

ALLELIC POLYMORPHISM OF EXON 3 OF LEPTIN GENE IN NILAGIRI SHEEP IDENTIFIED BY SEQUENCING AND PCR-RFLP

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Abstract: The discovery of hormone leptin had led to better understanding of the energy balance control. Leptin regulates feed intake, energy metabolism and body composition and plays a critical role in regulating body weight and growth in mammals. Characterization of Leptin gene (*LEP*) would not only help us understand the potential of this gene as marker but will also help us understand the genetic diversity of indigenous breeds and help in their conservation. In the present study the polymorphism in Exon 3 of *LEP* gene in Nilagiri sheep was done by sequencing and genotyping by PCR-RFLP. Five primers were designed to amplify the entire Exon 3 of *LEP* gene. Two polymorphisms 16973 G>A (SNP-L1) and 17476 C>T (SNP-L2) were detected in the Exon 3 in Nilagiri sheep. Animals of SNP-L1 were homozygous to the A allele. On genotyping the SNP-L2 by PCR-RFLP using restriction enzyme *BsrDI*, it was found that 77 per cent of the animals were of the CC genotype and 23 animals of the CT genotype. The allelic frequencies of C and T were 0.87 and 0.13 respectively. This shows that the *LEP* gene is polymorphic in Nilagiri sheep and PCR-RFLP is an appropriate tool to evaluate genetic variability.

Keywords: Nilagiri sheep, Leptin gene, SNP, PCR-RFLP.

Introduction

In areas where crop and dairy farming are not economical, sheep make a valuable contribution to the livelihood of economically weaker sections of the society with its utility for meat, wool, skin and manure. The discovery of hormone leptin had led to better understanding of the energy balance control. Leptin regulates food intake and controls how fats are distributed and stored in the body. While polymorphism in the leptin gene has been thoroughly investigated in bovine and swine, limited information is available on

polymorphism in the ovine *LEP* gene. In a first attempt for the identification of *LEP* (Exon 3) gene variation in Iranian Makoei sheep, Hashemi *et al.* (2011) investigated the polymorphism of the *LEP* gene by PCR–SSCP and five patterns were detected. To apply marker assisted selection efficiently, identification and validation of genetic markers is essential. Hence this preliminary study was undertaken to characterise the potential variation in Nilagiri sheep *LEP* gene using PCR-RFLP.

Materials and Methods

Blood samples were collected from 60 Nilagiri sheep and DNA was isolated. Five primers to amplify the entire Exon 3 of *LEP* gene based on the sequence from NCBI (Accession No. NC_019461 and Gene ID 443534). After standardisation of annealing temperature PCR was performed the product was sent for sequencing from twelve samples. Sequence data were analysed using the SeqMan program of LASERGENE software (DNASTAR Inc., USA) and SNPs were identified. The restriction site and the corresponding enzyme were analysed using the online tool NEB cutter (<http://tools.neb.com/NEBcutter2>) and *BsrDI* enzyme was used for genotyping the identified SNP in 60 samples.

Results and Discussion

LEP gene has three exons and two introns and the coding region starts from Exon 2 and ends in Exon 3. The Exon 3 is 2731 bp long and the open reading frame ends at 16245 bp. On analysing this Exon using five primers, two SNPs were identified in the Nilagiri breed both of which were in the untranslated region. The 16973 G>A (SNP-L1) was the first variation observed in which the reference sequence (from Texel an exotic breed) had a *G* allele whereas all the animals in Nilagiri had an *A* allele. No *GA* genotypes were found.

In SNP-L2, the mutation of *C* allele to *T* allele made the fragment devoid of a site for the restriction enzyme *BsrDI* and hence the 797 bp fragment which was cleaved into two fragments of 437 and 360 bp with *C* allele and remained un-cleaved in the presence of *T* allele. This enzyme was used to genotype all the samples in Nilagiri sheep. The frequency of *CC* genotypes was 0.77 and that of the *CT* genotypes was 0.23.

TT genotypes were absent in the population. The allelic frequencies were 0.87 for *C* and 0.13 for *T* in Nilagiri sheep. The chromatogram showing the SNP and the RFLP genotyping are shown in Fig. 1 and 2 respectively.

Both the SNPs identified in this study were transitions. PCR-SSCP protocol was used to detect polymorphism in the Exon 3 of *LEP* gene in Poll Dorsets, Suffolk, Texels and Tan

sheep which showed three SNPs, all of which resulted in amino acid changes (Li *et al.*, 2008). Polymorphism in the ovine *LEP* gene was reported by Zhou *et al.* (2009). The SNPs reported by Zhou *et al.* (2009) were absent in the present study and the first fragment was in complete homology with the reference sequence. The studies about *LEP* Exon 3 genotypes were carried out in Baluchi and Kermani sheep breeds (Tahmoorespur *et al.*, 2010; Tahmoorespur and Ahmadi, 2012 and Shojaei *et al.*, 2010) of Iran. While genotyping the same exon employing the same primers, three genotypes were found in the Baluchi and Kermani sheep. However in a later study five genotypes were detected for Exon 3 of *LEP* gene in Makoei sheep of Iran (Hashemi *et al.*, 2011) which concurs with the findings of Zhou *et al.* (2009). Genotyping of several Assaf, Awassi and Dorper breeds of sheep with fragments covering the Exon 3 of *LEP* gene, three synonymous and three non-synonymous mutations were found (Reicher *et al.*, 2010; Reicher *et al.*, 2011).

The SNPs identified in the Exon 3 of *LEP* gene in the present study are novel and reported for the first time. The previous reports were in the coding region alone and none of them included the entire Exon 3. Most of the previous reports include PCR-SSCP protocol and no sequencing and hence the exact position of nucleotide change and the exact nucleotide change could not be ascertained. As the polymorphism in the present study lie downstream the coding region no change in translation was seen. This study showed that Exon 3 of *LEP* gene is polymorphic in Nilagiri sheep. The present study was the first attempt for the identification of *LEP* (Exon 3) gene variation in Nilagiri sheep. Further studies are required to investigate the relationship between *LEP* gene polymorphisms and the performance traits, and the association of leptin concentration and genotypes in Nilagiri sheep.

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Fig 1. Chromatogram showing 17476C>T transition in Exon 3 of *LEP* gene in Nilagiri sheep

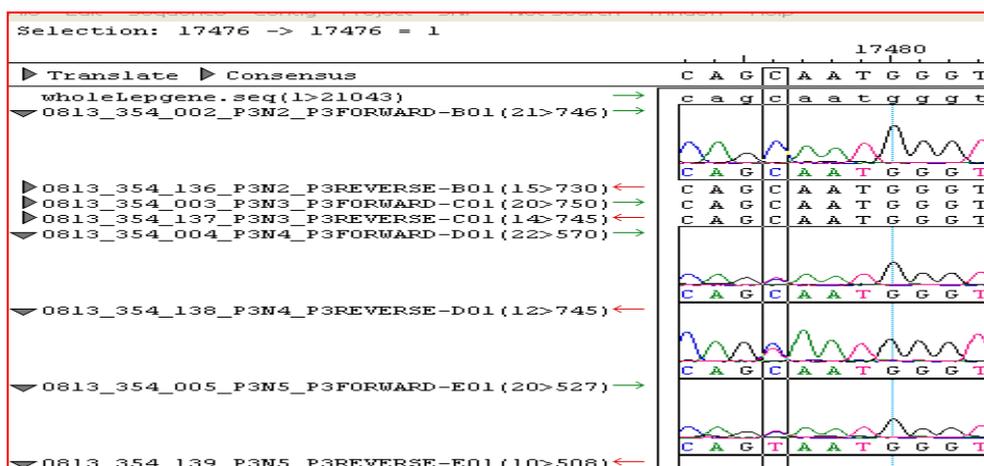


Fig. 2: PCR-RFLP genotyping of SNP-L2 in Nilagiri sheep

