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Sequence Variations of Mitochondrial DNA Displacement-Loop in Iranian Indigenous Sheep Breeds

Research Article

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ABSTRACT

Mitochondrial DNA (mtDNA) has been used extensively to study population genetics because it has the unique features of maternal inheritance, a relatively fast rate of evolution and lack of recombination. A total of 82 unrelated sheep from 10 Iranian indigenous sheep breeds were investigated to determine the maternal genetic diversity using a sequence of a 685 bp segment of the displacement loop (D-loop) of mtDNA. Analysis of this region revealed 74 haplotypes and 123 polymorphic sites. Haplotype diversity, nucleotide diversity and the average number of nucleotide differences were estimated to be 0.996 ± 0.003, 0.0372 ± 0.0001 and 25.23, respectively. The sequence analysis also revealed high level of genetic diversity among the native Iranian breeds. Analysis of molecular variance revealed that 3.43 percent of the variation is found among populations compared with 96.57 percent variation found within populations. The Neighbor-Joining (NJ) tree indicated four (A, B, C and E) of the five haplogroups described so far are present in Iranian sheep breeds. The phylogenetic tree did not show any distinct genetic structure among the studied populations, which suggested that there existed strong gene flow and intermixing among sheep populations probably caused by extensive transportation of sheep in history and similar maternal lineages among the regions.

KEY WORDS genetic diversity, Iranian sheep, mitochondrial DNA.

INTRODUCTION

Sheep domestication occurred about 10000 to 11000 years ago in an area extending from northern Zagros Mountains of Iran to South East Anatolia, Turkey. Asia Minor and the Middle East regions established an important geographical connection between Asia and Europe continents. For this reason, species in this area reflect a high-level of gene flow, mixing and differentiation from the time of the domestication event (Meadows *et al*. 2011). Sheep are one of the most economically important domestic animals in Iran, as well as in other parts of the world (Guo *et al*. 2005). The contribution of sheep in Iran can be categorized as a source of meat, wool, skin and milk. Genetic characterization of different sheep breeds is a first step to collect basic information for any breeding and conservation plan in order to maintain and improve production efficiency in this industry. Mitochondrial DNA (DNA) has shown good potential to study population genetics and evolution. MtDNA is the only extra-nuclear genome in the cytoplasm, and exists as multiple copies. It has a high mutation rate and the rate of mtDNA evolution is about 5 to 10 times faster than nuclear DNA (Xingbo *et al*. 2000; Reicher *et al*. 2012). MtDNA is inherited maternally, apparently lacks recombination and is highly variable within a species because of its elevated mutation rate that may be the result of lack of repair mechanisms and/or the presence of free radicals formed during the phosphorylation process. As a result, mtDNA is an important material for phylogenetic inference and for analyzing genetic diversity (Witas and Zawicki, 2004; Wang *et al.* 2007; Galtier *et al.* 2009). Studies performed on the control region fragment and / or the cytochrome b (cytB) gene of mtDNA of modern sheep from a wide geographical range divided domestic sheep's genetic diversity into five different haplogroups: A, B, C, D and E (Wood and Phua, 1996; Hiendleder *et al.* 1998; Hiendleder *et al.* 2002; Guo *et al.* 2005; Pedrosa *et al.* 2005; Meadows *et al.* 2005; Meadows *et al.* 2007).

The aim of present research was to study the genetic diversity within and between Iranian sheep populations. In this study, we investigated the mtDNA control region polymorphisms in 10 Iranian sheep breeds to obtain deeper insight into sequence variation, type of haplogroups and their diversity. The information for main use and population size of studied breed has been indicated in Table 1.

Table 1 Main use and population size per each breed in the distributed areas

Breed	Production type	Present census (2012)
Baluchi	Carpet wool	6300000
Ghareh-Gol	Fur-type	330000
Kalakuii	Carpet wool	190000
Lori-Bakhtiari	Meat-type	4000000
Moghani	Meat-type	3500000
Makuii	Meat-type and carpet wool	1400000
Naeeni	Carpet wool	700000
Shal	Meat-type	700000
Taleshi	Meat-type	470000
Zel	Meat-type	2810000

MATERIALS AND METHODS

Sample collection and DNA extraction

Blood samples were collected from 82 unrelated sheep from 10 Iranian indigenous sheep breeds including Balouchi, GharehGol, Kalakui, Lori-Bakhtiari, Moghani, Makui, Naini, Shal, Taleshi and Zel. The number of individuals per each breed is indicated in Table 2. Geographic distribution of the sampling sites of the 10 Iranian sheep breeds is shown in Figure 1. Genomic DNA was extracted by standard salting-out method (Mburu and Hanotte, 2005). Purity and concentration of DNA samples measured using NanoDrop and electrophoresis on 1% agar gel.

Polymerase chain reaction (PCR)

A 685 bp fragment from 15448 to 16127 bp of the mtDNA control region was amplified by using forward primer (15388F): 5' GCC CCA CTA TCA ACA CCC AAA G 3' and reverse primer (CD744R): 5' AAT GGG CGA TTT TAG ATG AGA TGG C 3' (Hiendleder, 1998). The PCR amplification reaction system consisted of genomic DNA 40 ng, dNTPs 0.08 m*M*, primers 0.2 M, $MgCl_2$ 1.25 m*M*, Taq DNA polymerase 0.05 U/µL. The PCR conditions were initial denaturation step at 95 ˚C for 5 min, 30 cycles of denaturation at 94 ˚C for 30 s, annealing at 59 ˚C for 60 s and extension at 72 ˚C for 60 s and a final extension step at 72 ˚C for 10 min. The PCR products were electrophoresed through 2.0% (wt/vol) agarose gel which was stained with ethidium bromide solution.

Sequencing analysis of mtDNA

The forward primer was used for sequencing PCR product using Genetic analyzer 3130X. All the sequences were edited and aligned using the BioEdit software (Hall, 1999). The polymorphism of the haplotypes was analyzed with the DnaSP 5.10.00 software (Librado and Rozas, 2009). The Neighbor-Joining phylogenetic tree based on pairwise distance between pairs of individual was constructed using the MEGA v.5.0program (Tamura *et al.* 2011). Analysis of molecular variance (AMOVA) was performed with ARLEQUIN version 3.11 to infer the amount of diversity between and within populations. The levels of statistical significance were tested by performing 1000 permutations (Excoffier *et al.* 2005). The representative sequences of each haplogroups A (HM236174, HM236175), B (HM236176, HM236177), C (HM236178, HM236179), D (HM236180, HM236181) and E (HM236182, HM236183) was retrieved from NCBI and added to the primary data set for performing phylogenic analysis (Neighbor-Joining tree) to identify type of haplogroups of Iranian sheep breeds (Meadows *et al.* 2011).

RESULTS AND DISCUSSION

In the present study, we identified 74 haplotypes in the analyzed 82 animals with 123 polymorphic sites. Estimates of genetic diversity within the sheep populations have been indicated in Table 2. The average of haplotype diversity, nucleotide diversity and the average number of nucleotide differences were estimated to be 0.996 ± 0.003, 0.0372 ± 0.0001 and 25.23, respectively. Breed-specific estimates of genetic diversity indicated that Kalakui contained the highest (0.04762) and Zel the lowest (0.00872) nucleotide diversity (π) within the Iranian populations (Table 2).

AMOVA allocates only a small portion of the total diversity to the between-breeds component. It reveals that mitochondrial diversity is mainly distributed within breeds (96.57 %) and only in part among regions (3.43 %) (Table 3).

To determine the clade membership of each haplotype, a Neighbor-Joining tree was constructed using the 92 haplotypes (Figure 2). The tree contained haplogroups of A, B, C and E (but not D).

Sequence analysis of the mtDNA from Iranian sheep showed high genetic diversity within the breeds.

Table 2 Estimates of genetic diversity within the sheep populations

Breed	ID	N	Hn	π	Hd
Baluchi	BAL	5	5	0.04412	1
Ghareh-Gol	GHR	16	13	0.04379	0.975
Kalakuii	KAL	4	4	0.04762	1
Lori-Bakhtiari	LOR	4	4	0.04118	1
Moghani	MOG	6	6	0.04075	1
Makuii	MUK	15	15	0.03652	1
Naeeni	NAI	3	3	0.03731	1
Shal	SHL	9	9	0.03698	1
Taleshi	TAL	12	11	0.03592	0.985
Zel	ZEL	8	4	0.00872	0.75

ID: identity document; N: number of individual sampled; Hn: observed haplotypes; π: population nucleotide diversity and Hd: haplotype diversity.

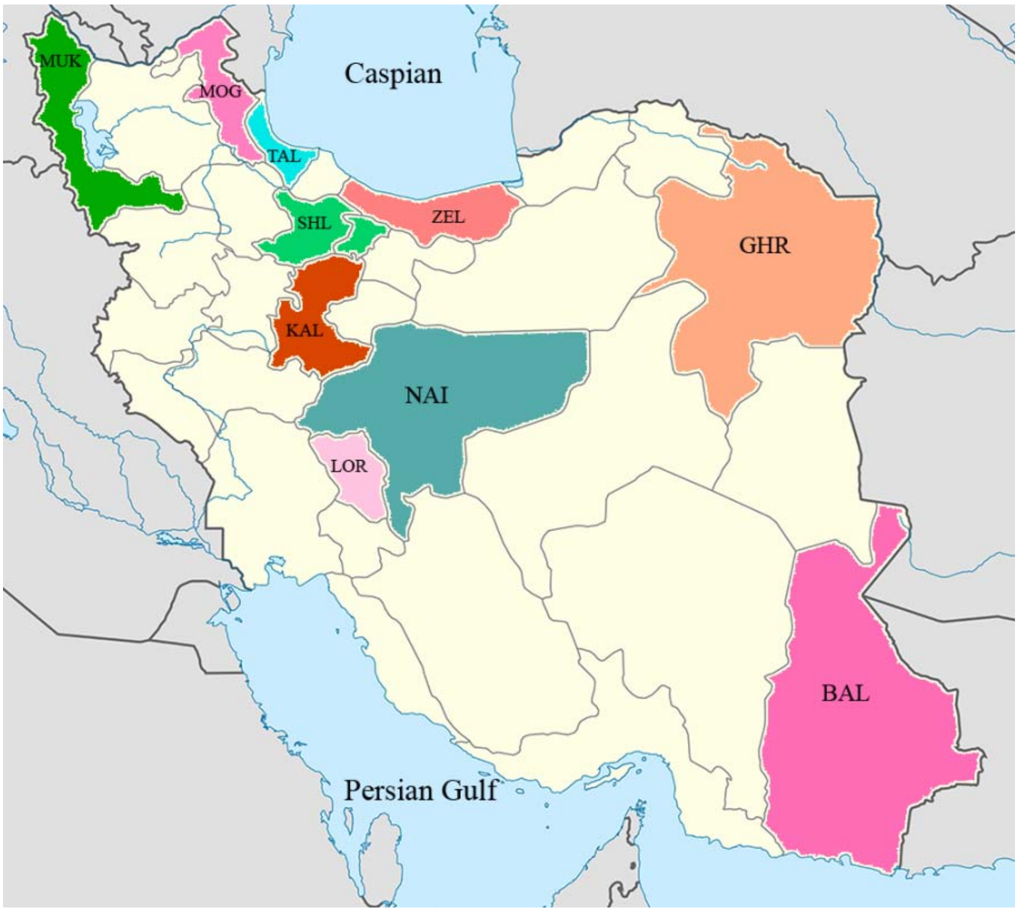

Figure 1 Geographic distribution of the sampling sites of the 10 Iranian sheep breeds
For breed abbreviations, see Table 2

The haplotype diversity in the analysed breeds was higher than that in other European breeds (Pariset *et al.* 2011).

The values of haplotype and nucleotide diversities observed in this study was higher than haplotype diversity of 0.792 ± 0.37 and nucleotide diversity of 0.00392 ± 0.00046 obtained from analysis of mtDNA variation and matrilineal structure in blue sheep populations of Helan Mountain, China (Wang *et al.* 2006).

Pariset *et al.* (2011) assessed genetic diversity of sheep breeds from Albania, Greece and Italy by mtDNA and nuclear polymorphisms (SNPs) and observed increased nucleotide diversity from the South to the East, which was consistent with the approach to the sheep domestication center. Lancioni *et al.* (2013) also assessed the genetic diversity of three Italian Merino-derived (IMD) breeds by mtDNA and observed three distinct subhaplogroups within B haplogroup.

Table 3 Analysis of molecular variance for 10 Iranian sheep breeds based on displacement-loop mitochondrial DNA sequence data

Source of variation	Degrees of freedom	Sum of squares	Mean square	Percentage of variation
Among populations	9	148.135	16.46	3.43^{ns}
Within populations	72	924.365	12.83	96.57**
Total	81	1072.500	13.24	

** (P<0.01).
NS: non significant.

Comparing the number of individuals sampled and the number of haplotypes (Table 2) indicates that each breed has its own haplotype that can be due to high diversity in Iranian sheep breeds and / or sampling strategy (collecting unrelated individuals).

Sequencing more individual may endorse this claim in future studies. In the present study, the analysis of molecular variance attributes most genetic variation to the within-population component (97%). These results were similar to the findings from an AMOVA analysis on Turkish sheep that showed the main genetic variation distributed within populations (95.61%) and the rest between populations and different geographical regions (Oner *et al.* 2013). Genetic diversity analysis of the mitochondrial D-loop of Nigerian indigenous sheep also showed 99.77% of genetic variation within populations and 0.32 % of variation between populations (Agaviezor *et al.* 2012).

Higher level of genetic diversity within rather than between Iranian sheep populations demonstrates that there is no clear structure between populations and also indicates a high level of gene flow through the maternal lineage between the populations (Tserenbataa *et al.* 2004). Phylogenic analysis did not yield distinct clusters representing the populations (Figure 2).

Low differentiation between populations can be attributed to limited pastures, random mating, lacking of appropriate breeding plans or management policies. Moreover, migration leading to natural gene flow between populations can also be important factors in reducing the differentiation between populations (Kantanen *et al.* 1995). However, Gizaw *et al.* (2011) declared that an important feature which should be noted about genetic diversity study of domesticated animal species is that genetic variation within populations is much higher that genetic variation between populations (Gizaw *et al.* 2011). In this study phylogenetic analysis demonstrated four major haplotypes (A, B, C and E) in 10 Iranian native breeds. Haplogroup A and haplogroup B are the most frequent and have been found in every geographic region where the domestic sheep have been sampled. Haplogroup A is mainly represented in Asian breeds, while haplogroup B is found in high frequency in breeds sampled in Europe (Wood and Phua, 1996; Hiendleder *et al.* 1998). Haplogroup C is less frequent and only few samples have been isolated in the Fertile Crescent (Asia), and the Caucasus and the Iberian Peninsula (Europe) (Guo *et al.* 2005; Pedrosa *et al.* 2005; Meadows *et al.* 2005; Tapio *et al.* 2006; Pereira *et al.* 2006).

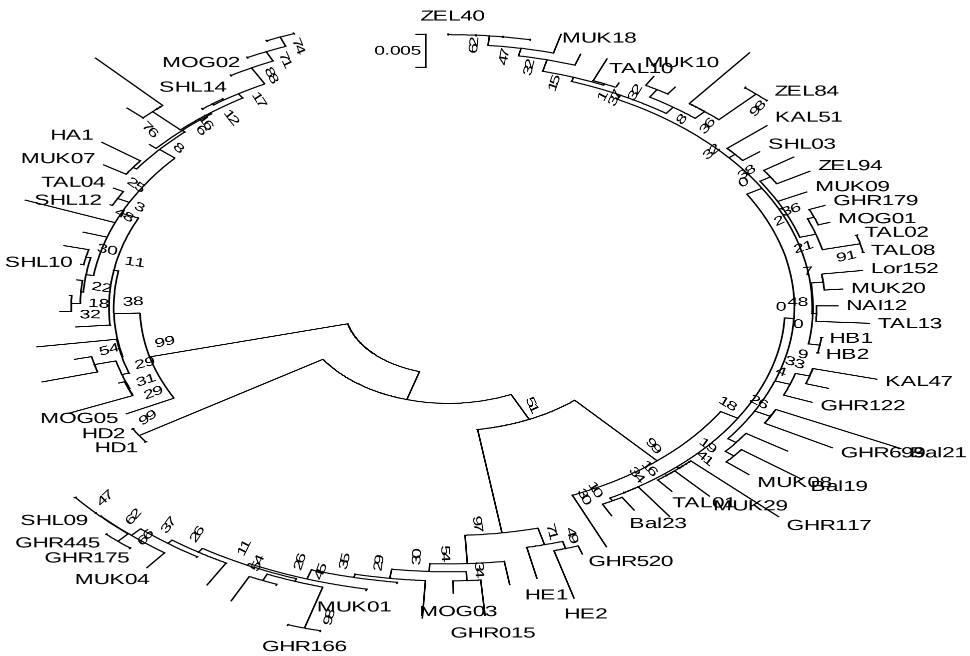

Figure 2 Phylogenetic relationship between maternal haplotype identified in the sheep populations (individual level)
For breed abbreviations, see Table 2

Haplogroup D and haplogroup E are the rarest and have only been found in the Caucasus and Turkey so far (Meadows *et al.* 2005). A recent study on the complete mitochondrial genome of ten domestic sheep and six wild sheep examined the relationship between domestic and wild sheep. The phylogenetic analysis confirms the division of domestic sheep into the five (A, B, C, D, E) haplogroups (Meadows *et al.* 2011). The only investigation on mtDNA sequence variation in Iranian sheep breeds was performed by Mohammadhashemi *et al.* (2012) on Moghani sheep breed. They obtained five haplotypes that were distributed within the 10 individuals analyzed. All the haplotypes of that study were subdivided into the haplogroup A.

CONCLUSION

High diversity, high gene flow, and low levels of breed differentiation are the characteristic of Iranian native sheep populations. There could be several reasons for this, including implementation of inappropriate breeding programs, random mating, and exchange of populations between different geographical areas. Alternatively, Iran's location close to or inside the domestication center has likely led to preservation of a large part of wild diversity in populations of this region and surrounding area (Benjelloun *et al.* 2011). Another explanation for the findings is Iran's location along the Silk Route that has been traversed by commercial caravans connecting China, India, Iran, Turkey and other locations for millennia. This route was important in trade of a variety of crops and animals and probably contributed to random mixing between different populations. So, the weak genetic structure of Iranian native sheep populations can be attributed to long-term severe genetic flow between Iranian native sheep, induced by the historical movement of humans.

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