Indian Journal of Animal Sciences **85** (1): 55–59, January 2015/Article

Identification of novel SNPs in *INHBB* gene of Indian goat

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Received: 25 July 2014; Accepted: 19 September 2014

ABSTRACT

Inhibin βB (*INHBB*) gene is considered as a possible candidate gene for the prolificacy trait based on the important role of inhibin in reproduction. A mutation (A782G) in exon 2 of *INHBB* gene has significant effect on litter size in Jining Grey goats. Thus the objective of the present study was to explore polymorphism (SNP) in exon 2 region of *INHBB* in a panel of Indian goats consisting of high, medium and low prolificacy goat breeds. Two pairs of primers, P1 and P2 were designed according to the exon 2 sequence of bovine *INHBB* with amplified product size of 244bp and 296bp respectively. Polymerase chain reaction followed by direct DNA sequencing revealed absence of already reported SNP (A782G) in Indian goats where favorable A allele was fixed. Two novel SNPs were identified in the amplified products of primer P2 in Indian goats. These included G→A mutation at base 693 and C→T mutation at base 840 of exon 2 (numbering is with respect to U16241 sequence) in Indian goats, which did not cause any amino acid change. Allele distribution was of similar magnitude in Indian goats with predominance of wild allele (G=0.93 and C=0.96). Heterozygote was observed for G693A mutation in one animal each of Beetal, Osmanabadi, Sangamneri and Ganjam breeds, however heterozygotes of C840T mutations were observed in 4 animals of Osmanabadi breed only. Novel SNPs need further investigations for association with prolificacy trait that would have important implications for the goat industry.

Key words: Goat, Inhibin βB (*INHBB*) gene, Polymorphism, Prolificacy

Inhibin is a glycoprotein hormone belonging to the transforming growth factor-β superfamily that suppresses follicle-stimulating hormone (FSH) synthesis and secretion (Robertson *et al.* 1985, Woodruff *et al.* 1996). It has 2 subunits, α and β, linked by disulphide bonds. Two inhibins, sharing a common α-subunit but different β-subunits (βA or βB) have been identified (Mason *et al.* 1985). Follicles are a major source of inhibin in sheep (Rodgers *et al.* 1989). *INHBB* gene were mapped to chromosome 2q31–33 in sheep and goat (Goldammer *et al.* 1995). Based on the important role of inhibin in reproduction, this gene was considered as a possible candidate gene for the prolificacy trait. In fact, inhibin βB (*INHBB*) gene has significant effect on litter size in some sheep breeds (Jaeger and Hiendleder 1994, Chu *et al.* 2011). Similarly, polymorphism (A872G) associated with prolificacy in *INHBB* exon 2 region was reported in the Jining Grey goat (Chu *et al.* 2012), a local breed of China that has significant characteristics of sexual precocity, year round oestrus, and high prolificacy (Tu 1989). The Jining Grey goat does with genotype AA had 0.73 or 0.84 kids more than those with genotype AB or BB, while the does with genotype AB had 0.11 kids more than those with genotype BB. These results provide further evidence that inhibin is an important functional factor in goat ovary.

No such information is available with respect to Indian goats. Prolificacy is an economically important trait which will play an important role in determining the efficiency of goat production in India. Our country is blessed with a rich repository of goat germplasm being home to more than 20 recognized breeds of goat. Indian goat breeds exhibit colossal variations in growth, production of meat, milk and fiber, disease resistance, heat tolerance and fecundity. Hence a study was conducted firstly to sequence characterize exon 2 of *INHBB* gene in Indian goats by selecting high prolificacy (Black Begal, Barbari, Beetal and Malabari), medium prolificacy (Osmanabadi, Sangamneri, Jakhrana) and low prolificacy (Ganjam, Sirohi) goat breeds and secondly to establish in Indian goats the status of SNP (A872G) reported to be associated with caprine prolificacy trait.

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MATERIALS AND METHODS

Animals and genomic DNA isolation: Nine well-recognized breeds of Indian goats differing in prolificacy (Barbari, Beetal, Black Bengal, Malabari, Jakhrana (Twinning>40%), Osmanabadi, Sangamneri (Twinning 20–30%), Sirohi and Ganjam (Twinning <10%) and geographic distribution were used for polymorphism scanning (Fig. 1). Five unrelated animals of each breed were selected from their breeding tracts. Blood was collected aseptically from the jugular vein in a vacutainer tube containing EDTA as anticoagulant. All samples were delivered back to the laboratory in an ice box. The genomic DNA was extracted from white blood cells using standard phenol-chloroform extraction protocol (Sambrook and Fristch 1989).

PCR amplification, sequencing and polymorphism detection: Two pairs of primers were designed according to the sequence of exon 2 (GenBank accession number U16241) of bovine *INHBB* gene published by Thompson *et al.* (1994). The expected amplification fragment size was 244 bp for primer P1 and 296 bp for primer P2. The primer sequences used were as follows: Primer P1: F: 5'- CGGGT-CCGCCTGTACTTCTT-3'; R: 5'- CCTGGATGGGTTCG-GTGAG-3'. Primer P2: F: 5'-CACCGGCTACTAT-GGGAACT-3'; R: 5'-ACTTGCCACACCCTGGTC-3'. Polymerase chain reaction was carried out in 25 µl reaction volume with about 50–100 ng genomic DNA using i-cycler. The reaction mixture consisted of 250 µM each of dATP, dCTP, dGTP, dTTP, 2.0 mM $MgCl_2$, 50 pmol primer, 1U *Taq* polymerase and corresponding *Taq* buffer. Amplification conditions were as follows: initial denaturation for 3 min at 95°C; followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 59.5°C for 30 sec, extension at 72°C for 1 min; and finally extension at 72°C for 10 min. The PCR products were separated by electrophoresis on 1.8% agarose gel in parallel with a 50 bp DNA ladder, enzymatically purified and sequenced using both primers (forward and reverse) by the dideoxynucleotide chain termination reaction (Sanger *et al.* 1977). Sequencing was performed in an automated ABI - 3100 sequencer using the terminator cycle sequencing ready reaction kit.

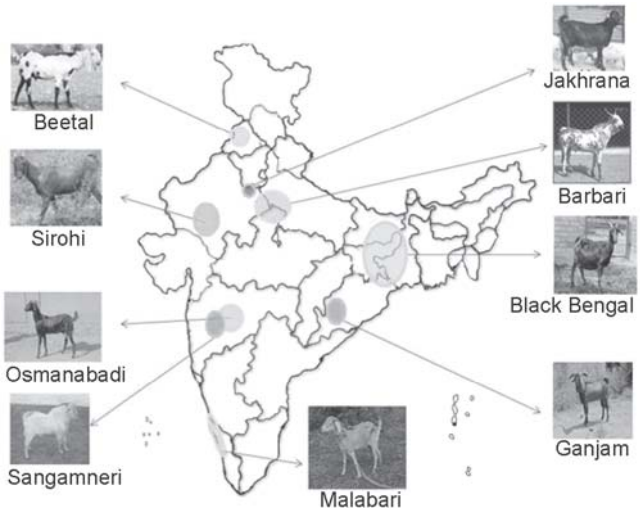

Fig. 1. Distribution of Indian goat breeds selected for characterization of *INHBB* gene.

Sequence data were edited manually using Chromas Ver. 2.33, (http://www.technelysium.com.au/chromas.html). Multiple sequence alignments were performed with MegAlign program of LASERGENE software version 5.07 to identify polymorphisms (mutations or single nucleotide polymorphisms). The coding DNA sequence was conceptually translated to amino acid sequences using Chromaspro software. Nucleotide BLAST program at NCBI

Table 1. Location of nucleotide substitutions and SNPs identified in goat *INHBB* gene

Nucleotide position: cattle/ sheep/goat*	Cattle (U16241)	Sheep (FJ167874.1)	Exotic goat (EF551362)	Indian goat	Type of change	Genotype frequency	Allele Frequency
129/71/105	G	A	A	A	Transition		
207/149/183	C	A	A	A	Transversion		
217/159/193	G	T	T	T	Transversion		
218/160/194	G	T	T	T	Transversion		
300/242/276	A	A	C	C	Transversion		
320/262/296	C	C	C	A	Transversion		
654/596/630	G	C	C	C	Transversion		
693/635/669	G	G	G	G/A	Transition(SNP)	GG=0.89 GA=0.09 AA=0.02	G=0.93 A=0.07
804/746/780	C	T	T	T	Transition		
831/773/-	T	A	-	A	Transversion		
839/-/-	G	-	-	A	Transition		
840/-/-	C	-	-	C/T	Transition(SNP)	CC=0.91 CT=0.09	C=0.96 T=0.04
847/-/-	G	-	-	A	Transition		
849/-/-	T	-	-	C	Transition		

*As per accession number: Cattle (U16241), sheep (FJ167874.1) and exotic goat (EF551362).

(http://www.ncbi.nlm.nih.gov/BLAST) was used for sequence homology searches in public databases.

RESULTS AND DISCUSSION

Partial *INHBB* gene of 9 Indian goat breeds was amplified using 2 pairs of primers. The amplified products were consistent with the target fragments and had a good specificity, which could be directly analyzed by sequencing. Sequences of exonic 2 region of *INHBB* gene in Indian goat were compared with goat (EF551362), sheep (FJ167874.1), as well as cattle (U16241). Sequences obtained from different Indian goat breeds exhibited 100% similarity with the exotic goat (EF551362). A single variation was recorded as compared to sheep whereas, maximum number of nucleotide changes (14) were observed in comparison to cattle (Table 1).

The polymorphic sites were explored in the 9 different goat breeds including the high fecundity and low fecundity goat breeds such as Black Bengal, a meat-type Indian goat breed, which is famous for its quality lean meat production and well known for giving multiple births with a prolificacy of above 180% (Zeshmarani *et al*. 2007) and Ganjam goat, a low prolificacy, late maturing, annual single kidding and slow growing goat (Acharya 1982). Sequencing revealed one nucleotide mutation (G→A) at base 693 and another (C→T) at base 840 (numbering as per cattle U16241) of exon 2 of *INHBB* gene in Indian goats. These mutations did not cause any amino acid change and were synonymous in nature.

No obvious difference could be established among the Indian goat breeds differing in the prolificacy, on the basis of allele distribution at 2 novel SNPs identified in the present study. Five goat breeds were monomorphic (GG) for mutation G693A whereas, Beetal, Sangamneri and Ganjam presented 2 (GG, GA) and Osmanabadi had all the 3 possible genotypes (Fig. 2).

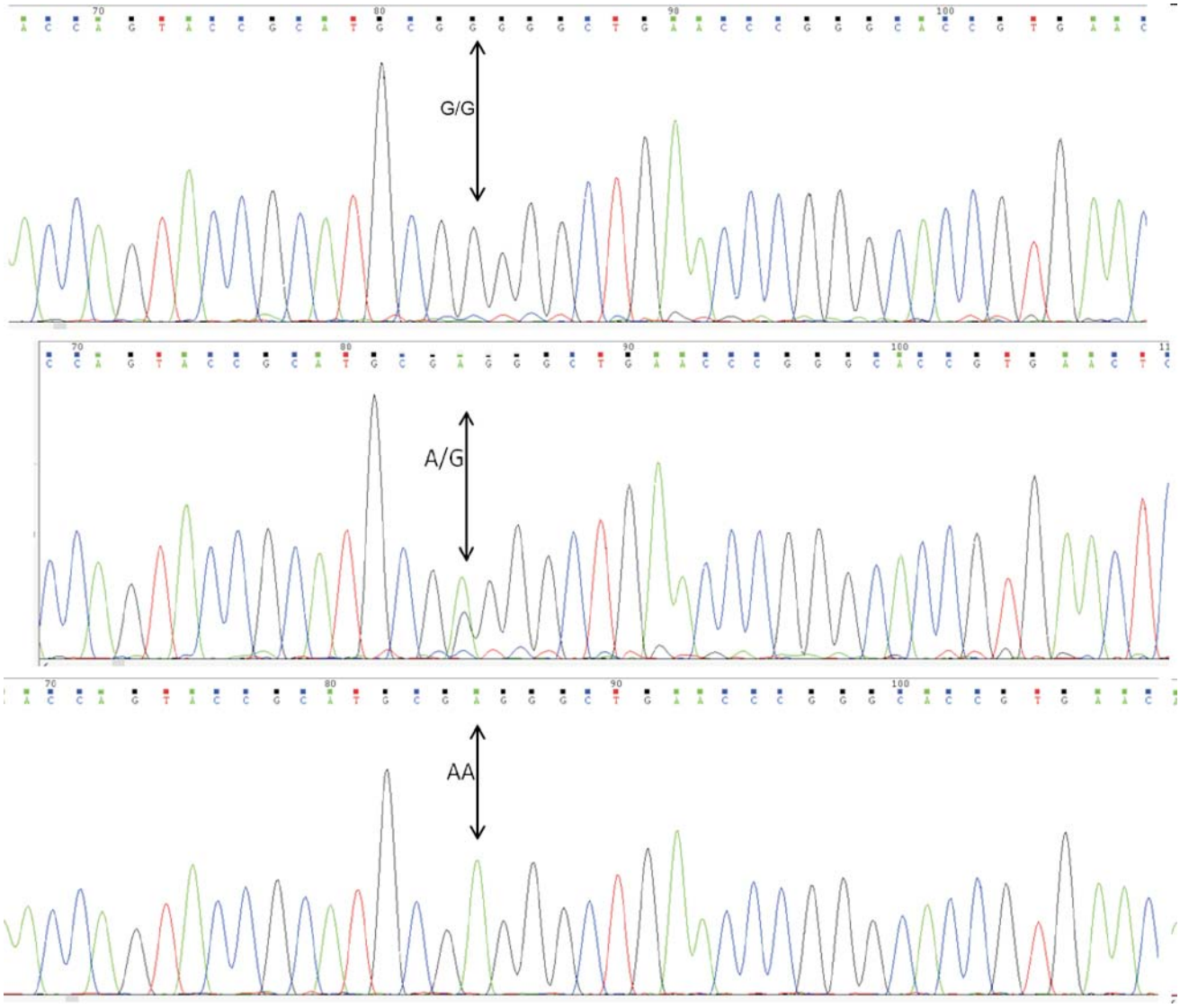

Fig. 2. Sequencing chromatogram of different genotypes at SNP G693A identified in Indian goats (arrow pointed to the mutation site).

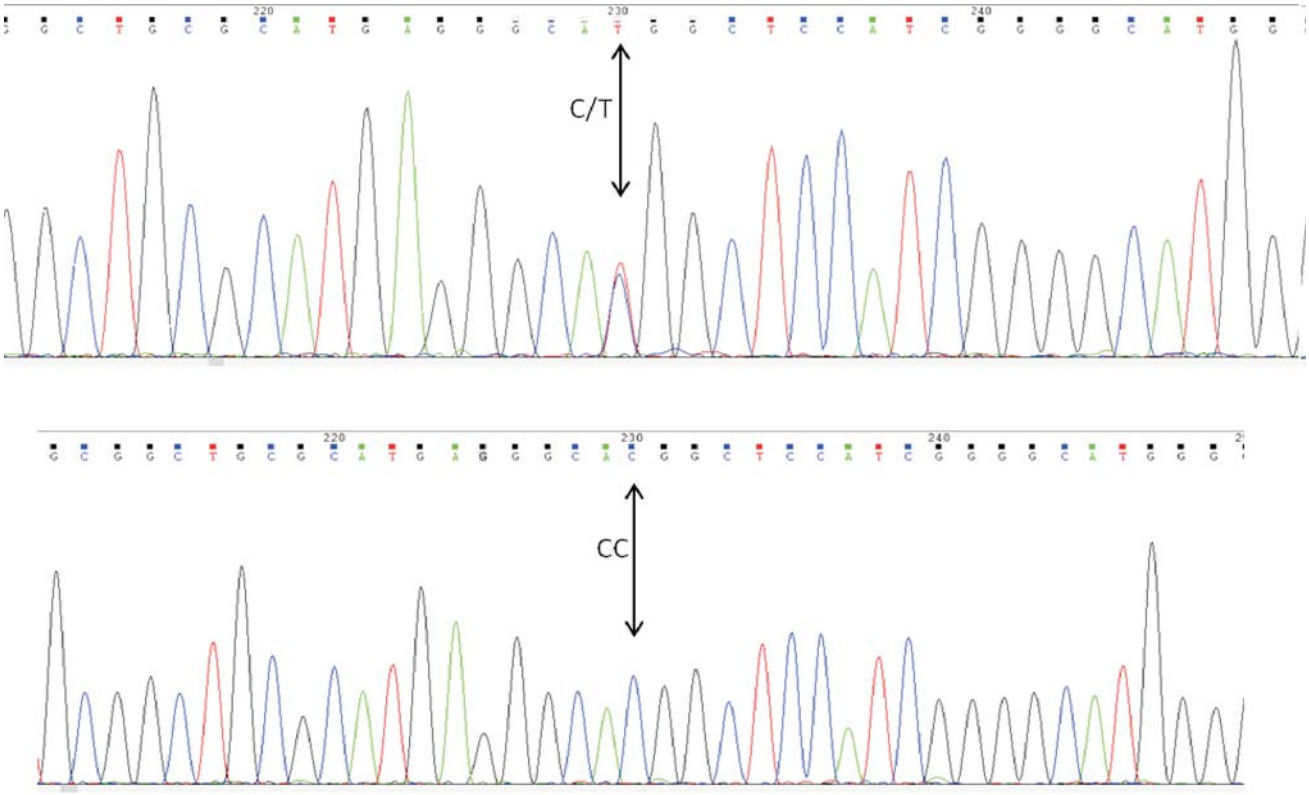

Fig. 3. Sequencing chromatogram of different genotypes at SNP C840T identified in Indian goats (arrow pointed to the mutation site).

On the other hand mutation C840T was monomorphic (CC) in all the investigated goat breeds except Osmanabadi where 80% animals were heterozygote (CT) and thus displayed polymorphism at this locus (Fig. 3).

In the present study homozygous does for the minor allele were rare for G693A locus and all together absent at C840T locus in the panel consisting of different Indian goats. This can be attributed to various reasons such as minor allele was rare so that homozygous individuals could not be detected in 45 Indian goats. The other reason could be that these homozygous does suffered from distinct developmental and reproductive defects, as well as from the embryonic death, or they were sifted out before arriving at reproductive age. Mating heterozygous bucks to heterozygous does, extensive sampling (larger goat samples of both sexes and more goat breeds), as well as DNA analysis would be required to establish the definite reason for this hypothesis.

Inhibin plays an important role in FSH regulation and acts as a growth factor in the ovary, thus it is proposed as a candidate gene for reproductive performance (Xue *et al.* 2004, Phillips 2005). Polymorphisms at the ovine *INHBB* gene were identified (Hiendleder *et al.* 1992, 1996, Jaeger and Hiendleder 1996, Chu *et al.* 2011) where *INHBB* gene had obvious genetic effect on litter size of sheep and litter size was significantly influenced ($P<0.05$) by sire, kidding season, parity and *INHBB* genotype. Similarly 3 genotypes (AA, AB and BB) were identified in Jining Grey and 2 (AB and BB) in Inner Mongolia Cashmere and Angora goats for the mutation (A→G) at base 782 of exon 2 of *INHBB* gene (Chu *et al.* 2013). This locus is associated with prolificacy trait in Jining Grey goats as genotype AA delivered 0.73 ($P<0.05$) or 0.84 ($P<0.05$) kids more than those with genotype AB or BB, while does of genotype AB delivered 0.11 ($P>0.05$) kids more than does of genotype BB. However, this is not the polymorphic locus in Indian goats where allele A, associated with the larger litter size has been fixed. On the contrary, 2 novel polymorphic loci (G693A and C840T) were identified in the exon 2 region of *INHBB* gene which implies that molecular genetic basis of hyperprolificacy in Jining Grey goat of China and Indian goats may be different. This is not the first instance of difference in SNPs of exotic and Indian goats. Similar differences in the loci of a gene were reported earlier for *GDF9* (Ahlawat *et al.* 2012), *BMP4* (Sharma *et al.* 2013) and *BMPR1B* (Ahlawat *et al.* 2014) genes. Identification of novel SNPs in Indian goats may be attributed to the fact that goat population presents a different molecular spectrum than exotic goat breeds. Further investigations involving extensive sampling from larger number of goat breeds should be taken up to establish the dispersal of A allele (A782G) as well as positive effect of favorable allele on prolificay in Indian goats as in Chinese Jining Grey goat. To our knowledge, this is the first report of mutations in the Indian goat *INHBB* gene. But whether the reproduction trait of goat is associated with the 2 novel polymorphisms needs to be defined by association studies with the reproductive traits.

ACKNOWLEDGEMENTS

This work was financially supported by the Network Project on Animal Genetic Resources (ICAR).

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