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COMPARISON OF GENETIC DIVERSITY OF LEPTIN GENE BETWEEN WILD GOAT AND DOMESTIC GOAT BREEDS IN IRAN

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ABSTRACT

Leptin is a polypeptide which is mostly secreted by the white adipose tissue and a little by gastrointestinal tract and placenta, plays an important role for controlling body weight, feed intake, immunity, milk production, and reproduction. The aim of this study was to investigate the exon 2 of the *leptin* gene polymorphism in Iranian wild and domestic goats using PCR-RFLP. Blood samples were collected from 14 wild and domestic goat breeds including (Cashmere Abadeh, Torki-Ghahghaei, Naeini, Robati, Nadoushani, Adani, Shahrbabaki, Birjandi, China goat, Sannen, Pakistani, Raeini cashmere, Najdi, and Wild goat) and then genomic DNA was extracted. A 152 bp fragment from exon 2 of the *leptin* gene was amplified. PCR products were digested with *Hinf I* restriction enzymes and was separated and visualized on the agarose gels. From possible three genotypes (TT, TC, and CC), only two genotypes TT and CC were observed in 14 studied domestic and wild populations with the genotype frequency of 95% and 5% respectively. The number of observed alleles, number of effective alleles, Nei's Index and Shanon's Index were 2, 1.10, 0.10 and 0.20 respectively. The studied populations were not found to be in Hardy-Weinberg equilibrium. Our investigation demonstrated that TT genotype and T allele had a very high frequency (0.95) in studied goats. Hence, it can be concluded that this finding can provide the basis for selection when considering evolution and differentiation among breeds, however, further studies should be carried out on a larger population of different domestic and wild breeds to verify the final conclusions.

Key words: Diversity, domestic goat, leptin gene, PCR-RFLP, wild goat

INTRODUCTION

Goat breeding is growing in the world and its products have a desirable landscape (Moghadaszadeh *et al*., 2015). Although in countries with high incomes, there have been many improvements and changes in agricultural technology and knowledge, the number of goats has increased in these countries as well (Shamsalddini *et al*., 2016). The first reason for breeding goats in the world is because of meat production and dairy production is in the next priority. The most important goat breeding continents are Asia and Africa (Mohammadabadi & Tohidinejad, 2017). In developing countries, 96% of the milk and meat producing goat populations are found and 4% are found in developed countries (FAO, 2008). There are 30 million heads of cashmere goats around the world and 4.5–5 million heads of them are in Iran that is 20% of all in the world (Baghizadeh *et al*., 2009). For farmers in the arid and semi-arid regions, goat breeding plays an important and effective economic role. Increasing meat production using scientific, accurate, and precise selective programs are one of the most important goals for genetic improvement of goats. This can be done by identifying the genotype of reproductive and productive traits of animals and their relationships, through determining the polymorphism and finding the phylogenetic relation-

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ships of domestic animals (Soufy *et al*., 2009; Ruzina *et al*., 2010; Moghbeli *et al*., 2013; Mohammadabadi & Tohidinejad, 2017).

One of the important genes is *leptin.* Leptin was derived from *leptos* (Greek), its weight is 16 KD, encodes for a 167 amino acid, is the product of the *ob* gene, is mainly produced by white adipose tissue, to a lesser extent, by muscle cells, stomach epithelium, placenta, fetal tissues, and mammary glands (Gregorio *et al*., 2014) and plays important role for regulating of feed intake, energy balance, fertility, and immune functions (Javanmard *et al*., 2008). This gene has 3 exons and 2 introns, however only 2 of its exons are translated to protein (Shojaei *et al*., 2010). The *leptin* gene is located on chromosome 4 in cattle, sheep and goat (Gregorio *et al*., 2014), and on chromosome 8 in water buffalo (Gregorio *et al*., 2014). This gene has both endocrine performances, in the brain and in the various peripheral tissues and autocrine/paracrine efficiency into tissues (Zieba *et al*., 2003). It is demonstrated that leptin is expressed in adipocytes (Chilliard *et al*., 2001), fetus (Yuen *et al*., 2002), breast (Bartha *et al*., 2005), rumen (Yonekura *et al*., 2002), small intestine (Yonekura *et al*., 2002) and hypophysis (Yonekura *et al*., 2003) of ruminants. Some effects of the expression of this gene are decreasing in feed intake, losing body weight, losing fat deposit weight and increasing metabolism energy (Javanmard *et al*., 2008). In addition, *leptin* may be required for controlling the reproduction and in this way, *leptin* gene may also operate as the symptom for the reproductive system to produce adequate body fat for assisting to prosperous conception and pregnancy (Liefers & Veerkamp, 2002). It has been demonstrated that there is a direct correlation between plasma level of leptin and body fat mass and energy balance in cattle and sheep (Shojaei *et al*., 2010). Leptin gene effects on milk performance in cattle and reproduction in beef cattle (Shojaei *et al*., 2010). Expression of this gene is changed in various physiological and growth stages in an animal, hence leptin can be used as a marker for growth, feed efficiency and health of the animal (Bartha *et al.*, 2005). Celi *et al*. (2008) showed that plasma and milk *leptin* concentrations will not be affected by maternal food variation in dairy goat and milk *leptin* concentration has a negative correlation with kids' liveweights and average daily growth rate. However, there is a relatively limited number of studies have been conducted on leptin in goats, compared to cattle and in particular in Iranian wild goat, hence the aim of this study was to study the exon 2 of *leptin* gene in some Iranian native goat breeds, some Iranian imported goat breeds and wild goat.

MATERIALS AND METHODS

In this study, 546 blood samples were collected from different individuals of fourteen goat breeds (Figure 1) in Iran (Cashmere Abadeh (CAB), n=40; Torki-Ghahghaei (TOG), n=34; Naeini (NAE), n=30; Robati (ROB), n=28; Nadoushani (NAD), n=38; Adani (ADA), n=14; Shahrbabaki (SHB), n=28; Birjandi (BIR), n=34; China goat (CHI), n=34; Sannen (SAN), n=60; Pakistani (PAK), n=60; Raeini cashmere (RAC), n=60; Najdi (NAJ), n=28; and Wild goat (*Capra aegagrus*) (WIL), n=28). Wild goat (*Capra aegagrus*) samples were collected from 3 protected region and Zoos of Iran (Sistan and Baluchistan, Kerman and Fars provinces). The wild goat is a widespread species of goat, that distributes from Europe and Asia Minor to Central Asia and the Middle East. It has been listed as vulnerable on the IUCN Red List since 1996 (Figure 2). Wild goat is a species of the mountain goat. Males are commonly called Kal and have long and sword horns. Five mL blood sample of animals were gathered through the jugular vein in K3 and stored in EDTA containing tubes to prohibit coagulation. The modified salting-out method (Abadi *et al*., 2009) was employed to extract genomic DNA. Both spectrophotometry and agarose gel (1%) was applied to determine the quality of the extracted DNA. A 152-bp fragment within the exon 2 of the leptin gene was amplified using PCR primers 5'- TGCAGTCTGTCTCCT CCAAA -3' and 5'- CGATAATTGGATCACATTT CTG -3' (Singh *et al*., 2009). CinnaGen PCR Master Kit was used to perform PCR amplification in a 25 µL reaction volume, according to the instructions by the manufacturer (CinnaGen Co., Iran). PCR protocol was done at 3 steps: step 1; 5 min at 94°C, step 2 had 34 cycles consisted of 3 stages; 30 s at 95°C, 30 s at 55°C, 30 s at 72°C and step 3 for final extension 5 min at 72°C. Electrophores on 1% agarose gel at constant voltage and 1× TBE for approximately 2 hours was used for visualization of PCR products. The gels were visualized by staining with ethidium bromide and photographed under ultraviolet light, and then all PCR products were digested with 5 U of *Hinf*I restriction enzyme (Fermentas) at 37°C overnight, and the resulting products were separated by the 3% agarose gel and visualized by ethidium bromide staining. Diversity indices including gene diversity (H), the observed number of alleles (Ne), Shannon's information index and Nei's Index were calculated using PopGen software (McNally *et al*., 2014). The POPGEN 3.2 software (Yeh *et al*., 1999) was used to construct the dendogram of un-weighted pair group with arithmetic mean (UPGMA). All animals were cared for according to the Animal Care and Use Committee (ICAC, 1995).

Fig. 1. Locations of the fourteen study sites in Iran. The putative subspecies are indicated as Cashmere Abadeh (CAB), Torki-Ghahghaei (TOG), Naeini (NAE), Robati (ROB), Nadoushani (NAD), Adani (ADA), Shahrbabaki (SHB), Birjandi (BIR), China goat (CHI), Sannen (SAN), Pakistani (PAK), Raeini cashmere (RAC), Najdi (NAJ), and Wild goat (WIL-1, 2 and 3).

Fig. 2. Iranian wild goat.

RESULTS

The extracted DNA had good quality (Figure 3) and its concentration was approximately 100 nanograms per microliters. The tested DNA of the goat used in the present study was amplified using the specific primers and yielded PCR products at the expected size, 152 bp (Figure 4).

Amplification of the *leptin* gene produced 152 bp fragments; when these fragments were digested with the restriction enzyme *HinfI*, the CC genotype produced two bands: 84 and 68 bp (one restriction site in the C allele), the TT genotype produced one band: 152 bp (no restriction sites in the T allele), and the TC produced three bands: 152, 84 and 68 bp (heterozygote genotype). The different alleles resulting from the digestion of the PCR products with the *HinfI* restriction enzyme after running on the agarose gel electrophoresis are presented in Figure 5. The genotypic and allelic frequencies of the *leptin* gene in studied goats have been shown in Table 1. In this study, the Hardy Weinberg equilibrium was estimated with Chi-square and G-square tests. The studied populations were not found to be in Hardy-Weinberg equilibrium ($P<0.05$).

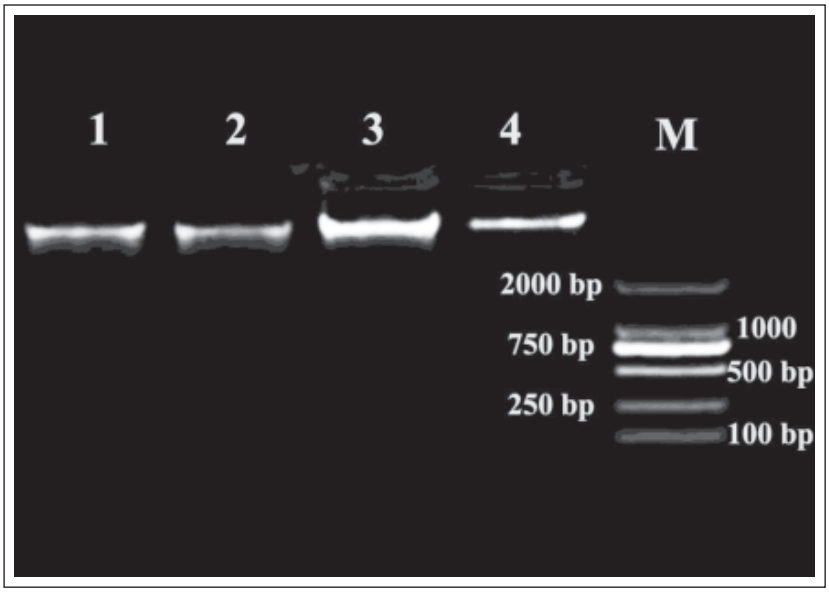

Fig. 3. Some samples of the extracted DNA from the studied animals on the 1% agarose gel. From left to right: line 1: Raeini cashmere (RAC), line 2: Najdi (NAJ), line 3; Wild goat (WIL) and line 4 Torki-Ghahghaei (TOG), line 5 (M) is size marker.

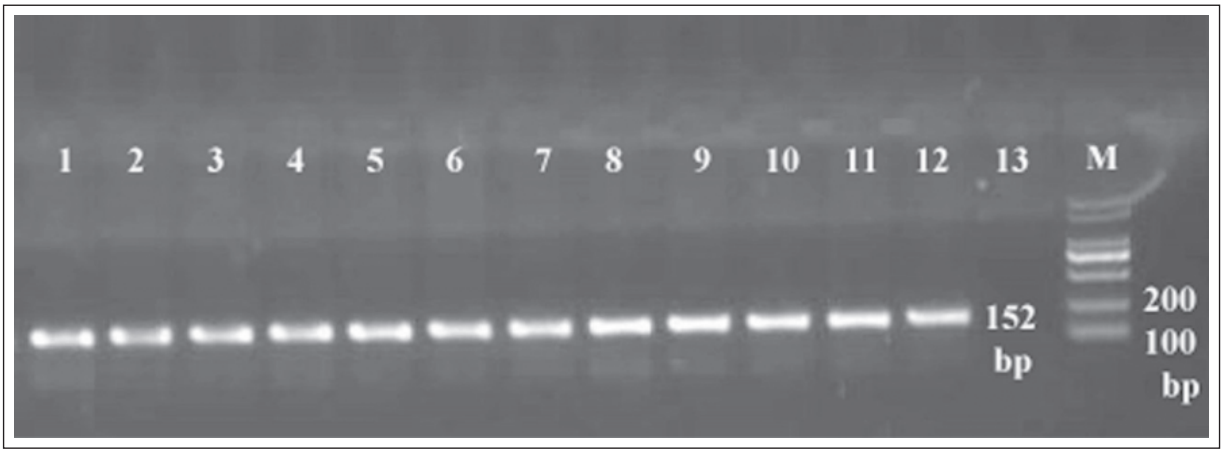

Fig. 4. Ethidium bromide-stained agarose gel of amplified PCR products representing amplification of exon 2 of *leptin* gene in some studied goats. From left to right: Line 1: Cashmere Abadeh (CAB), line 2: Torki-Ghahghaei (TOG), line 3: Naeini (NAE), line 4: Robati (ROB), line 5: Nadoushani (NAD), line 6: Adani (ADA), line 7: Shahrbabaki (SHB), line 8: Birjandi (BIR), line 9: China goat (CHI), line 10: Sannen (SAN), line 11: Pakistani (PAK), line 12: Raeini cashmere (RAC), line 14 (M) is size marker: 100 bp, 200 bp, 400 bp, 500 bp, 600 bp, 800 bp, 1000 bp and line 13 is negative control.

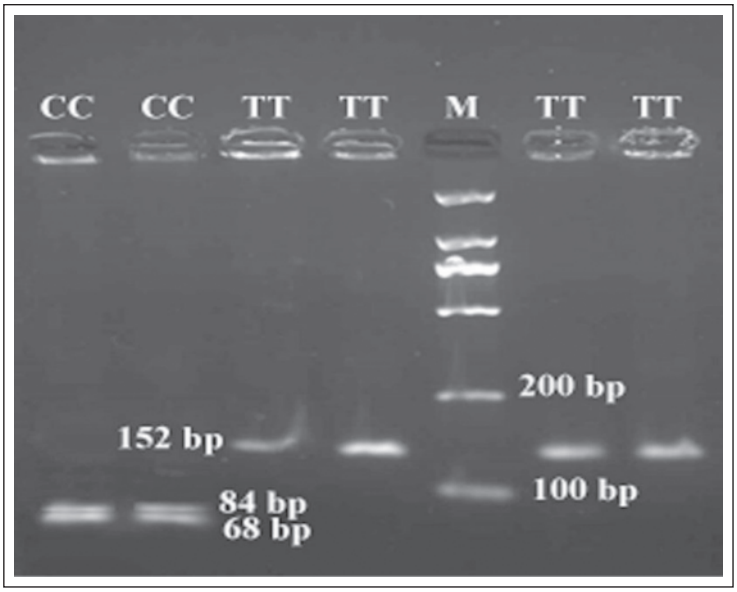

Fig. 5. Ethidium bromide-stained agarose gel of amplified PCR products representing amplification of exon 2 of leptin gene in some studied goats. From left to right: Line 1 is Torki-Ghahghaei (TOG) with CC genotype, line 2 is Birjandi (BIR) with CC genotype, line 3 is Raeini cashmere (RAC) with TT genotype, line 4 is Pakistani (PAK) with TT genotype, line 6 is Sannen (SAN) with TT genotype, line 7 is Nadoushani (NAD) and line 5 (M) is size marker: 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp.

Table 1. Genotypic and allelic frequencies of exon 2 of *leptin* gene in studied goat breeds

Breed	Genotype	N	Genotypic frequency	Allele	Allelic frequency
Cashmere Abadeh (CAB)	TT	40	1.00	T	1.00
Torki-Ghahghaei (TOG)	TT	30	0.88	T	0.88
	CC	4	0.12	C	0.12
Naeini (NAE)	TT	30	1.00	T	1.00
Robati (ROB)	TT	28	1.00	T	1.00
Nadoushani (NAD)	TT	34	0.89	T	0.89
	CC	4	0.11	C	0.11
Adani (ADA)	TT	14	1.00	T	1.00
Shahrbabaki (SHB)	TT	28	1.00	T	1.00
Birjandi (BIR)	TT	32	0.94	T	0.94
	CC	2	0.06	C	0.06
China goat (CHI)	TT	34	1.00	T	1.00
Sannen (SAN)	TT	60	1.00	T	1.00
Pakistani (PAK)	TT	60	1.00	T	1.00
Raeini cashmere (RAC)	TT	60	1.00	T	1.00
Najdi (NAJ)	TT	10	0.36	T	0.36
	CC	18	0.64	C	0.64
Wild goat (WIL-1, 2 and 3)	TT	28	1.00	T	1.00
All	TT	518	0.95	T	0.95
	CC	28	0.05	C	0.05

The values of the population genetics parameters in studied polymorph breed have been shown in Table 2. Cashmere Abadeh, Naeini, Robati, Adani, Shahrbabaki, China, Sannen, Pakistani, Raeini cashmere, and Wild breeds were monomorphic based on exon 2 of the leptin gene. A UPGMA dendrogram based on the Nei's standard genetic distance among studied animals has shown in Figure 6.

Table 2. The values of the population genetics parameters in polymorphic studied goats

Breed	Number of observed alleles	Number of effective alleles	Nei's Index	Shanon's Index
Torki-Ghahghaei (TOG)	2	1.21	0.18	0.33
Nadoushani (NAD)	2	1.14	0.13	0.25
Birjandi (BIR)	2	1.12	0.11	0.22
Najdi (NAJ)	2	1.99	0.50	0.69
All	2	1.10	0.10	0.20

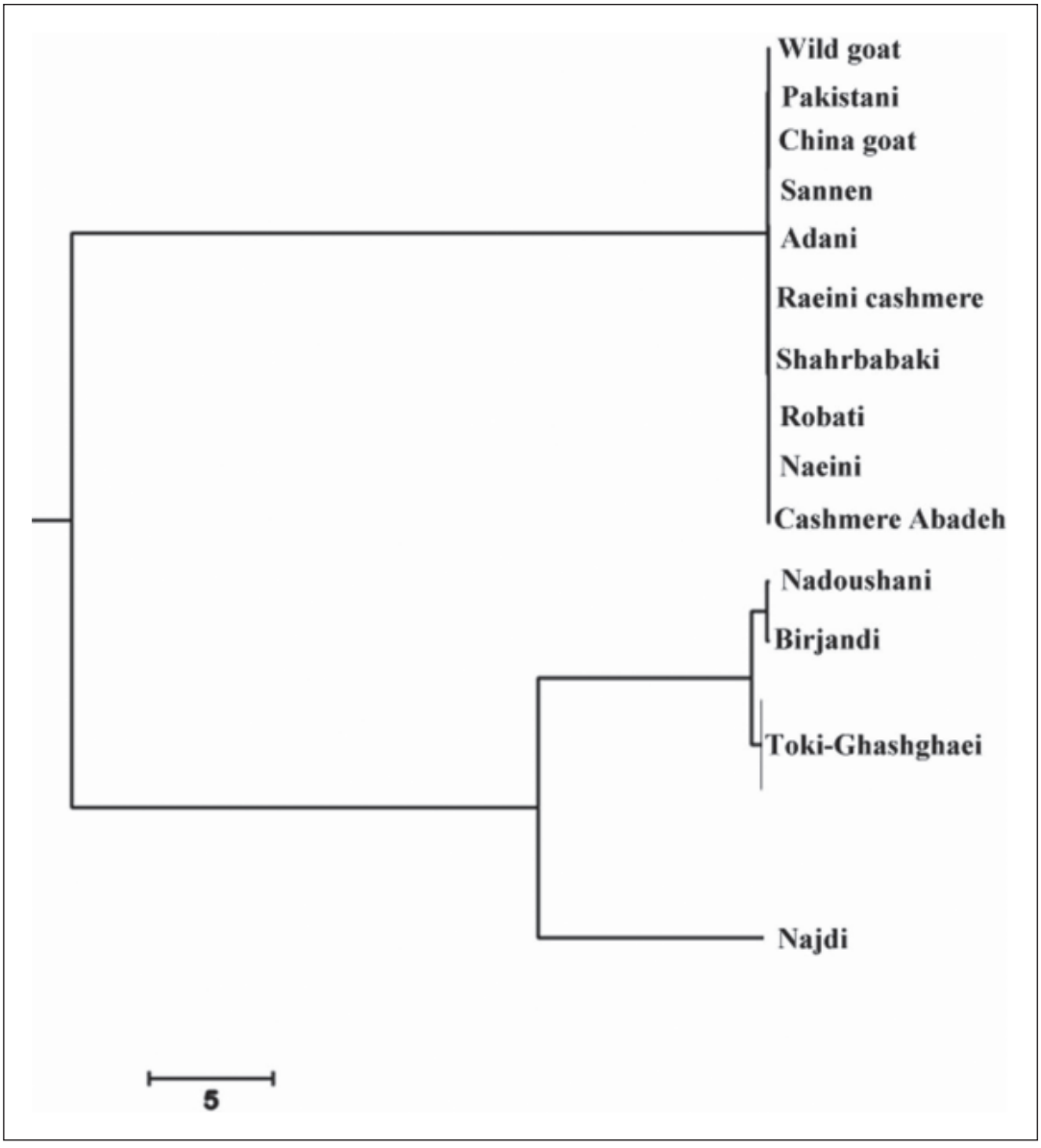

Fig. 6. UPGMA phylogenetic tree based on Nei genetic distance.

Based on UPGMA phylogenetic tree Cashmere Abadeh, Naeini, Robati, Adani, Shahrbabaki, China, Sannen, Pakistani, Raeini cashmere and Wild breeds were clustered in the same branch and Torki-Ghahghaei, Nadoushani, Birjandi and Najdi breeds were clustered apart from ten other breeds.

DISCUSSION

Genetic variation is a basic requirement for animal breeding, whereas a high genetic variation is needed for genetic improvement of domestic animals (Askari *et al*., 2011; Khodabakhshzadeh *et al*.,

2016). The number of alleles at different marker loci serves as a measure of the genetic variability having a direct impact on the differentiation of breeds within a species (Vajed Ebrahimi *et al*., 2017). Since 50% of the native breeds exhibited 2 observed alleles and more than 1.1 effective alleles, the studied locus screened in this study was appropriate in expressing the molecular characteristics and or genetic variation in the populations. The effective number of alleles at each locus provides information on predominant alleles. Since, diversity (Shanon's Index) in the studied polymorphic native breeds displays moderate (0.22 for Birjandi breed) to high (0.69 for Najdi breed) genetic variation; these breeds have a good gene pool for breeding programs, as sufficiently high genetic diversity is the basis for preserving the adaptive abilities of a population. As for some limitation on the number of population and loci, further research needs to be done on the correct evaluation of the genetic relationship among the populations. The level of variation depicted by the number of alleles and other diversity indices were similar to earlier reports (Ko³odziej *et al*., 2009; Gregorio *et al*., 2014). The studied breeds deviated from Hardy-Weinberg equilibrium. This can be attributed to the excess of heterozygote individuals than homozygote individuals, migration, high mutation rate, artificial selection in some studied breeds and the low number of animals in any studied population (Vajed Ebrahimi *et al*., 2017).

Results of UPGMA phylogenetic tree indicated that four breeds; Torki-Ghahghaei, Nadoushani, Birjandi and Najdi breeds that clustered separately from other ten breeds were genetically different from the rest breeds. The reason for this could be a mutation, selection, or even migration. This tree revealed that the ten monomorphic breeds were clustered together. Among four polymorphic breeds, the smallest distance was observed between Birjandi and Nadoushani breeds and the largest distance were seen between Najdi and Nadoushani breeds. These genetic distances are in agreement with their geographic distances of these breeds and confirm them. Rout *et al.* (2008) studied Indian domestic goats and clustered these animals and showed that clustering according to genetic information and confirmed based on their geographic origin. A similar observation of population clustering according to their geographic origin has been reported in cattle (MacHugh *et al.*, 1997). This shows that geographically adjacent populations are more genetically related. Al-Atiyat *et al.* (2012) studied goat breeds in Jordan and clustered them in three clusters where the first showed the closeness of Dihawi and Black Mountain, then both are closer to Desert in the second cluster. Whereas the third cluster group those three breeds together with Damascus. Therefore, their finding undoubtedly confirmed the breed similarity and differentiation among the studied goat breeds in term of morphology (Zaitoun *et al.*, 2005) and production traits (Tabbaa & Al-Atiyat, 2009) and heat tolerance traits (Al-Tamimi *et al*., 2013). These previous findings confirm the results of current research.

The frequencies of leptin alleles in 14 studied breeds showed that allele frequency of T was higher than frequency for allele C. Low frequency of allele C in the studied breeds of the 546 different domestic and wild goats is consistent with the results of other researchers (Kulig *et al*., 2001; Korwin-Kossakowska *et al*., 2002; Kmieć *et al*., 2003; Kołodziej *et al*., 2009).

Our results, in terms of genotype number, were similar to results of other researchers (Křenková *et al*., 1999; Kulig *et al*., 2001; Korwin-Kossakowska *et al*., 2002; Kmieć *et al*., 2003; Kołodziej *et al*., 2009), as they observed only two genotypes TT and TC. In the current study, we observed only one genotype (TT) in the ten of 14 studied breeds, similar to the wild breed. This might be explained by the origination of Iranian domestic breeds from the wild breed, which has conserved their genotype for intron 2 of *leptin*. Furthermore, the low number of the studied samples might also influence the distribution of TT genotype as observed in our study. So it is suggested to investigate more samples from any intact breeds which were kept separately. Confirming this conclusion, Gregorio *et al*. (2014) studied *leptin* gene and reported that further analyses, based on a large population of animals and in different breeds, are needed to increase the body of knowledge on the role of leptin and its association with health and production traits in goats. Moreover, breeds containing TT genotype are carrying the best gene pool for selection and breeding programs to improve desired traits. According to Askeri *et al*. (2011), high genetic diversity is the most important factor for animal genetic improvement. In the other study, Maitra *et al*. (2014) identified and characterised *leptin* gene polymorphism in Indian goats and reported whole gene sequence of this gene and its diversity including SNP in native goats and detected seven new SNPs which were never presented formerly by direct gene sequencing in a group containing seven goat breeds with different phenotype (weight and size) and geographical distribution. They analysed obtained sequences using bioinformatics procedures and reported 22 variations in comparison with an exotic goat. From these 22 detected variations seven were SNPs found in exon 2 (g.1029T_C), intron 2 (g.1621G_A) and 3'UTR (g.3968T_C, g.3971C_T, g.4026G_A, g.4105G_A and g.4225T_C). They demonstrated that these recognized seven novel

SNPs in the *leptin* gene could illustrate a percent of meat quality and tenderness polymorphism in Indian goat populations that can supply a foundation for the subsequent precise investigation of *leptin* genotype correlation with performance (meat quality trait). Gregorio *et al*. (2014) compared goat, sheep, cattle and water buffalo leptin (LEP) genes and effects of the Intron 1 microsatellite polymorphism in goats and showed that leptin gene plays a role as a marker for metabolism and mammary gland health in dairy goats.

CONCLUSIONS

The results of our investigation demonstrated that TT genotype and T allele have a very high frequency (0.95) in goats bred in Iran, hence this finding could provide basic information for animal selection when considering evolution and differentiation among breeds. Taking into account all aspects and results, goat *leptin* gene can be useful for achieving genetic relationship of goat breeds and for selection of economic traits, but considering some differences between the results of current research and results of various investigates, it can be concluded that more investigations need to perform in leptin gene polymorphism and its effects on the important traits using more samples from each goat breed to verify the final conclusions.

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