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

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Received: 9 December 2021; Accepted: 28 July 2022

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 β (TGF-β) 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

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
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'-CTGGGGAAATGTGTTGGTA-3' and Reverse: 5'-GCTAAGAGTTGGTTGATGAG-3') were used to amplify the intron-2 region of the BMP-4 gene with 380 bp size (Ariyarathne et al. 2016). The PCR reaction mixture of a total 25 μl consisted of 1 μl of each primer (1.0 mM), 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/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-Document 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).

![](image1)

**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)**

![](image2)

**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 (Ariyarathne 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
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 deoxy 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 1. Frequencies of genotypes and alleles at BMP4 gene locus

<table>
<thead>
<tr>
<th>Genotype / Allele</th>
<th>Barbari</th>
<th>Sirohi</th>
<th>Black Bengal</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (14)</td>
<td>0.47±0.08</td>
<td>0.67±0.20</td>
<td>0.50±0.15</td>
</tr>
<tr>
<td>BB (16)</td>
<td>0.53±0.16</td>
<td>0.33±0.10</td>
<td>0.50±0.15</td>
</tr>
<tr>
<td>A (20)</td>
<td>0.47±0.07</td>
<td>0.67±0.26</td>
<td>0.50±0.15</td>
</tr>
<tr>
<td>B (14)</td>
<td>0.53±0.07</td>
<td>0.33±0.26</td>
<td>0.50±0.15</td>
</tr>
</tbody>
</table>

Chi-square value 31.04** 31.31** 31.03**

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

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

<table>
<thead>
<tr>
<th>Breed</th>
<th>Genotype</th>
<th>BW (0D)</th>
<th>BW (3M)</th>
<th>BW (6M)</th>
<th>BW (9M)</th>
<th>BW (12M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbari</td>
<td>AA (14)</td>
<td>1.83±0.08</td>
<td>9.02±0.25</td>
<td>11.51±0.28</td>
<td>13.94±0.25</td>
<td>16.44±0.39</td>
</tr>
<tr>
<td></td>
<td>BB (16)</td>
<td>1.89±0.07</td>
<td>9.46±0.23</td>
<td>12.04±0.26</td>
<td>14.41±0.25</td>
<td>17.53±0.37</td>
</tr>
<tr>
<td></td>
<td>Mean (30)</td>
<td>1.85±0.05</td>
<td>9.24±0.25</td>
<td>11.78±0.19</td>
<td>14.18±0.18</td>
<td>16.99±0.27</td>
</tr>
<tr>
<td>Sirohi</td>
<td>BB (10)</td>
<td>1.79±0.09</td>
<td>10.47±0.29</td>
<td>14.10±0.33</td>
<td>17.36±0.29</td>
<td>21.95±0.47</td>
</tr>
<tr>
<td></td>
<td>Mean (30)</td>
<td>1.88±0.06</td>
<td>10.57±0.13</td>
<td>14.20±0.20</td>
<td>17.49±0.19</td>
<td>22.11±0.29</td>
</tr>
<tr>
<td>Black Bengal</td>
<td>BB (15)</td>
<td>1.35±0.08</td>
<td>4.95±0.24</td>
<td>7.29±0.27</td>
<td>9.71±0.26</td>
<td>13.49±0.38</td>
</tr>
<tr>
<td></td>
<td>Mean (30)</td>
<td>1.32±0.05</td>
<td>5.00±0.07</td>
<td>7.31±0.19</td>
<td>9.73±0.18</td>
<td>13.61±0.27</td>
</tr>
</tbody>
</table>

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.

ACKNOWLEDGEMENTS

The authors are highly thankful to the authority of Nanaji Deshmukh Veterinary Science University, Jabalpur for granting financial support to carry out this research work.

REFERENCES


