*Genetics and Molecular Biology*, 28, 4, 740-742 (2005)

www.sbg.org.br

Short Communication

# DNA polymorphism of leptin gene in *Bos indicus* and *Bos taurus* cattle

Vivek Choudhary, Pushpendra Kumar, Tarun K. Bhattacharya, Bharat Bhushan and Arjava Sharma

*Indian Veterinary Research Institute, Animal Genetics Division, Molecular Genetics Lab, Izatnagar, India.*

## Abstract

Leptin plays an important role in the regulation of feed intake, energy metabolism, growth and reproduction of cattle. We used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique to screen for DNA polymorphisms of the leptin gene in 403 cattle belonging to various breeds of *Bos indicus* (Hariana, Sahiwal, Gir and Nimari cattle), *Bos taurus* (Holstein Friesian (HF) and Jersey cattle) as well as *Bos taurus* x *Bos indicus* crossbreds (½ HF x ½ Hariana*)*. In all the cattle we amplified two regions of the leptin gene, a 522 bp fragment comprising the partial intron 2 and exon 3 and another 94 bp fragment consisting of part of exon 2. Digestion of 522 bp PCR products with the *Bsa*AI restriction enzyme revealed three genotypes in all the breeds of cattle studied. This is the first report of the presence of leptin gene polymorphism in purebred *Bos indicus* cattle of Indian origin (indicine cattle). Almost similar gene and genotype frequencies were observed in all the breed groups, while the frequency of mutant homozygotes (AA) was very low (0.03 to 0.07). On digestion of the 94 bp fragment with the *Kpn*2I restriction enzyme, three genotypes were observed in HF, Jersey and crossbred cattle. The CC genotype had the highest frequency (0.68) in crossbreds whereas the frequency of CT heterozygotes was highest (0.69) in HF cattle. This mutation was absent in all the breeds of indicine cattle. The results suggest that the *Bsa*AI-RFLP mutation has occurred far back in evolution before the divergence of taurine and indicine cattle while the *Kpn*2I mutation has occurred recently as indicated by the fact that this mutation was only detected in taurine cattle.

*Key words:* leptin gene, PCR-RFLP, polymorphism, *Bos indicus*, *Bos taurus*.

Received: August 24, 2004; Accepted: June 14, 2005.

Variations at DNA level contribute to the genetic characterization of livestock populations and this may help to identify possible hybridization events as well as past evolutionary trends. Variation in the exonic region of a gene may lead to changes in amino acids which alter the expressed protein, and although intronic variation does not change the amino acid sequence of the protein it may play a significant role in gene splicing or the binding of regulatory proteins during transcription. In livestock, such variations in DNA may also be associated with, or linked to, economic traits, which are governed by many genes each having a small effect (Gelderman, 1997). However, the major gene model suggests that only a few genes may account for relatively large proportion of the genetic variation (Lande, 1981), such major genes being the genes usually involved in the biology of a trait and are the candidate genes for marker identification. There is also the possibility that major genes may be linked with some quantitative trait loci (QTL) contributing to a major part of the variation in traits. Leptin is a protein involved intricately in the growth and metabolism of animals and which plays an important role in the regulation of feed intake, energy metabolism, growth and reproduction of cattle (Ramsay and Cranwell, 1999) and thus the leptin gene is a potential candidate gene for QTL studies. Although many polymorphic studies on the bovine leptin gene have been reported (Pomp *et al.,* 1997; Lien *et al*., 1997; Wilkins and Davey, 1997; Konfortov *et al.* 1999; Haegeman *et al.,* 2000; Buchanan *et al*., 2002), to our knowledge there has been no reported polymorphic study involving cattle originating in the Indian subcontinent (indicine cattle, *Bos indicus*). The objective of the present investigation was to study genetic variations in the leptin gene of the *Bos indicus*, *Bos taurus* and their crossbred cattle.

Our study was conducted on a total of 403 animals from seven different breed groups of cattle maintained at Indian livestock farms as follows: 60 Hariana cattle at the Dairy Farm, Veterinary College, Mathura; 32 Sahiwal cattle at the Livestock Research Center, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar; 20 Gir cattle at the Kandiwali Gausala, Mumbai; 29 Nimari cattle at the Cattle Breeding Farm, Khandwa; 40 Jersey cattle at the Dairy Estate, Bhopal and Bull Mother Farm, Lucknow; 17 Holstein Friesian (HF) cattle and 205 ½ Holstein

Send correspondence to Pushpendra Kumar. Senior Scientist, Indian Veterinary Research Institute, Animal Genetics Division, Molecular Genetics Lab, Izatnagar, Bareilly-243 122, India. E-mail: pushpendra@ivri.up.nic.in; pushpendraivri@rediffmail.com.

Friesian x ½ Hariana crossbred cattle maintained at Cattle and Buffalo Farm, Indian Veterinary Research Institute, Izatnagar. Hariana, Sahiwal, Gir and Nimari cattle are *Bos indicus* (indicine) breeds while Holstein Friesian and Jersey cattle are *Bos taurus* (taurine) breeds.

From each animal, about 15 mL of venous blood was collected from the jugular vein and placed into a sterile 15 mL polypropylene centrifuge tube containing 0.5 mL of 2.7% EDTA solution as an anticoagulant and transported in an icebox to the laboratory and stored at 4 °C until needed for DNA extraction. Genomic DNA was isolated by phenol-chloroform extraction method (Sambrook and Russell, 2001) and the purity assessed by spectrophotometry; samples showing an optical density (OD) ratio (260 nm/ 280 nm) of between 1.7 and 1.9 being used for further analysis while samples outside this range were reprocessed. From the purified genomic DNA, a 522 bp region spanning over a part of intron 2 and exon 3 and a 94 bp region of exon 2 of the leptin gene was amplified using two different sets of primers (Table 1). PCR was carried out for both fragments in a final volume of 25 µL containing 100 µM dNTPs mix, 10 pmol of each primer, 2.5 µL of 10x PCR assay buffer containing 1.5 mM $MgCl_2$, 1.0 unit of *Taq* DNA polymerase and 80-100 ng of the purified bovine genomic DNA. The amplification of the 522 bp region was carried out using a thermal cycler (PTC-200, M J Research) pre-programmed for the following conditions: initial denaturation for 5 min at 94 °C followed by 35 cycles (denaturation at 94 °C for 15 s, annealing at 64 °C for 30 s and extension at 72 °C for 1 min) and a final extension at 72 °C for 5 min. The amplification program for the 94 bp fragment consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles (denaturation at 94 °C for 30 s, annealing at 54 °C for 45 s and extension at 72 °C for 30 s) and a final extension at 72 °C for 5 min. The PCR products were separated by 2% (w/v) agarose gel electrophoresis at 6 V $cm^{-1}$ for one hour. A 20 µL aliquot of the 522 bp PCR product was digested with 5 Units of *Bsa*AI restriction enzyme and 15 µL of 94 bp PCR product was digested with 5 Units of *Kpn*2I restriction enzyme at 37 °C overnight in a water bath, the reaction being stopped by adding 6x loading dye (bromophenol blue 0.25% (w/v) in 40% (w/v) sucrose) to the digested product. The digested PCR products were subjected to 3.5% (w/v) agarose gel electrophoreses and stained with ethidium bromide, the banding being visualized and documented using a gel documentation system. The gene and genotype frequencies were calculated according to the method of Falconer and Mackay (1996).

For the 522 bp PCR leptin fragment we found three *Bsa*AI digestion patterns in all the breed groups (*B. indicus*, *B. taurus* and crossbreds) indicating three genotypes; an intact 522 bp fragment as AA genotype; 441 and 81 bp fragments as GG genotype; and 522, 441 and 81 bp fragments as GA genotype. Our results agree with those of Lien *et al.* (1997) who for the first time described a guanine (G) to adenine (A) substitution (G → A substitution) in intron 2 of the leptin gene of Norwegian cattle. Although Konfortov *et al*. (1999) have reported that some of the *B. indicus* crossbred cattle have leptin gene polymorphisms that are not found in *B. taurus* breeds, our findings are the first report of leptin gene polymorphisms in purebred *B. indicus* cattle. Taken together, the present results suggest that leptin gene mutation might have occurred far back in evolution before the divergence of cattle into taurine (*B. taurus*) and indicine (*B. indicus*) cattle.

Buchanan *et al*. (2002) described a cytosine (C) to thymine (T) substitution (C → T substitution) in intron 2 of the leptin gene of the *B. taurus* breeds *i.e.* Angus, Charolais, Hereford and Simmental, suggesting the existence of C and T alleles and therefore CC, TT and CT genotypes. In our work involving the 94 bp leptin fragment we identified three *Kpn*2I digestion patterns in the Bos *taurus* and crossbreds indicating three genotypes: an intact 94 bp fragment as TT genotype; 75 and 19 bp fragments as CC genotype; and 94, 75 and 19 bp fragments as CT genotype. Konfortov *et al*. (1999) also reported this mutation in both *B. taurus* (Limousin, Charolais, Charolais x Friesian, Simmental x Friesian, Limousin x Friesian, Charolais x Hereford/Friesian, Jersey, Simmental breeds) and its crossbreds with *Bos indicus*. In our study, the Hariana, Sahiwal, Gir and Nimari breeds of *B. indicus* cattle showed only the 75 and 19 bp (CC genotype) restriction pattern, indicating absence of the C → T mutation. We also know that the TT polymorphism observed in our Holstein Friesian x Hariana crossbred population was inherited from Holstein Friesian sires. This C → T mutation may be used as a marker to differentiate the two subspecies of cattle along with many other markers.

The genotype frequencies of the *Bsa*AI-RFLP genotypes are presented in Table 2, from which it can be seen that the AA homozygote genotype frequency was very low (0.03 to 0.07) in all the indicine and exotic cattle populations. Of all the breeds, the Holstein Friesian population showed the highest GG homozygote genotype frequency

**Table 1** - Primer sequences used to amplify fragments of the leptin gene.

| | | | |
|---|---|---|---|
| *Bsa*AI-RFLP | L1: 5'-GTCTGGAGGCAAAGGGCAGAGT-3'<br>L2: 5'-CCACCACCTCTGTGGAGTAG-3' | 522 bp | Lien *et al*., 1997 |
| *Kpn*2I-RFLP | L5: 5'-ATGCGCTGTGGACCCCTGTATC-3'<br>L6: 5'-TGGTGTCATCCTGGACCTTCC-3' | 94 bp | Buchanan *et al.*, 2002 |

**Table 2** - Genotype frequency of the *Bsa*AI-RFLP genotypes in different cattle breeds.

| | Genotype frequencies | | |
|---|---|---|---|
| Breeds/ Genotypes | GG | GA | AA |
| Crossbreds | 0.54 | 0.43 | 0.03 |
| Holstein Friesian | 0.67 | 0.30 | 0.03 |
| Jersey | 0.57 | 0.38 | 0.05 |
| Hariana | 0.40 | 0.53 | 0.07 |
| Sahiwal | 0.47 | 0.50 | 0.03 |
| Gir | 0.55 | 0.40 | 0.05 |
| Nimari | 0.48 | 0.45 | 0.07 |

(0.67) and the lowest GA heterozygote frequency (0.30). The G and A allele frequency was 0.76G and 0.24A in the crossbreds, 0.82G and 0.18A in the Holstein Friesian cattle, 0.76G and 0.24A in Jersey cattle, 0.66G and 0.34A in Hariana cattle, 0.72G and 0.28A in Sahiwal cattle, 0.75G and 0.25A in Gir cattle and 0.71G and 0.29A in Nimari cattle. It can be seen that in all the populations the frequency of the A allele was low (0.18 to 0.34), suggesting that there may be a selection force acting against the A allele or favoring the G allele in these populations.

In case of the *Kpn*2I-RFLP, the CC, CT and TT genotype frequency was 0.68CC, 0.27CT and 0.05TT in the crossbreds, 0.25CC, 0.69CT and 0.06TT in the Holstein Friesian cattle and 0.18CC, 0.52CT and 0.30TT in Jersey cattle. The C and T allele frequency was 0.82C and 0.18T for crossbreds, 0.60C and 0.40T for Holstein Friesian cattle and 0.44C and 0.56T in Jersey cattle. The TT genotype was present at very low frequency in crossbred and Holstein Friesian cattle but was comparatively higher in Jersey cattle. The T allele frequency was comparatively low in crossbred population as compared to the two pure breeds of taurine cattle, possibly due to the absence of this mutation in Hariana cattle which contributed only the C allele to the crossbred population. Our findings for taurine cattle are similar to those of Buchanan *et al*. (2002) who reported T allele frequencies of 0.58 in Angus cattle, 0.34 in Charolais cattle, 0.55 in Hereford cattle and 0.32 in Simmental cattle (all taurine cattle) and Konfortov *et al*. (1999) who found the T allele frequency to be 0.41 in taurine cattle. In all the four breeds of indicine cattle, the C allele frequency was 1.0, possibly due to the absence of the V allele (valine) in these populations.

Summarizing, we found that the 522 bp leptin gene fragment is polymorphic in all the breeds studied, while the 94 bp leptin gene was polymorphic only in taurine cattle and *B. taurus* x *B. indicus* crossbreds. In all the polymorphic breeds the frequencies of mutant homozygotes were very low for both the fragments. The results also suggested that the *Bsa*AI-RFLP mutation has occurred far back in evolution before the divergence of taurine and indicine cattle while the *Kpn*2I mutation has occurred recently as indicated by the fact that it was only detected in taurine cattle.

## Acknowledgments

The authors are thankful to the Director of this institute for providing the necessary facilities for carrying out this work. We are also thankful to those in charge of the various livestock farms for giving permission to collect the blood samples.

## References

Buchanan FC, Fitzsimmons CJ, Van Kessel AG, Thue TD, Winkelman Sim DC and Schmutz SM (2002) Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet Sel Evol 34:105-116.

Falconer DS and Mackay TFC (1996) Introduction to Quantitative Genetics. 4th edition. Addison Wesley Longman Ltd., Essex, England, pp 1-2.

Gelderman H (1997) Investigations on inheritance of quantitative characters in animals by gene markers 1. Methods Theor Appl Gen 46:319-330.

Haegeman A, Van Zeveren A and Peelman LJ (2000) New mutation in exon 2 of the bovine leptin gene. Anim Genet 31:79.

Konfortov BA, Licence VE and Miller JR (1999) Re-sequencing of DNA from a diverse panel of cattle reveals a high level of polymorphism in both intron and exon. Mamm Genome 10:1142-1145.

Lande R (1981) The minimum number of gene contributing to qualitative variation between and within populations. Gene 99:541-553.

Lien S, Sundvold H, Klungland H and Vage DI (1997) Two novel polymorphisms in the bovine obesity gene (OBS). Anim Genet 28:245.

Pomp D, Zou T, Clutter AC and Barendse W (1997) Mapping of leptin to bovine chromosome 4 by linkage analysis of a PCR based polymorphism. J Anim Sci 75:1427.

Ramsay TG and Cranwell PD (1999) A review - Leptin: A regulator of feed intake and physiology in swine. Proceeding of the Seventh Biennial Conference of the Australasian Pig Science Association (APSA), 28 November - 1 December, Adelaide, Australia, pp 157-170.

Sambrook J and Russell DW (2001) Molecular Cloning - A Laboratory Manual. 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 5.61-5.90.

Wilkins RJ and Davey HW (1997) A polymorphic microsatellite in the bovine leptin gene. Anim Genet 28:376.

*Associate Editor: Pedro Franklin Barbosa*