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Identification of Point Mutation in Exon 3 of Leptin Gene in Munjal Sheep

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ABSTRACT

Background: Leptin is a varied hormone which plays vital role in body development by regulating the balance between food intake and energy expenditure by signaling to the brain. Leptin has diverse effect on controlling appetite, energy metabolism, growth, reproduction, body composition and immunity. The present study was aimed to screen candidate point mutation (g.332G>A) in the targeted genomic region of leptin gene in Munjal sheep.

Methods: A total of 50 Munjal sheep were selected and genomic DNA was isolated in Automated Maxell RSC DNA/ RNA purification system by using Maxwell RSC whole blood DNA kit. Reported set of primers was used to amplify 463 bp fragment encompassing targeted region (exon 3) of leptin gene. PCR-RFLP was performed to genotype targeted point mutation in our resource population. PCR products were digested by *Cail* 1 restriction enzyme to genotype g.332G>A (at 332th nucleotide of exon 3 leptin gene) non-synonymous mutation (Arg to Gln).

Result: All studied samples resolved into monomorphic banding pattern, revealed only AA (463 bp single band bp) genotype. The absence of candidate mutation in our resource population might be due to small sample size.

Key words: Leptin gene, Munjal sheep, PCR-RLFP, Polymorphism.

INTRODUCTION

Sheep, with its multi-faceted utility for meat, milk, skin, wool and manure, form a vital part of rural economy in most areas of the country particularly in the arid, semi-arid and mountainous areas. India is rich source of sheep genetic resources having 43 breeds. As per 20th Livestock Census (2019), India stands at number three in world sheep population with 74.26 million sheep. Munjal sheep is large in size, tall and rectangular in shape. The origin of Munjal sheep are not known exactly but it is supposed to have originated in India through the sheep breeders of Rajasthan, Punjab and Haryana breeding Nali with Lohi sheep (Arora *et al.* 1986; Mason, 1988). Munjal sheep is very popular among farmers of Haryana, Punjab and Rajasthan for their height and heavy weight.

Leptin, a diverse hormone which plays vital role in body growth by regulating the balance between food intake and energy expenditure by signaling to the brain. Leptin has diverse effect on controlling appetite, energy metabolism, growth, reproduction, body composition and immunity. Leptin is produced by white adipocytes and also from the tissues of stomach, skeleton, muscle and placenta (Friedman and Halaas, 1998). Leptin has a huge effect in controlling whole body energy metabolism and can be regarded as a "metabolism modifier." Leptin is encoded of three exons and two introns and is found on fifth chromosome in sheep (Pomp *et al*. 1997). Leptin gene polymorphism has drawn much attention in biomedical research particularly from animal scientists for their possible role in economically important production and reproduction traits. Zhou *et al.* (2009) found four SNPs in the exon 3 of Leptin gene of which three were non-synonymous and resulted in amino acid changes. Boucher *et al*. (2006) conducted a study to identify the SNPs in leptin gene in sheep and their associations with growth traits in a population set of Dorset and Suffolk lambs. Barzehkar *et al*. (2009) demonstrated the polymorphism of the Leptin gene and its association with growth and carcass traits in three Iranian sheep breeds. Shojaei *et al*. (2010) also reported association of leptin gene polymorphism and growth traits in Kermani sheep and found that the growth traits were significantly affected by the genotypes. Cauveri *et al.* (2014) reported two SNPs (PCR-RFLP), 16973 G>A (SNP-L1) and 17476 C>T (SNP-L2) in the Exon 3 in Nilagiri sheep. Animals of SNP-L1 were homozygous to the A allele. Bakhtiar *et al*. (2017) also explored exon 3 region of leptin gene to explore g.332G>A in Sanjabi sheep. Similarly, many researchers targeted exon 3 of Leptin gene to screen candidate marker associated with various economic traits in sheep (Meena *et al.* 2017; Kaplan *et al.* 2018; Saleem *et al*. 2018). Taking into the consideration these facts, the study was carried with

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the objective to explore candidate point mutation in exon 3 of Leptin Gene in Munjal Sheep.

MATERIALS AND METHODS

The study was carried on randomly selected Munjal sheep maintained at Sheep Breeding Farm, Department of Animal Genetics and Breeding, Lala Lajpat Rai University of Veterinary and Animal sciences, Hisar, India. A total of 50 animals were taken to detect polymorphism in genomic region of Leptin gene. 5 ml of blood was aseptically collected from the jugular vein in vacutainer tube containing EDTA (2.7%). DNA was extracted in Automated Maxell RSC DNA/RNA purification system (Promega) by using Maxwell RSC whole blood DNA kit and quality of DNA was also assesed (Fig 1). DNA samples were dissolved in 0.1X TE buffer (pH 8.0).

DNA amplification and genotyping

Reported set of primers was used to amplify exon 3 coding sequence of the Leptin gene (Bakhtiar *et al*. 2017) (Table 1). PCR amplification was carried out in a total volume of 25 ml with 100 ng DNA template, Dream Taq Green PCR Master Mix (Promega). Polymerase chain reaction (PCR) was carried out in thermal cycler (T-100 BIO-RAD) as per the conditions given in Table 2. The PCR product was checked on 2.5% agarose gel. PCR-RFLP was also performed to genotype animal for reported 332G>A candidate SNP (Bakhtiar *et al*. 2017), amplified PCR products (10 µl) of all animals were digested with 2 U Cail *1* restriction enzyme (Thermo Scientific) at 37°C for 10 h and were subsequently resolved in 2.5% agarose gel stained with ethidium bromide.

RESULTS AND DISCUSSION

In the present study, the PCR product (463 bp) of the Leptin gene was amplified encompassing exon 3 in Munjal sheep (Fig 2). The PCR product was digested with *Cail* 1 restriction enzyme and digested products were resolved on 2.5% agarose gel, revealed uncut single band (Fig 3). The PCR-RFLP demonstrated the existence of only one allele A, showing single band consisting of 463 bp, was assigned as the AA genotype corresponding to Glutamin homozygote for Munjal sheep. Thus, the animals under study were found to be monomorphic, which was reported first time in Munjal Sheep. Contrary to our study, Bakhtiar *et al*. (2017) observed all the three possible genotyope at targeted loci with predominance of G allele and reported that polymorphism in 332G>A locus had a significant effect on viability trait,

Table 1: Primer sets designed for amplification of the target region of LEP Gene.

Gene	Primer sequences (5′-3′)	A.T.	Product size (bp)	Region
LEP F	TGTTGTCCCCTTCCTCCTG	63°C	463	Exon 3
LEP R	CCCACATAGGCTCTCTTCTGC			

A.T.: Annealing temperature; bp: Base pair.

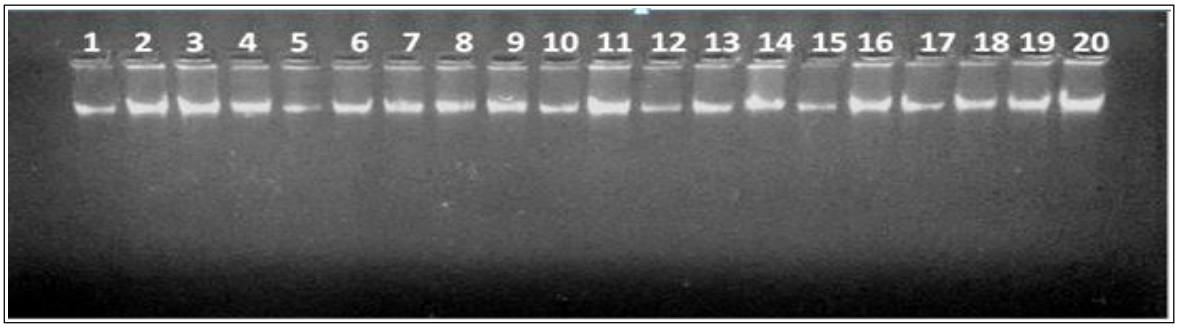

Fig 1: Quality checking of the genomic DNA.

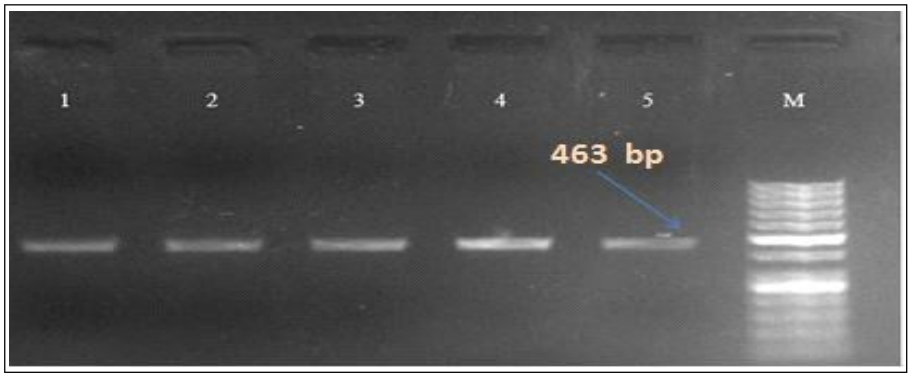

Fig 2: PCR amplicons of Leptin gene in Munjal sheep.

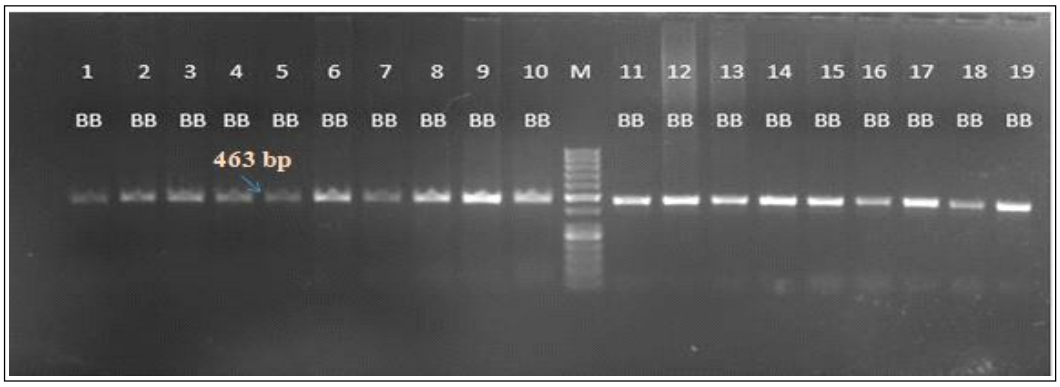

Fig 3: PCR-RFLP genotypes of Leptin gene using *BcnI* RE in Munjal sheep.

Table 2: PCR protocol.

Steps		Temperature	Time
1	Initial denaturation	95°C	5 min.
2	Denaturation	95°C	30 sec.
3	Annealing	63°C	30 sec.
4	Extension	72°C	1 min.
5	Go to step 2, (35 X)		
6	Final extension	72°C	5 min.

water test and scrotal circumference as GA genotypes had the highest amounts for these traits compared with GG genotypes (P<0.05) in Sanjabi rams. Our observations are in agreement with those of Gregorio *et al.* (2014) who observed uniform pattern in the partial genomic sequence of part of intron-1 to part of exon-3 of ovine leptin gene. The results are also in agreement with Cauveri *et al.* (2014) that studied polymorphism in exon 3 of leptin gene in Nilagiri sheep. Qureshi *et al.* (2015) reported monomorphic pattern of leptin gene in three sheep breeds of Pakistan using PCR RFLP technique. However, they observed polymorphism at position C1467T and A3050- in intron 2 of Ovine leptin gene. In an earlier attempt, Li *et al.* (2008) also did not find polymorphism in exon 2 using PCR-SSCP protocol in 358 sheep including Poll Dorset, Suffolk, Texel and Tan sheep. Study of polymorphism in exon 2 of LEP gene have been done in Dorset and Suffolk breeds (Boucher *et al.* 2006) and Shal, Zandi and Zel breeds (Barzhekar *et al.* 2009) but no polymorphism was detected by them in this region. However, leptin gene polymorphism in sheep were reported by many scientists like Zhou *et al.* (2009), Shojaei *et al.* (2010), Hajihosseinlo *et al.* (2012), Bahrami *et al.* (2013), Mahmoud *et al.* (2014), Meena *et al.* (2016), Maitra *et al.* (2014), Quirino *et al.* (2016) in different sheep breeds.

CONCLUSION

This study insight an evidence that Munjal sheep have no variability in targeted leptin locus. All animals were found to be monomorphic with respect to g.332G>A SNP. As a result, the targeted loci in Munjal sheep is largely conserved. It is possible that this monomorphism is a breed-specific trait. Therefore, reported SNPs were not considered to be a universal marker for a specific feature across all breeds. As a result, there is a need to investigate before implementing selection criteria.

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