang et al., 2013) was used to obtain a list of nucleotide differences from the reference mtDNA. HaploGrep 2 web tool was used for the mitochondrial haplogroup determination based on the observed SNPs (Weissensteiner et al., 2016).

Only Illumina reads that were filtered by PALEOMIX (Schubert et al., 2014) with mapDamage2 tool (Jonsson et al., 2013) were used for subsequent haplogroup analysis. BWA with the *-rescale* parameter (Li and Durbin, 2009) was used for the mapping against the rCRS. Sequence variant detection was conducted using the VarScan software (v 2.3.9) with $p\text{-}value < 0.01$ (Koboldt et al., 2012).

We used pmdtools (threshold that equals at least to 0) for modern reads elimination from all sequenced data. SNPs with lower *p-value* or with more stringent threshold (up to three) were used to avoid conflicting SNPs (belonging to different haplotypes) (Skoglund et al., 2014).

These filtered reads were then used for mitochondrial (with HaploGrep 2) haplogroup determination. Unlike with Sanger sequencing, here we used SNPs from the whole mitochondrial genome.

Y-chromosome haplogroup determination was conducted in Yleaf tool (Ralf et al., 2018) with the following parameters:

- Base quality threshold (-q) – 20;
- Read number threshold (-r) – 1;
- Base distribution threshold (-b) – 90.

3. Results

Sanger and high-throughput Illumina DNA sequencing platforms were used for maternal haplogroup identification for 11 out of 14 Koban culture human remains from Klin-Yar 3 (two individuals) and from Zayukovo-3 (ten individuals) archaeological sites. Illumina DNA reads were also used to identify Y-chromosomal haplogroup for 5 out of 14 Koban culture individuals (one from Klin-Yar 3 and five from Zayukovo-3 archaeological sites). In addition, one sample of the Sarmatian individual (Zayukovo-3, grave 91 of the 2nd – 3rd centuries AD) was also sequenced to compare with the other Zayukovo-3 specimens.

Illumina reads (ranging from 7,041,263 to 42,755,534 reads per sample) were generated for DNA-libraries that were constructed from DNA extracts, and then were used for maternal and paternal haplogroup identification. Despite using ancient DNA facilities, the significant percentage of sequencing reads related to environmental (bacterial) DNA fraction and only a few of them were suitable for haplogroup identification (Table 2). Only PALEOMIX filtered reads that contained specific to ancient DNA postmortem cytosine deamination patterns (Der Sarkissian et al., 2015) were used for haplogroup identification (Table 2). Postmortem DNA damage patterns that were created using MapDamage2.0 tool (Jonsson et al., 2013) for the specimens are presented in Supplementary (Figs. S1–S15). Following Illumina read alignment against the rCRS, we analyzed the whole mtDNA sequences (coverage from 8,84 × to 22.83 ×) from five Koban individuals (specimens: 2, 7, 8, 9, 11) (Table S2).

Sanger sequencing was also used for mitochondrial haplogroup identification, and its results corresponded well with Illumina analysis data (see Table 3).

Table 2
Illumina generated reads and number (%) of mapped reads to the human reference mitochondrial genome (rCRS).

Library name	No. Illumina reads generated	No. Illumina reads filtered by PALEOMIX	No. reads mapped on mitogenome	% mapped reads
1	21,805,253	9,218	24	0.04
2	7,041,263	1,862,453	2,067	26.45
3	24,531,374	360,746	133	1.47
4	31,057,158	11,146	4	0.04
5	23,668,256	23,057	27	0.10
6	32,260,771	15,739	64	0.05
7	39,035,217	3,791,961	2,146	9.71
8	32,909,845	166,182	1,045	0.50
9	32,605,058	3,049,707	1,322	9.35
10	33,826,912	17,080	9	0.05
11	33,990,235	3,485,107	1,388	10.25
12	42,755,534	1,044,937	423	2.44
13	30,802,279	195,629	94	0.64
14	20,495,659	564,777	555	2.76
15	42,588,503	408,931	474	0.96

Table 3
Mitochondrial and Y-chromosomal haplogroups of the Koban culture samples.

Sample number	Burial site	Mitochondrial haplogroup (Sanger sequencing)	Mitochondrial haplogroup (Illumina sequencing)	Y-chromosomal haplogroup (Illumina sequencing)
1	Klin-Yar-3	H20a	–	–
2	Klin-Yar-3	J1c	J1b1	E1a2a1b1b
6	Zayukovo-3	N	–	–
7	Zayukovo-3	U5a1a1h	U5a1a2	G2a1a1a2
8	Zayukovo-3	HV1	HV1a1a	D1a2a1
9	Zayukovo-3	T1a	T1a1	G2a1a
10	Zayukovo-3	H1e	–	–
11	Zayukovo-3	W5a	N	R1b1a1b
12	Zayukovo-3	R6/H1e	–	–
13	Zayukovo-3	–	–	R1a
14	Zayukovo-3	R6	–	–
15	Zayukovo-3	I1	–	–

Here we report the results of Y-chromosomal and mitochondrial DNA haplogroup analysis of ancient Koban and one Sarmatian culture individuals that lived during the Iron Age in the North Caucasus (9th – 5th centuries BC and 2nd – 3rd centuries AD). The ancient DNA analysis based on revealed SNPs (please see the Supplementary file: Table S3; Table S4) indicate that mtDNA haplogroups of humans that have been buried in the Klin-Yar 3 and Zayukovo-3 cemeteries belong to H20a, J1c, N, HV1, T1a, H1e, W5a, R6, I1.

Out of the fifteen studied samples, six specimens yielded Y chromosome results (Table 3). The haplogroup of each sample was detected by Y chromosome sequencing (Supplementary Dataset).

Basic Y-chromosomal haplogroups, which have been described in this study, are common for the Caucasus and Europe during the Iron Age period - E1a2a, G2a1a, R1b, and R1a; moreover, R1a and R1b haplogroups have been usually associated with the Indo-European migrations (Haak et al., 2015; de Barros Damgaard et al., 2018). Previously published archeological data show that the Scythian invasions had a significant influence on cultural legacy of Koban archeological culture (Kozenkova, 1989). This point of view can be confirmed by our Y-chromosomal data (see Y-haplogroup identification results for samples 11 and 13 in Table 3); the Y-haplogroups R1a and R1b were frequently described in both Scythians and Sarmatians (Underhill et al., 2010; Juras et al., 2017; de Barros Damgaard et al., 2018; Krzewinska et al., 2018; Mary et al., 2019). The G1a haplogroup is usually associated with Near Eastern Neolithic cultures (Lazaridis et al., 2016), but it was also presented in Neolithic Europe (Lacan et al., 2011a; Lacan et al., 2011b). Nowadays G2a has low frequencies in Central Europe; however, it is widespread in modern Ossetians (Balanovsky et al., 2011), Balkars, and Karachays (Dzhaubermezov et al., 2017). R1a, R1b, and E1a2a haplogroups can be identified in other modern North Caucasian ethnic groups – Balkars, Karachays, Dargins, Lezghins, and Abkhaz (Nasidze et al., 2004; Balanovsky et al., 2011; Khusnutdinova et al., 2012; Dzhaubermezov et al., 2017). The frequencies for several found haplogroups (G2a1a, R1a, and R1b) in the modern North Caucasian ethnic groups based on Balanovsky with colleagues (Balanovsky et al., 2011) are presented in Supplementary (Figs. S16–S18).

At the same time, the Eastern Asian D1a2a1 (Gayden et al., 2007; Wang et al., 2014) Y-haplogroup was found in the Zayukovo-3 cemetery and the same specimen has rare HV mitochondrial haplogroup, which apparently spread to Europe through the Caucasus territory for the period after Last Glacial Maximum (Shamoon-Pour et al., 2019).

4. Discussion

The last years have been marked by steady growth in genomic analysis data from the Neolithic, Bronze and Iron Ages, and early medieval cultures of the Caucasus. These studies have had a significant impact on the modern archaeology of the ancient cultures of the Ponto-Caspian region. However, there are pieces missing from this puzzle. The Koban archaeological culture, which is considered to be a link between the Bronze Age cultures and the modern populations and ethnic groups have not been genetically explored until now.

In our study, Sanger and Illumina sequencing were used for the better understanding of the mtDNA and Y-chromosome diversity and ancestry of Koban archaeological culture of the North Caucasus.

Despite the significant conformity, we found several differences in sub-haplogroup designation using two sequencing methods. This disagreement may be related to different SNPs found by these methods because Sanger sequencing was limited to the HVR1 region while Illumina sequencing allowed analyzing the whole mitochondrial genome.

In the Koban culture, we identified the dominancy of several mitochondrial and Y-chromosomal haplogroups distributed in ancient Europe and the Caucasus (Damgaard et al., 2018; Wang et al., 2019). Our data show relative genetic continuity of ancient Caucasus cultures, which were occasionally influenced by other cultures. Mitochondrial haplogroups H, J, N, T, W, I, U, which were described in the Koban culture representatives, had been also previously observed in ancient (Nedoluzhko et al., 2014; Sokolov et al., 2016; Wang et al., 2019) and modern North Caucasian ethnic groups (Nasidze et al., 2004; Khusnutdinova et al., 2012; Dzhaubermezov et al., 2017); moreover, these haplogroups are presented in Western Eurasia for thousands of years (Khusnutdinova et al., 2012; Allentoft et al., 2015). At the same time, the presence of R1b Y-chromosome haplogroup in the specimen that has been found on the Koban culture cemeteries might be the genetic footprint of Scythian invasions to the North Caucasus during Iron Age.

Surprisingly, one sample discovered in the Zayukovo-3 cemetery had a rare HV mitochondrial haplogroup that migrated to Western Europe from the Near East and, possibly, through the Caucasus after Last Glacial Maximum (Shamoon-Pour et al., 2019); moreover, this person had an interesting paternal ancestry – the Y-chromosomal haplogroup of this specimen (D1a2a1) has been widely described in East Asia.

Other Y-chromosome haplogroups that were observed in our study are very common for modern North Caucasian ethnic groups; particularly, G2a1a haplogroup from specimens 7 and 9 (Zayukovo-3 cemetery) is widely presented in modern Ossetians (Balanovsky et al., 2011), Balkars, and Karachays (Dzhaubermezov et al., 2017).

Finally, this pilot study is the first step towards unraveling the genetic ancestry of the unique Iron Age and medieval cultures of the North Caucasus, and understanding the genetic heritage of the modern local Pontic-Caspian populations and ethnic groups.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Sanger sequences of D-loop region and all SRA sequence data are publicly available at the NCBI BioProject: PRJNA589514.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasrep.2020.102357.

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