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Characterization of leptin gene and analysis of genetic polymorphism in mithun

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Mithun (*Bos frontalis*) is having important role in socio-cultural and economic life of tribal people for meat purpose. The quality of meat is good and is liked by the tribal people, which has protein percentage of 14–19% and crude fat and carbohydrate of 0.4 to 3.58 and 0.06 to 4.97%, respectively (Heli *et al*. 1994). Leptin, a hormone encoded by the leptin (obese) gene and mainly produced by white adipose tissue, plays a significant role in regulation of growth and metabolism of animals (Hossner 1998). The physiological role and biology of Leptin (Houseknecht *et al.* 1998) is well reviewed and a number of genetic studies were conducted in cattle which showed that leptin as an important gene controlling various economic traits like growth, feed intake, marbling of meat etc in beef cattle (Buchanan *et al.* 2002, Carolyin *et al.* 1998, Haegeman *et al.* 2000, Legonigro *et al.* 2003, Lien *et al.* 1997, Nkrumah *et al.* 2006, Pomp *et al.* 1997 and Vallinoto *et al*. 2004). Thus looking to the importance of this gene in growth and development and scarcity of research work in mithun, this present investigation was conducted to find out the extent of variability in the mithun population through PCR-RFLP study of a leptin gene fragment reported previously in bovine (Lien *et al.* 1997) and characterized by nucleotide sequencing.

Mithuns (126) belonging to 4 strains (Nagaland, Arunachal, Manipur and Mizoram) were screened under this present investigation to find out the reported polymorphism. These animals belonged to the Institute farm as well as those collected from the breeding tract of mithuns. Genomic DNA was isolated from the blood samples by DNA isolation kit following manufacturer's instructions. DNA was isolated from fresh, refrigerated (4°C for 1–2 days) and frozen (–20°C) blood. The DNA samples so isolated were checked for quality in 2% agarose gel and concentration in a nano-drop spectrophotometer.

The PCR reaction mixture was prepared using dNTPs of 100 mm (each), primers of 10 pm (each), Mg Cl_2 1.5 mM and 1 ml of Taq DNA polymerase of 1 unit/ml concentration. The standardized PCR programme was comprised following steps - 95°C for 5 min (step 1- initial denaturation), 95°C for 30 sec (step 2 - denaturation), 60°C for 45 sec (step 3 - annealing), 72°C for 1 min (step 4 - extension), 72°C for 210 sec (step 5 - final extension). Step 2 to step 4 repeated for 30 cycles in a gradient thermal cycler.

A 522 bp fragment was amplified and digestion with *Bsa*AI generated fragments 441 and 81 bp (Fig. 2) with G in position 1620 whereas undigested single fragment of 522 bp was obtained with A in the same position. Here the bovine leptin gene sequence (Fig. 1) was used as reference sequence (Y11369). A 522 bp fragment of mithun leptin gene (GenBank Acc. No. GQ411537) spanning over a part of intron II (Fig. 1) to intron III was amplified using the primers reported by Lien *et al.* (1997).

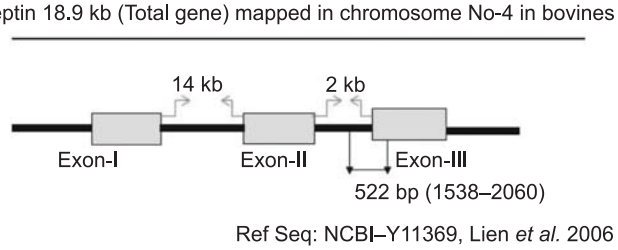

Fig. 1. Gene structure and area of amplification.

Table 1. Gene and genotype frequency

Genotypes	No of animals	Genotype frequency	Gene frequency	
			A allele	B allele
AA	04	0.03		
AB	42	0.34	0.20	0.80
BB	80	0.63		
Total	126	1.0		

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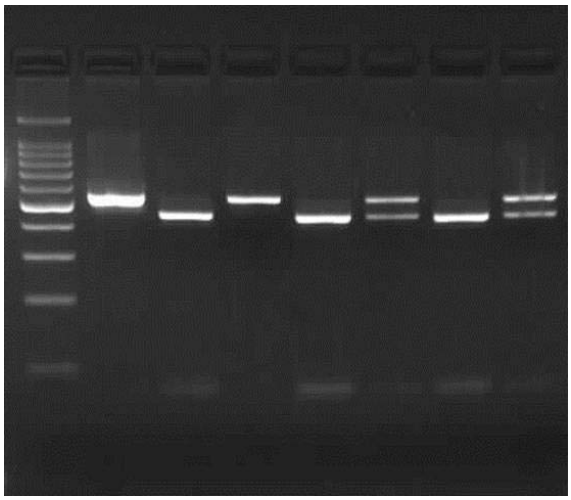

Fig. 2. PCR-RFLP product using BsaAI. M = 100 bp DNA marker, AA = uncut band at 522 bp, BB = two bands at 441 bp and 81 bp, AB = three bands at 522 bp, 441 bp and 81 bp.

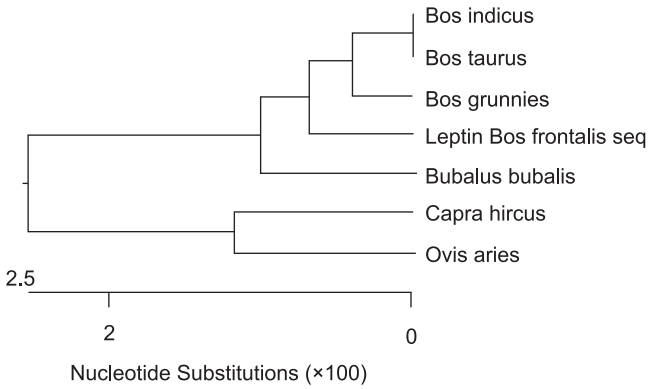

Fig. 3. Phylogenetic tree showing relationship among related species based on leptin sequence comparison.

The result of PCR-RFLP study revealed 3 BsaAI digestion pattern in 126 mithuns. The result suggested an intact 522 bp fragment as AA genotype; 441 and 81 bp fragments as BB genotype and 522, 441 and 81 bp fragments as AB genotype similar to the findings of Lien *et al.* (1997). The gene and genotype frequencies of the BsaAI-RFLP genotypes are presented in Table 1. The A and B allele frequency was 0.20 and 0.80 respectively. This result indicated the frequencies of allele A was low, suggesting that there may be a selection force acting against allele A and favouring allele B in Mithun. The similarity to the present finding was also seen in different breeds of *Bos indicus, Bos Taurus* and *Bos taurus* × *Bos indicus* crossbreed with low allele frequencies of allele A (Choudhary *et al.* 2005). Taniguchi *et al.* (2002) have isolated the bovine leptin gene including its promoter region. They had shown the exon-intron organization of the bovine leptin gene, which consisted of three exons and two introns and spanned about 18.9 kb, equivalent to that of human or mouse gene. There are 3 exons and 2 introns in the leptin gene of animals (Bidwel *et al.* 1997). A short untranslated sequence is exon 1, and the amino acid sequence located in the second and third exons. Lien *et al*. (1997) has identified 2 polymorphisms in the intron 2 of bovine leptin gene in Norwegian breed.

The amplified fragment of 522 bp of leptin gene was sequenced in an Automated DNA sequencer. The obtained sequence was subjected to NCBI/BLAST which confirmed the amplification of 522 bp fragment of leptin gene in mithun. The sequence was submitted to Gen bank (Acc No GQ 411537) and aligned using MEGALIGN program of DNASTAR software with the corresponding gene fragments of other ruminants available in public domain. The paired distances were presented (Table 2). The comparison of nucleotide sequence revealed high similarity (98.9%) with *Bos indicus* and *Bos taurus* followed by *Bos grunniens* (98.5%) *Bubalus bubalis* (97.9%), *Capra hircus* (95.0%) and *Ovis aries* (94.4%). From the phylogenetic tree revealed that the *Bos taurus and Bos indicus* formed a cluster with a closer similarity to mithun followed by *Bos grunniens, Bubalus bubalis, Capra hircus* and *Ovis aries* respectively (Fig. 3). In the coding sequence (> 230<522) only 4 mutations, viz. 446 (A/G), 485 (C/T), 497 (C/T) and 512 (A/C) which differentiate this fragment from that of related large ruminants like *Bos grunniens, Bos taurus and Bos indicus.*

This is the first report on the characterization and analysis of genetic polymorphism in the leptin (obese) gene of Mithun. Future work may be taken up to associate this polymorphism in leptin gene of Mithun with any economic trait like growth or meat quality in this animal.

Table 2. Paired distances of leptin gene fragments of mithun with other ruminants (ClustalW method)

	Bos frontalis	*Bos grunniens*	*Bos indicus*	*Bos taurus*	*Bubalus bubalis*	*Capra hircus*	*Ovis aries*
Bos frontalis	***	98.5	98.9	98.9	97.9	95.0	94.4
Bos grunniens	1.6	***	99.2	99.2	98.1	94.3	94.3
Bos indicus	1.2	0.8	***	100.0	98.1	94.6	94.6
Bos taurus	1.2	0.8	0.0	***	98.1	94.6	94.6
Bubalus bubalis	2.0	1.9	1.9	1.9	***	95.4	94.6
Capra hircus	5.0	5.6	5.2	5.2	4.8	***	97.3
Ovis aries	5.2	5.2	4.8	4.8	5.2	2.3	***

*Per cent similarity in upper triangle and percent divergence in lower triangle.

SUMMARY

The present study was designed to characterize leptin gene and to search polymorphism in this gene of mithun. This is the first report on the identification of polymorphism in the leptin gene of mithun. The leptin gene was found to be polymorphic with respect to the locus studied in a sample size of 126 animals. The BB genotype was highly prevalent in the population with a allelic frequency of 0.8 of B allele. The comparison of nucleotide sequence revealed high similarity (98.9%) with *Bos indicus* and *Bos taurus* followed by *Bos grunniens*, *Bubalus bubalis*, *Capra hircus* and *Ovis aries*. Phylogenetic tree revealed that the *Bos taurus and Bos indicus* formed a cluster with a closer similarity to mithun followed by *Bos grunniens, Bubalus bubalis, Capra hircus* and *Ovis aries* respectively. The comparison of DNA sequences of 522 bp leptin gene fragment of mithun with other bovine species revealed 4 mutations, viz. 446 (A/G), 485 (C/T), 497 (C/T) and 512 (A/C) which differentiate this fragment from that of related large ruminants like *Bos grunniens, Bos taurus and Bos indicus.*

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