

## MOLECULAR IDENTIFICATION OF *CAPRA HIRCUS* IN EAST CHIA SABZ, AN IRANIAN PRE-POTTERY NEOLITHIC SITE, CENTRAL ZAGROS, BASED ON MTDNA

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### ABSTRACT

Since the beginning of livestock domestication in Near East in Neolithic era, human communities have benefited from goat (*Capra hircus*). Central Zagros has been identified as an independent center of Neolithization and goat domestication. The aim of this study was to assess the molecular analysis of five ancient goat samples belonged to East Chia Sabz, Central Zagros, Iran. Control region (HV1) of mitochondrial DNA was partially amplified and sequenced to compare the Neolithic goat haplotypes with modern goat haplotypes. In phylogenetic analysis, the five Neolithic sequences were grouped into A haplogroup which shows the early population expansion of dominant A lineage within Zagros in 9<sup>th</sup> millennium BC.

**Keywords:** *Capra hircus*, Central Zagros, mtDNA, Neolithic haplotypes.

### INTRODUCTION

In recent decades, zooarchaeological evidence has revealed that the Central Zagros was one of the most important origins of livestock throughout the Neolithic period. The archaeological traces show that amongst the first domesticated ungulates, goats (*Capra hircus*) were domesticated 8000–7000 cal. BC in Zagros Mountains, Iran (Zeder and Hesse, 2000; Zeder, 1999). It is widely accepted that the wild goat (*C. aegagrus*) is the wild ancestor of modern domestic goats (Meadow 1996; Porter 1996). In recent years, in addition to archaeological and zooarchaeological evidence, molecular studies have become one of the most powerful tools for the study of livestock origin in the process of domestication (Zeder, 2006). Mitochondrial DNA plays a key role in the study of evolution and phylogeny and has been widely used to study goat domestication process and origin (Han *et al.*, 2010). Mitochondrial DNA shows maternal lineage inheritance with relatively rapid evolution rate and no recombination system (Liu *et al.*, 2006); therefore, these genetic features have made mtDNA an appropriate tool for the investigation of origin of species. One of the most important parts of the mtDNA for this purpose is HV1 (hypervariable 1 of control region). Many early studies of the livestock origin focused on this part of the genome (Luikart *et al.*, 2006). Furthermore, it has been commonly used to describe the genetic polymorphism in goats (Naderi *et al.*, 2007; Luikart *et al.*, 2001). The aim of this paper was to analyze

the mtDNA (D-Loop) of five goat samples belonged to East Chia Sabz, an aceramic Neolithic site in the Central Zagros, Iran.

### MATERIALS AND METHODS

**DNA extraction, amplification and sequencing:** DNA extraction was carried out according to special requirements (Cooper and Poinar, 2000). All the steps and processes were performed in a dedicated laboratory. The pre-extraction preparations were carried out as follows: briefly, samples were cleaned up by UV-irradiated water and were soaked in 10% bleach for five minutes. To avoid contamination from prior handling, the exterior layer of the bones was removed using a rotary tool (approx. 5 mm in depth). Then, a small cube of the bones was removed and irradiated in all dimensions under shortwave-UV light for 30 minutes. Samples were powdered in a sterile porcelain mortar. Ancient DNA was extracted using a GeneClean Kit for Ancient DNA (MP Biomedicals, USA). DNA extraction was carried out in a clean area, where personal and sample protections were rigorously observed. Two pairs of primers were used to amplify HV1 of control region of the mitochondrial DNA in Simplex-PCRs as follows: CAP-F (5'-CGTGTATGCAAGTACATTAC-3') and CAP-R (5'-CTGATTAGTCATTAGTCCATC-3') (Naderi *et al.*, 2008), and Caprine-F (5'-ACAACACGGACTTCCCCTACT-3') and Caprine-R (5'-CATGGTGAACAAGCTCGTGA-3') (Horsburgh and

Rhines, 2010). Five  $\mu\text{l}$  of the extracted DNA were amplified in a 25 $\mu\text{l}$  reaction containing 1 U of DNA Taq polymerase (Qiagen, Germany), 2 mM of  $\text{MgCl}_2$ , 0.4 mM of each dNTP and 1  $\mu\text{l}$  of each primer in 10 pM concentration. To make the final volume, sufficient amount of sterile double-distilled water was added to the reaction. The amplification was carried out using the following cycling conditions: one cycle of initial denaturation at 95°C for 10 min; then 30 cycles of denaturation at 95°C for 50 sec, annealing at 60°C for 50 sec and elongation at 72°C for 50 sec. The amplification was completed with one cycle of final elongation at 72°C for 10 min. Amplified DNA was electrophoresed on 3% polyacrylamide gel. Positive PCR reactions were sequenced by Macrogen Inc. (South Korea) using Sanger method.

**DNA analysis:** Raw sequences data were corrected using Seqman II software v5.00. Trimmed sequences were aligned with other deposited goat sequences in GenBank and then the phylogenetic tree was constructed by MEGA4 software v4 (Tamura *et al.*, 2007) using neighbor-joining method with 1000 bootstrap replications (see Fig. 1).

**Authentication of ancient DNA:** The current study has been supported by evidence for ancient DNA authenticity: 1) samples were chosen from a systematic excavation under strict protocols; 2) all preparations (e.g. washing and powdering), DNA extraction and PCR analysis were performed in different isolated places where no modern or ancient DNA had been used previously; 3) no contaminations with goat DNA were detected during the extractions and amplifications. Furthermore, PCR reactions included negative controls to monitor possible contaminations; and 4) all equipment used in this study were dedicated to ancient DNA research.

**Archaeological data:** The archaeological site of Chia Sabz is located on the bank of Seymareh River, Lorestan Province, Western Iran (see Fig. 2). The altitude of this site is 663 meters above the sea level. In 2010, the systematic excavation in the site yielded 14 pre-pottery Neolithic layers. The relative and absolute chronologies have shown that the site was occupied from middle 9<sup>th</sup> to middle 8<sup>th</sup> Millennium BC (Darabi *et al.*, 2011). From a

stratigraphical point of view, all layers were continuous and no gaps had been attested between the layers (Hessari, 2010).

**Zooarchaeological data:** Although most of the animal bones were fragmented, some were successfully identified to the species and elements. Samples were chosen from the lower layers since the upper layers might contain the residues of the domesticated goats. Of the whole assemblage, five well-preserved samples were selected randomly by the specialists, including metapodial, radius, humerus, ulna and calcaneus bones.

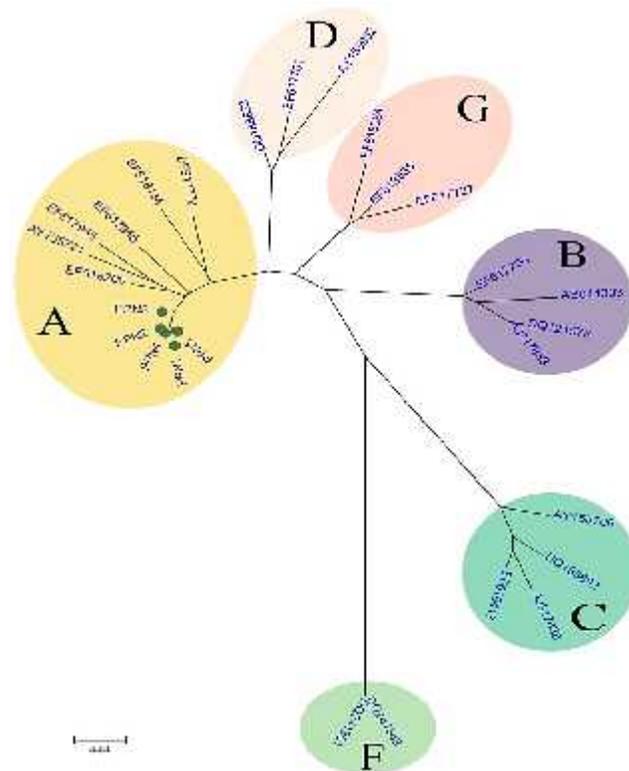
## RESULTS

The HV1 of control region (fragment of mtDNA) was generated successfully, encompassing between 534 and 632 base pairs. The sequences of mtDNA HV1 of control region have been annotated in NCBI GenBank database and the accession numbers were addressed in the paper (KC404854, KC404855, KC404856, KC404857 and KC404858). The comparison of the Neolithic HV1 sequences with GenBank HV1 sequences revealed high rates of identity (up to 99%) which shows good preservation of DNA in Neolithic samples. Furthermore, 22 reference sequences from GenBank database were chosen for phylogenetic comparison with Neolithic HV1 sequences. The information for reference sequences is shown in Table 1. These reference sequences were belonged to six main well-defined haplogroups named A, B, C, D, F and G which were identical to those from reference studies (Han *et al.*, 2010; Naderi *et al.*, 2008, 2007). Following construction of the phylogenetic tree, the modern haplotypes were clustered in the six main haplogroups, while all five Neolithic sequences were grouped in the A haplogroup. The validity of the main haplogroups was strongly approved by the bootstrap values of >90%. The A haplogroup was the largest, including ancient and modern haplotypes with 11 individuals. The other five haplogroups were only consisted of modern sequences and lined up thereafter by three, three, four, four and two individuals for D, G, B, C and F haplogroups, respectively (see Fig. 1).

**Table 1.** The haplogroup specification of 22 modern reference goats.

Accession no.	H <sup>a</sup>	Region	Original work	Ref. 1	Ref. 2
AY155721	A	India	Joshi <i>et al.</i> , 2004	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
EF618134	A	Italy	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
EF617779	A	France	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
EF618200	A	Jordan	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
EF617945	A	Iran	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
EF617965	A	Iran	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
AB044303	B	Laos	Mannen <i>et al.</i> , 2001	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
EF617706	B	Azerbaijan	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
AJ317833	B	Mongolia	Luikart <i>et al.</i> , 2001	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
DQ121578	B	China	Liu <i>et al.</i> , 2006	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
AY155708	C	India	Joshi <i>et al.</i> , 2004	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
AJ317838	C	Switzerland	Luikart <i>et al.</i> , 2001	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
EF618413	C	Spain	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
DQ188892	C	China	Liu <i>et al.</i> , 2005	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
AY155952	D	India	Joshi <i>et al.</i> , 2004	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
EF617701	D	Austria	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
DQ188893	D	China	Liu <i>et al.</i> , 2005	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
DQ241349	F	Sicily	Sardina <i>et al.</i> , 2006	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
DQ241351	F	Sicily	Sardina <i>et al.</i> , 2006	Naderi <i>et al.</i> , 2007	Han <i>et al.</i> , 2010
EF618084	G	Iran	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
EF618535	G	Turkey	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010
EF617727	G	Egypt	Naderi <i>et al.</i> , 2007	NA	Han <i>et al.</i> , 2010

<sup>a</sup>H, haplogroup; NA, not announced or applicable



**Figure 1.** Phylogenetic tree of domestic goat (*C. hircus*) mtDNA HV1 of control region sequences, constructed by MEGA4 software v4 using neighbor-joining method. Bootstrap resampling was calculated 1000. The five Pre-Pottery Neolithic haplotypes (abbreviated as PPN1–PPN5) are green spotted and grouped into the A haplogroup. Twenty-two modern reference mtDNA HV1 sequences were retrieved from GenBank database to identify A, B, C, D, F and G mitochondrial lineages.



**Figure 2.** The appearance of ancient A haplogroup in Central Zagros, Iran, belonged to Neolithic Era (East Chia Sabz site shown by star) and in Qazvin Plain belonged to Chalcolithic Era (Qabrstan site). Modern wild A haplogroup (as the strongest candidate for the ancestors of modern domestic goats) is only distributed in Eastern Iran and Eastern Anatolia.

## DISCUSSION

Mitochondrial DNA is one of the most common genomic tools for the evolutionary studies of goat species. In previous research on mtDNA (control region and cytochrome b), analysis of the modern goat samples have revealed six various mitochondrial groups known as A, B, C, D, F and G. Luikart *et al.* (2001) found the first three haplogroups spread around the world; of which the A lineage is the most widespread group within all continents, while the B lineage was detected mainly in Southern and Eastern Asia including China, Mongolia, Laos, Pakistan, India and Malaysia. C lineage was only present in Mongolia, Switzerland and Slovenia with a very small population (Luikart *et al.*, 2001). Recent studies have investigated other maternal lineages, termed D, F and G. D lineage was rare and only detected in Pakistan, India and China, whereas F and G lineages were identified in goats from Pakistan, India, Spain and Italy with a few total number (Han *et al.*, 2010; Naderi *et*

*al.*, 2007; Sultana *et al.*, 2003; Sardina *et al.*, 2006; Joshi *et al.*, 2004).

The genetic comparison of Neolithic sequences (from this study) with modern reference sequences showed five Neolithic haplotypes categorized into the A haplogroup. This haplogroup was regarded as a relatively ancient population expansion which has corresponded to the first domestication event of goats in nearly 10,000 years ago (Luikart *et al.*, 2001). The appearance of A haplogroup in Chia Sabz (see Fig.2) can be linked to the A lineage originating in the Fertile Crescent, especially in Taurus Mountains in Turkey (Peters *et al.*, 2005) and Zagros Mountains in Iran (Zeder, 2005; Zeder and hesse, 2000). Studies on the modern goat mtDNA have revealed that the A lineage contains the most number of individuals and has the widest geographical distribution within the world (Joshi *et al.*, 2004; Mannen *et al.*, 2001; Luikart *et al.*, 2001). Relatively, two newly investigated ancient goat haplotypes from Qabrestan Site in Qazvin Plain (Chalcolithic Era, 3782–3361 cal. BC) belonged to the A lineage (Fernandez *et al.*, 2005). Although

Qabrestan is far from Eastern Anatolia and Central Zagros, where the most evidence of goat domestication and herding exist (e.g. Ganj Dareh, Guran, Cayonu and Nevali Cori), it shows that the A lineage was expanded into the Central Plateau of Iran after the initial domestication of goats. This fact is due to movements after the first domestication events, since human always translocated livestock animals to different regions.

The Genetic research has previously proposed that the wild goat (*C. aegagrus*) is the most likely ancestor of modern domestic goats (Mannen *et al.*, 2001). The mtDNA data of modern wild (*C. aegagrus*) and domestic goats (*C. hircus*) showed that the origin of A lineage most likely lied in Eastern Anatolia (Naderi *et al.*, 2008). The archaeological evidences have revealed the origin of goat domestication in Nevali Cori in nearly 10,500 years ago (Peters *et al.*, 2005; Peters *et al.*, 1999) and in Cayonu in the same region (Hongo and Meadow, 2000). Naderi *et al.* (2008) have proposed that the A haplogroup is missing amongst the population of bezoars in Zagros Mountains and Iran Plateau and the presence of the A lineage in Eastern Iran could be an introgression from or feralization of domestic goats (see Fig.2). Based on the five haplotypes of the A lineage from this study, it can be proposed that the Central Zagros has the potency of being one of the origins of modern A lineage goats and could have given rise to the Neolithic expansion of A haplogroup since the initial phases of domestication. However, three highly divergent A, B and C lineages were diverged more than 200,000 ya, the expansion of population have suggested that the A lineage has experienced a relatively ancient expansion but B and C lineages underwent a recent expansion and were domesticated 2,130 and 6,110 ya, respectively (Luikart *et al.*, 2001). The large A lineage possibly correspond to an initial domestication events that occurred about 10,000 ya. Nowadays, approximately 90% of domestic goats are included in the A haplogroup (Naderi *et al.*, 2008).

The zooarchaeological analysis of Chia Sabz has not been published fully yet, but the preliminary studies suggest herding management and domestication in this pre-pottery Neolithic site, since 22% of the bone assemblage were belonged to immature young animals at the time of death (Darabi *et al.*, 2011). The second excavation season in this aceramic Neolithic site has shown that Chia Sabz is a rich site for zooarchaeological and archaeogenetic studies of important livestock species in Seymareh Valley and Central Zagros (Hessari 2010). The current study presents the first molecular research on ancient goats in Central Zagros and can provide novel information on domestication and origin of modern domestic goats. In summary, this study proposes that Central Zagros has possibly played a key role in beginning of domestication and population expansion of the goat A lineage. Additional molecular data from important Neolithic sites in Zagros will result in a better

understanding of A lineage domestication and its ancient expansions in Western Iran.

**Acknowledgments:** This project was a part of a MA thesis in Islamic Azad University, Tehran Markaz Branch and financially supported by a grant from Tehran Medical Genetics Laboratory (grant no. 91003). The authors would like to express their gratitude to Dr. S. Zare Karizi who kindly helped with the laboratory analysis. Many thanks to L. Mottaghi, Z. Bahmani, S. Sorahi and M. Khalili for great help in laboratory works. We are grateful to A. Aliyari, the deputy within the Institute of Archaeology, University of Tehran, for facilitating access to the bone complex of Chia Sabz site.

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