



Evaluation of Bone Morphogenetic Protein-4 gene polymorphism for growth traits in Indian goat breeds

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ABSTRACT

The genetic improvement of production traits can be made through marker assisted selection using a candidate gene approach. Bone morphogenetic proteins (BMPs) are multifunctional growth factors that belong to the transforming growth factor b (TGF-b) superfamily. This study aimed to detect the genetic polymorphism of BMP-4 in different goat breeds by polymerase chain reaction-single strand confirmational polymorphism (PCR-SSCP) and association of polymorphic variants with growth traits. The amplified fragments of BMP4 gene of 380 bp size were analysed using SSCP in non-denaturing PAGE and the results showed the presence of two genotypes: AA (47-67%) and BB (33-53%) in Barbari, Sirohi and Black Bengal breed. The association of BMP-4 polymorphism with different growth trait parameters showed non-significant effect of genotypes. However, some genotypes showed non-significant superiority over others. Further research on a large population is required to validate the role of the BMP-4 gene in goat growth traits.

Keywords: BMP-4, Goat, Growth traits, PCR-SSCP

Molecular genetics techniques are of great interest in the identification of genetic variations in candidate genes which are associated with different productive and reproductive traits in farm animals (Missohou *et al.* 2006). These genetic variations affect the physiological pathways that consequently lead to quantitative variations in different phenotype characteristics (Lan *et al.* 2007). In quantitative genetics, there are a number of genes which are associated with growth, development and physiological function which were studied as excellent candidates for linkage relationships with quantitative traits of economic importance. Bone morphogenetic protein 4 (BMP4) is a BMP family member. This protein has anabolic and pleiotropic functions that are assumed to play crucial roles in skeletal development (Hogan 1996); bone formation (Bellusci *et al.* 1996); and white, beige, and brown adipogenesis regulation (Elsen *et al.* 2014). During embryonic development, BMP4 is involved in many important processes, such as mesoderm progenitor and endothelial induction, mesodermal differentiation, somite formation, ectodermal differentiation, and myogenesis induction (Vainio *et al.* 1993, Graham *et al.* 1994, Graff 1997). In India, there is a shortage in meat production comparing to the nutritional requirements of people and this

gap is increasing day by day. To reduce this gap between demand and supply, production potential of indigenous goat breeds needs to be increased. This improvement in meat production potential of goats can be achieved by using new genetic technology for selection of growth traits. There are several well recognised indigenous goat breeds as well as large population of non-descript goats but suffer lack of knowledge about the genetic polymorphism of growth-related genes and their association with growth traits in Indian goat breeds. This study aimed to detect the genetic polymorphism of BMP-4 gene by PCR-SSCP and association of polymorphic variants with growth traits in Barbari, Sirohi and Black Bengal goats.

MATERIALS AND METHODS

Sample collection and DNA isolation: A total of 90 goats, comprising 30 each of Barbari, Sirohi and Black Bengal, were investigated for association of the BMP-4 gene with growth traits. About 5 ml of blood was collected from the external jugular vein in a vacutainer tube containing anticoagulant from each goat and kept in an ice box until delivered back to the laboratory. Records of birth weight and body weight at birth and at 3, 6, 9, and 12 months of age were collected from Amnala goat farm, Jabalpur. The genomic DNA was extracted from white blood cells using a standard phenol-chloroform extraction protocol (John *et al.* 1991). The DNA concentration was determined using a NanoDrop-1000 Spectrophotometer and then diluted to the working concentration of 30 ng/L. The DNA samples

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were stored at -20°C for further use.

PCR amplification of targeted gene fragment of BMP-4 gene: A set of the following published primers (i.e. Forward: 5'-CTGGGGAAATGTTTGGTA-3' and Reverse: 5'-GCTAAGAGTTGGGTGATGAG-3') were used to amplify the intron-2 region of the BMP-4 gene with 380 bp size (Ariyaratne *et al.* 2016). The PCR reaction mixture of a total 25 µl consisted of 1 µl of each primer (1.0 µM), 12.5 µl PCR master mix, 3 µl of genomic DNA and 7.5 µl of nuclease free water. The PCR cycling protocol was standardized as initial denaturation at 95°C/5 min, 35 cycles of denaturation at 94°C/45 s annealing at 57°C/60 s and extension at 72°C/60 s, with a final extension at 72°C/10 min. The amplification was verified by electrophoresis on 2% agarose gel in 1× TBE buffer using 100 bp DNA ladder as a molecular size marker for confirmation of the size of the PCR products. The gel was stained with ethidium bromide and visualized on UV trans-illuminator under Gel-Documentation System (Bio-Rad).

Single strand conformation polymorphism (SSCP): PCR products (5 µl) were mixed with 15 µl of denaturing solution (95% formamide, 25mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 5 min at 95°C and then snap chilled on ice for 15 min. Denatured DNA samples were subjected to PAGE in 1× TBE buffer at a constant voltage of 200 V for 7 hrs. The gel (29:1 Acrylamide/Bisacrylamide) was stained with 0.1% silver nitrate. The SSCP gel was visualized to see the polymorphic patterns and genotyped accordingly.

DNA sequencing and Sequence analysis: Representative samples of amplified BMP-4 gene were sequenced by Sanger's dideoxy chain termination sequencing method in an automatic ABI Prism DNA Sequencer. Sequence analysis and alignment were carried out using Mega-6 software. The nucleotide sequence of the tested gene in goat were submitted to GenBank (NCBI, BankIt).

Statistical analysis: The population genetics parameters like genotypic frequencies, allele frequencies and Chi-square test for HWE were estimated using Popgene-32. Association of the genotypes with growth traits of goats were determined by the analysis of variance of quantitative traits using SPSS-16 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Bone Morphogenetic Protein 4 (BMP4) gene belongs to TGF-β (Transforming Growth Factor-beta) super family. Bone Morphogenetic Proteins (BMPs) play important role in embryonic development, homeostasis, repairing of various tissue, cell differentiation and apoptosis (Wozney *et al.* 1988). BMPs are important due to their crucial role in follicular growth and differentiation, cumulus expansion and ovulation (Shimasaki *et al.* 1999). So far, more than 30 members have been identified in BMP family of which BMP4 is the most important one. Many studies have revealed a positive correlation of metabolic rate with growth rate and body mass in guinea fowl and Japanese quail (Dietz and Drent 1997), growth rate in salmonids

(Rosenfeld *et al.* 2015), growth rate in beef cattle (Ellenberger *et al.* 1989) and growth and body composition in growing lambs (Zhang *et al.* 2017).

PCR-SSCP of BMP-4 gene: The amplified fragment of 380 bp of the BMP4 gene was obtained from all tested DNA samples of goats (Fig. 1). The 380 bp PCR products were resolved on 6% PAGE and the major bands in the upper region of the gel were scored. The PCR-SSCP analysis of the intron-2 region of the BMP4 gene (380 bp) revealed two polymorphic patterns, named AA and BB genotypes, in all the three breeds of goat (Fig. 2).

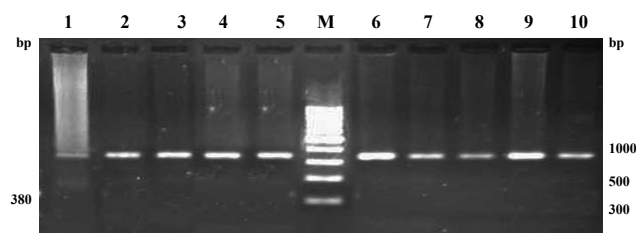


Fig. 1. Ethidium bromide-stained gel of PCR products representing amplification of BMP-4 gene in goats. (M: 100 bp ladder; Lanes: 1-3, BMP4 gene PCR product in Barbari; Lanes 4-7: Sirohi; Lanes 8-10: Black Bengal goats)

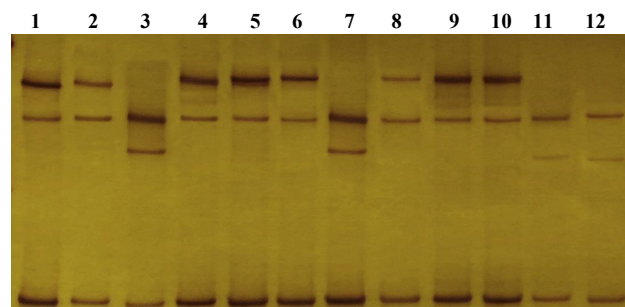


Fig. 2. Polymorphic SSCP patterns of BMP4 gene. (Lane 1,2, 4-6, 8-10: AA Genotype; Lane 3,7,11-12: BB Genotype)

Genotype frequencies in Barbari, Sirohi and Black Bengal goats were found to be 0.47, 0.67 and 0.50 for the AA genotype and 0.53, 0.33 and 0.50 for the BB genotype, respectively. Whereas, the allelic frequencies in Barbari, Sirohi, and Black Bengal goats were 0.47, 0.67 and 0.50 for A allele and 0.53, 0.33 and 0.50 for B allele, respectively. The highly significant ($p < 0.01$) chi-square values in Barbari, Sirohi and Black Bengal goats showed that the populations were not in Hardy Weinberg equilibrium at this locus (Table 1).

The current findings of PCR-SSCP polymorphism in BMP4 gene are in agreement with previous finding in Xuhuai White goat, Boer goat and Haimen goats (Fang *et al.* 2010), nine different Indian breeds of goat (Sharma *et al.* 2013), Jinning Grey goats (Chu *et al.* 2011) and in Srilankan non-descript, crossbreds and Jamunapari goats (Ariyaratne *et al.* 2016). However, Sarma *et al.* (2019) reported a monomorphic pattern in Assam hill goats.

The populations of Sirohi, Barbari and Black Bengal goats were found in HW disequilibrium at the BMP4 gene locus. However, Hardy-Weinberg equilibrium was reported

Table 1. Frequencies of genotypes and alleles at BMP4 gene locus

Genotype / Allele	Breed		
	Barbari	Sirohi	Black Bengal
AA	0.47 (14)	0.67 (20)	0.50 (15)
BB	0.53 (16)	0.33 (10)	0.50 (15)
A	0.47	0.67	0.5
B	0.53	0.33	0.5
Chi-square value	31.04**	31.31**	31.03**

**, Significant ($p < 0.01$); Figures in the parenthesis shows number of observations.

at this locus in Xuhuai White goat, Boer goat, Haimen goats and Jinning Xuhuai White goat, Boer goat, Haimen goats and Jinning Grey goats (Fang *et al.* 2010, Chu *et al.* 2011). The Hardy Weinberg disequilibrium observed in present study may be due to recent selection, mutation, migration, or small sample size of goats on the farm.

Association of BMP4 gene polymorphic variants with growth traits: The results of the analysis of variance for growth traits of different goat breeds have been presented in Table 2. The body weight at birth, 3 months of age, 6 months of age, 9 months of age and 12 months of age showed significant ($p < 0.01$) differences among breeds, whereas the effect of genotypes and breed $\times$ genotype interaction was found to be non-significant ($p > 0.05$) for all these traits (Table 2).

It has been reported that morphogenetic proteins (BMPs) have multiple roles in skeletal development, homeostasis and regeneration in mammals and livestock (Shimaski *et al.* 1999), but very few traceable information are available regarding the association of BMP4 gene variants with body weight of indigenous goat breeds. In contrast to the current findings, significant associations of body weight and conformational traits with BMP4 variants have been reported in goats (Fang *et al.* 2009 and 2010), sheep (Ibrahim 2019) and cattle (Zhong *et al.* 2010). In the current study, no significant association between body weight parameters and genotypes was recorded, but considering the important

role of the BMP4 gene on muscle and bone growth, further study on a larger population can be done to establish the effect of the BMP4 gene on growth.

Sequencing and sequence analysis of BMP4 gene: The representative samples of BMP4 gene were sequenced by Sanger's dideoxy chain termination sequencing method in Automatic ABI Prism DNA sequencer (Eurofin Pvt. Ltd. Bengaluru). The obtained sequences were subjected to NCBI BLAST for sequence analysis. The amplified fragments were confirmed to be of the BMP4 gene after comparison with goat sequences. Sequencing confirmed the amplification of the BMP4 gene in Sirohi, Barbari and Black Bengal.

Comparative study of BMP4 gene fragment among different goat breeds: A comparative study of the BMP4 gene fragment among different goat breeds was carried out by ClustalW method of sequence alignment using MEGA6 software. A total of seven sequences (1 new sequence from present study and six sequences retrieved from NCBI) were aligned using ClustalW method. The nucleotide sequence of the BMP4 gene obtained in the present study showed 99% similarity with the other goat and sheep BMP4 gene locus. The BMP4 sequences of present study showed similarity of 99% with *Capra hircus* (NCBI accession no. XM_18053541, XM_18053542, XM_13967192, XM_13967193, EU_104684) and similarity of 99.84% with *Ovis aries* (NCBI accession no. NM_001110277), respectively.

Phylogenetic study among goat breeds: The phylogenetic tree for the BMP4 gene was constructed using the Neighbor-Joining (NJ) method based on the Maximum Composite Likelihood model. Phylogenetic analyses were conducted using MEGA6 software. The nucleotide sequences of BMP4 of Barbari in the present study and different goat sequences of the same gene locus retrieved from NCBI were used for the construction of phylogenetic tree. Phylogenetic analysis revealed that the goat sequences retrieved from NCBI was close while the Barbari sequence from present study was in different clade.

Table 2. Mean body weight (kg) from birth to 12 months of age in goat breeds

Breed	Genotype	Growth trait				
		BW (0D)	BW (3M)	BW (6M)	BW (9M)	BW (12M)
Barbari	AA (14)	1.83 ^a ±0.08	9.02 ^c ±0.25	11.51 ^b ±0.28	13.94 ^b ±0.25	16.44 ^b ± 0.39
	BB (16)	1.89 ^a ±0.07	9.46 ^{bc} ±0.23	12.04 ^b ±0.26	14.41 ^b ±0.25	17.53 ^b ±0.37
	Mean (30)	1.85 ^a ±0.05	9.24 ^b ±0.25	11.78 ^b ±0.19	14.18 ^b ±0.18	16.99 ^b ± 0.27
Sirohi	AA (20)	1.97 ^a ±0.07	10.68 ^a ±0.21	14.30 ^a ±0.23	17.63 ^a ±0.22	22.28 ^a ± 0.32
	BB (10)	1.79 ^a ±0.09	10.47 ^{ab} ±0.29	14.10 ^a ±0.33	17.36 ^a ±0.29	21.95 ^a ± 0.47
	Mean (30)	1.88 ^a ±0.06	10.57 ^a ±0.13	14.20 ^a ±0.20	17.49 ^a ±0.19	22.11 ^a ±0.29
Black Bengal	AA (15)	1.30 ^b ±0.08	5.06 ^d ±0.24	7.33 ^c ±0.27	9.77 ^c ±0.26	13.72 ^c ± 0.38
	BB (15)	1.35 ^b ±0.08	4.95 ^d ±0.24	7.29 ^c ±0.27	9.71 ^c ±0.26	13.49 ^c ±0.38
	Mean (30)	1.32 ^b ±0.05	5.00 ^d ±0.07	7.31 ^c ±0.19	9.73 ^c ±0.18	13.61 ^c ±0.27

Figures in parenthesis show number of observations, values with different superscript in columns differ significantly ($p < 0.01$). BW(0D), Birth weight; BW(3M), Body weight at 3 Months; BW(6M), Body weight at 6 Months; BW(9 M), Body weight at 9 Months; BW(12M), Body weight at 12 Months.

Evolutionary divergence of BMP4 gene: Evolutionary divergence of BMP4 gene sequences was estimated using a Maximum Composite Likelihood model by a bootstrap procedure (1000 replicates) in MEGA6 software. The below diagonal value showed the per cent divergence, while the above diagonal values showed the per cent similarity between goat and sheep sequences at the BMP4 gene locus. The result showed 99% similarity within the compared goat sequences.

In conclusion, the currently screened goat population showed only two genotypes (AA and BB) at the BMP4 gene locus. The association of the BMP-4 gene with growth traits at different intervals showed a non-significant effect of genotypes. However, some genotypes showed non-significant superiority over others. Further research on a large population is required to validate the role of the BMP-4 gene in goat growth traits.

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