Polymorphism of fecundity genes (*BMP15 and GDF9*) and their association with litter size in Bangladeshi prolific Black Bengal goat

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Abstract

The primary objective of this study was to identify polymorphisms in two major fecundity genes (*BMP15* and *GDF9*) and their association with litter size in Black Bengal goat, a prolific goat breed in Bangladesh. Total 40 blood samples were collected from Black Bengal does with twining records in the first three parities. All sampled animals were genotyped for fragments of exon 2 of

BMP15 gene and exon 1 and 2 of *GDF9* gene using DNA sequencing. The results of DNA sequence analysis revealed six polymorphic loci (g.735G>A, g.743C>A, g.754G>T, g.781C>A, g.808C>G and g.1061C>T) in *BMP15* gene and three (g.118C>T, g.302_303insT and g.1173_1174insA) in *GDF9* gene. Association analysis for polymorphic loci showed litter size in Black Bengal goat significantly varied between genotypes at g.735G>A and g.781C>A loci of *BMP15* gene. Further studies with a high number of genetically unrelated animals for assessing the association of these loci and others in the fecundity genes with litter size may be useful.

Keywords: Black Bengal goat; fecundity genes, polymorphisms, litter size

Introduction

Prolificacy (increase in litter size) is an essential economic trait in farm animals. This trait abides vital attention to the animal breeders since a small increase in offspring size can yield substantial gains in profit. Prolificacy in domestic species genetically influenced by multiple genes called fecundity genes.[1] Studies on the genetics of prolificacy in goat detected several candidate fecundity genes.[2] Bone Morphogenetic Protein 15 (*BMP15*), and Growth Differentiation Factor 9 (*GDF9*) are two of them.[2] Several studies reported the correlation between prolificacy and genetic polymorphisms in *BMP15* and *GDF9* genes in different goat breeds around the globe. [2-11]

In Bangladesh, with a census size of 25.7 million goat stands for the third largest livestock species. Most of the goats in Bangladesh are Black Bengal goat (>90%), reared mainly by landless, small-scale farmers. [12] Black Bengal goat is a dwarf goat breed and is well-known for its high prolificacy, meat quality and skin quality.[13] The average litter size of this breed ranges from 1.93 to 2.33. [12,14,15] This breed also renders a significant source of red meat (25%) production in Bangladesh [16]. Considering its importance, increasing the numbers of black Bengal

goat has invariably been the central breeding goal for selective goat breeding program in Bangladesh.

The use of molecular markers in animal breeding has added benefits over conventional breeding techniques. The advancement in molecular genetics has led to the detection of DNA markers with considerable effects on the traits of economic importance. To the best of our knowledge, no screening attempted to identify polymorphisms in fecundity genes in goats of Bangladesh, using DNA based technologies. Therefore, this study aimed at the detection of genetic variants in fecundity genes (*BMP15* and *GDF9*) and their relationship with litter size in the only recognized native goat breed in Bangladesh, Black Bengal.

Materials and Methods

Sample acquisition

In the present study, a total of forty (40) prolific Black Bengal does (with twining in the first three parities) were selected from two Upazilas (a sub-district unit) from Chattogram district, Bangladesh. We selected unrelated animals at random from their breeding tracts by picking up only two samples per smallholding and only five herds per village. Demographic data for all selected animal were collected using a predefined questionnaire.

Blood sample collection and DNA extraction

In the present study, a total of forty (40) prolific Black Bengal does (with twining in the first three parities) were selected from two Upazilas (a sub-district unit) from Chattogram district, Bangladesh. We selected unrelated animals at random from their breeding tracts by picking up

only two samples per smallholding and only five herds per village. Demographic data for all selected animal were collected using a predefined questionnaire. Blood samples were collected aseptically from the jugular vein in a vacutainer tube containing ethylene diamine tetraacetic acid (EDTA) as the anticoagulant. All samples were delivered to the Poultry Research and Training Center (PRTC) laboratory at Chattogram Veterinary and Animal Sciences University using an icebox. Genomic DNA was extracted from the blood samples using GeneJET Genomic DNA Purification kit (Thermo Scientific, Waltham, Massachusetts, USA) according to the manufacturer's instructions. The quality of isolated DNA was investigated using agarose gel electrophoresis (0.8%).

Polymerase chain reaction (PCR) amplification

All selected goats were genotyped for segments of *BMP15* (Gene ID: 100861233) and *GDF9* (Gene ID: 100860859), respectively, associated with fecundity in sheep and goat [3,17-19]. To amplify exon 1 of *GDF9* gene and, exon 2 of *BMP15* gene, we used two sets primers reported in the literature (Table 1).

Polymerase chain reaction (PCR) was performed in a final reaction volume of 25 µL on a Thermo-cycler (2720 Thermal cycler, Applied Biosystems, USA). The PCR reaction was run under the following thermal condition: initial denaturation for 1 min at 94 °C; 30 cycles of denaturation at 94 °C for 45 s, annealing at 60°C for 45 s, extension step at 72 °C for 45 s with a final extension at 72 °C for 5 min. The amplified PCR product was electrophoresed by running 10 µl of through a 2% agarose gel stained with ethidium bromide (0.5µg/ml) (Sigma Aldrich, USA). The specific sizes of the fragments were distinguished by using 1kb plus DNA ladder (O,GeneRuler, 1 kb Plus, Thermo Scientific Fermentas) in the gel and visualized by a UV transilluminator gel-documentation system (BDA digital, Biometra GmbH, Germany).

Sequencing and analysis

Amplified PCR products were bidirectionally sequenced in Macrogen Co., Korea. Nucleotide sequence data were edited and analysed by MEGA version 10.0.5.[20] The detected polymorphisms (SNPs) were compared to the *Capra hircus* nucleotide database in NCBI database using BLAST.[21] The sequences were submitted with the accession number of MN401415 for *BMP15* and MN401414 for *GDF9* gene, respectively.

Phylogenetic analysis

We performed a phylogenetic analysis of exon 2 of *BMP15* gene and exon 1 of *GDF9* gene with accessible published sequences of the same regions of genes in different goat breeds to identify the genetic diversity of Black Bengal goat. CLUSTALW[22] was used for multiple alignment and iTOL[23] to visualize the phylogenetic trees.

Statistical analysis

We used SHEsis online platform (http://analysis.bio-x.cn) [24] to calculate genotype frequencies, allele frequencies and $\chi 2$ values for Hardy–Weinberg equilibrium (HWE) test. The deviation from HWE for each polymorphism was tested using the Hardy–Weinberg law.[25,26] Heterozygosity (He), polymorphism information content (PIC) and effective allele number (Ne) for each polymorphism were estimated employing an online computing software (http://www.msrcall.com/Gdicall.aspx). For PIC, following classifications was used i) low polymorphism if PIC value <0.25, ii) moderate polymorphism if PIC value ≥ 0.25 to ≤0.50 and iii) high polymorphism if PIC 0.50.

A generalized linear model was used to analyse the association of polymorphisms in *BMP15* and *GDF9* gene with litter size by applying the least-squares method in SPSS 25 statistical software, SPSS Inc., Chicago, IL, USA.

$$Yi = \mu + Pi + eij$$

where, *Yi* is the phenotypic value of litter size; μ, is the overall population mean; *Pi*, is the genotype and *eij*, is the random error.

Results

Sequence analysis of the BMP15 gene exon 2

In a comparison of caprine *BMP15* gene sequence, we identified six single nucleotide polymorphisms (SNPs): g.735G>A, g.743C>A, g.754G>T, g.781C>A, g.808C>G (Fig.1) and g.1061C>T in the exon 2 of *BMP15* gene (Fig.1 and Supplementary Fig. S1). Five of these polymorphisms leading to amino substitutions (Table 2). The predicted possible effect of identified polymorphism on the structure and function of the functional protein revealed two mutations: g.754G>T and g.1061C>T have a significant impact on the coding BMP15 peptide (Fig. 2) indicating a substantial phenotypic effect. The g.781C>A mutation deduced to cause a moderate change while remaining two mutations did not show significant effects on the functional BMP15 protein.

Sequence analysis of the GDF9 gene exons 1 and 2

The sequence of the exon 1 of *GDF9* gene showed a SNP g.118C>T and an insertion mutation g.302_303insT. The g.302_303insT mutation causes a frameshift in the reading frame of the *GDF9* gene which leads to a premature stop codon at third codon downstream of the mutated

codon (Supplementary Fig. S2). We identified a one bp insertion in between nucleotide 1173 and 1174 in the exon 2 of *GDF9* gene, which deduced a premature stop codon at 16th codon downstream of the mutated codon (Supplementary Fig. S3).

Genetic parameters for the detected polymorphisms in BMP15 and GDF9 gene

The genotypic and allelic frequencies, PIC, HE, Ne and $\chi 2$ values for detected polymorphisms in two fecundity genes are presented in Table 3. The g.735G>A of *BMP15* showed all the three possible genotypic combinations while remaining SNPs recorded with two genotypes. All 40 tested individuals had only homozygous mutant genotypes for g.1061C>T of *BMP15* and g.118C>T of *GDF9* gene. All tested goats expressed heterozygous genotype at g.808C>G and homozygous mutant genotype at g.1061C>T of *BMP15* gene. Genotype frequencies for most of the detected polymorphisms were significantly different from the expectations of Hardy-Weinberg equilibrium in Black goat population in this study ($P < 0.01$), except g.735G>A and g.754G>T in *BMP15* gene. Three polymorphisms in *BMP15* genes (g.743C>A, g.781C>A and g.808C>G) and two in *GDF9* gene (g.302_303insT and g.1173_1174insA) were found to moderate polymorphic according to the classification of PIC. The remainder of the detected polymorphisms was found to be low polymorphic.

Phylogenetic analysis

Results of phylogenetic analysis show that the sequences of the exon 2 of *BMP15* gene in this study clustered in a shared cluster with previously published sequences for Black Bengal goat, however, diverges from that of other goat breeds (Fig. 4). On the other hand, sequences of the exon 1 of *GDF9* gene did not form a breed-specific cluster (Fig. 5).

Association between SNPs of BMP15 and GDF9 and litter size trait

The litter size of AA genotype individuals at G735A locus of *BMP15* was significantly higher than that of GG genotype ($p<.05$), however, there is no significant difference between individuals with GA and GG genotypes at this locus. Individuals with heterozygous CA genotype at C781A locus of *BMP15* had a significantly higher ($p<.01$) litter size than those of CC genotype. Individuals with different genotypes at other loci in both *BMP15* and *GDF9* gene did not show any significant difference for litter size. In this study, the mean litter size was highest (3.04±0.45) in individuals with heterozygous CA genotype at C781A locus of *BMP15* (Table 4).

Discussion

We herein reported nine polymorphic sites in two well-studied fecundity genes namely *BMP15* and *GDF9* in Black Bengal goat, the only prolific goat breed in Bangladesh. The genetic parameters of the detected loci and their association with the litter size in the Black Bengal goat were also analysed. The g.735G>A locus of *BMP15* recorded with three possible combinations of genotypes while the remaining loci expressed either two or one genotypes in the study population. The frequency distribution of genotype in a population is the simplest way to describe mendelian variation[27], which might not be the fact in this study since we sequenced only 40 goats for each locus. However, other factors such as such matting pattern, random genetic drift, individual survival, reproductive success, and migration also generate genetic variation in a population.[28]

In this study, four SNPs in *BMP15* gene viz. g.743C>A, 754G>T, g.781C>A and g.1061C>T were found to be novel based on an extensive literature search. Remaining two SNPs identified by us in *BMP15* gene viz g.735G>A and g.808C>G have also been reported in Black

Bengal goats in the neighbouring country Indian.[2,4,18] None of the polymorphisms in *GDF9* gene detected by us was reported so far in goat. However, several studies reported polymorphisms in the flanking sequences of *GDF9* gene. [2,25,29]

The diversity of the impact on molecular function determines the effect of a mutation. Mutational effects can be neutral, harmful or beneficial depending on their context or location[30]. In our study, two of the identified polymorphisms in *BMP15* gene predicted to have a significant effect on resulting protein sequence, hence might contribute to phenotypic change. An advantageous phenotypic effect of two insertions detected by us in *GDF9* gene might be excluded since frameshifting generally assume to cause a loss of function resulting from degrading mutant mRNA by nonsense-mediated or non-stop-mediated mRNA decay[31-33]. However, any harmful or neutral effect[34] of these frameshift mutations on GDF9 protein function cannot be ruled out.

Phylogenetic analysis of *BMP15* gene revealed Black Bengal goat including the present study assorted in a common cluster which differs from that of other goat breeds. The *GDF9* gene sequences show more genetic diversity in different goat breeds. A similar trend of genetic divergence in the coding region of *BMP15 and GDF9* gene has reported by Xue-qin et al.[35]

Results of association study showed that goat with AA genotype at G735A of *BMP15* had significantly higher litter size than that of GG genotypic individuals ($p = <0.05$). The effect of G735A in *BMP15* gene on litter size also reported in Indian Black Bengal goat[2,4]. Besides, the phenotypic effects of this synonymous mutation could not be ruled out since there are many mechanisms exist by which a synonymous mutation can affect a phenotype.[36] In this study, Black Bengal goat with genotype CA at C781A of *BMP15* gene had significantly higher litter size than those with CC at the same locus. Ahlawat et al[2,4] and Feng et al[37] have reported a

significant effect of allelic variants in *BMP15* gene on the litter size in different goat breeds including Black Bengal.

Variations of a quantitative trait are controlled by several genes, genetic variants and their interactions. Hence, detection of polymorphisms that are underlying the differences in a quantitative trait, for instance, litter size remains as a challenge in modern genetics. *BMP15*, *GDF9* and *BMPR1B* are three well-documented candidate genes for litter size in sheep and goat. Till date, no association of *BMPR1B* gene with litter size in goat has been established. However, researchers have explored the association between litter size in goat and polymorphisms in *BMP15* and *GDF9* genes. These two genes are part of the ovary-derived transforming growth factor β (TGF β) that have an integral role as growth factors and receptors in follicular development in the ovaries.[38] Both bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs) have critical role in follicle growth and cell-survival signalling hence causal mechanism underlying the high prolificacy or fertility in female animal.[39] Considering the biological importance of *BMP15* and *GDF9* genes, this study investigated polymorphisms in these genes and their association with the litter size in Bangladeshi prolific Black Bengal goat.

Conclusion

This is the first study exploring polymorphisms in *BMP15* and *GDF9* genes and investigating their association with litter size in Bangladeshi goat. The findings of the study reveal that different genotypes at two loci in *BMP15* gene had significant ($p \leq 0.05$) effect on litter size in the prolific Black Bengal breed of Bangladesh. Hence, there is a need for further research with a substantially large number of animals across a wide range of geographically divergent populations of this breed. Our results enrich the repository of molecular markers database of

caprine fecundity genes which pave the way for association studies with fecundity trait, hence contribute to molecular breeding in goat.

Data availability

The assembled and annotated sequences for *BMP15* and *GDF9* genes were deposited to GenBank database under accession numbers MN401414 and MN401415, respectively.

Conflict of Interest

The authors declare that they have no competing financial interests.

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Author contributions

A. Das conceived this project. M. Shaha performed sample collection. M. Shaha and A. Dutta extracted genomic DNA and performed PCR. A. Das and M. Das Gupta performed data curation and formal data analysis. A. Das and O. F. Miazi involved in funding acquisition, project administration and supervision. A. Das drafted the original manuscript. All authors read and approved the final manuscript.

References

1. Drouilhet L, Mansanet C, Sarry J, et al. The Highly Prolific Phenotype of Lacaune Sheep Is Associated with an Ectopic Expression of the B4GALNT2 Gene within the Ovary. *PLOS Genet*. 2013;9(9):e1003809.

2. Ahlawat S, Sharma R, Roy M, Tantia MS, Prakash V. Association analysis of novel SNPs in BMPR1B, BMP15 and GDF9 genes with reproductive traits in Black Bengal goats. *Small Ruminant Res*. 2015;132:92-98.

3. Ahlawat S, Sharma R, Maitra A. Screening of indigenous goats for prolificacy associated DNA markers of sheep. *Gene*. 2013;517(1):128-131.

4. Ahlawat S, Sharma R, Roy M, Mandakmale S, Prakash V, Tantia MS. Genotyping of Novel SNPs in BMPR1B, BMP15, and GDF9 Genes for Association with Prolificacy in Seven Indian Goat Breeds. *Anim Biotechnol*. 2016;27(3):199-207.

5. Deldar-Tajangookeh H, Shahneh AZ, Zamiri MJ, Daliri M, Kohram H, Nejati-Javaremi A. Study of BMP-15 gene polymorphism in Iranian goats. *Afr J Biotechnol*. 2009;8(13).

6. Feng T, Geng C, Lang X, et al. Polymorphisms of caprine GDF9 gene and their association with litter size in Jining Grey goats. *Mol Biol Rep*. 2011;38(8):5189-5197.

7. Hadizadeh M, Mohammadbadi MR, Niazi A, Esmailizadeh A, Gazooei YM. Search for polymorphism in growth and differentiation factor 9 (GDF9) gene in prolific beetal and tali goats (Capra hircus). *J Biodiver Environ Sci*.2014; 4(4):186-191

8. He Y, Ma X, Liu X, Zhang C, Li J. Candidate genes polymorphism and its association to prolificacy in Chinese goats. *J Agril Sci*. 2010;2(1):88.

9. Hua GH, Chen SL, Ai JT, Yang LG. None of polymorphism of ovine fecundity major genes FecB and FecX was tested in goat. *Anim Reprod Sci*. 2008;108(3-4):279-286.

10. Jalbani MA, Kaleri HA, Baloch AH, et al. Study of BMP15 gene Polymorphism in Lehri goat breed of Balochistan. *J Appl Environ Biol Sci*. 2017;7(2):84-89.

11. Polley S, De S, Batabyal S, et al. Polymorphism of fecundity genes (BMPR1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. *Small Ruminant Res*. 2009;85(2-3):122-129.

12. Amin M, Husain S, Islam A. Reproductive peculiarities and litter weight in different genetic groups of Black Bengal does. *Asian-Australas J Anim Sci*. 2001;14(3):297-301.

13. Choudhury M, Sarker S, Islam F, et al. Morphometry and performance of Black Bengal goats at the rural community level in Bangladesh. *Bangladesh J Anim Sci*. 2012;41(2):83-89.

14. Akhtar F, Islam A, Amin M. Effect of Selection for Growth on Production Performance in Black Bengal Goats. *Pakistan J Biol Sci*. 2006;9(2):182-185.

15. Islam M, Amin M, Kabir A, Ahmed M. Comparative study between semi-intensive and scavenging production system on the performances of Black Bengal goat. *J Bangladesh Agril Uni*. 2009;7(452-2016-35476).

16. Black Bengal – a promising goat genetic resource of Bangladesh. Vol 2019: Food and Agricultural Organization of United Nations.; 2006.

17. Javanmard A, Azadzadeh N, Esmailizadeh AK. Mutations in bone morphogenetic protein 15 and growth differentiation factor 9 genes are associated with increased litter size in fat-tailed sheep breeds. *Vet Res Commun*. 2011;35(3):157-167.

18. Maitra A, Sharma R, Ahlawat S, Borana K, Tantia MS. Fecundity gene SNPs as informative markers for assessment of Indian goat genetic architecture. *Indian J Anim Res*. 2016;50(3):349-356.

19. Abdoli R, Mirhoseini SZ, Hossein-Zadeh NG, Zamani P. Screening for causative mutations of major prolificacy genes in Iranian fat-tailed sheep. *Int J Fertil Steril*. 2018;12(1):51.

20. Kumar S, Stecher G, Li M, Knyaz C, Tamura KJMb, evolution. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. 2018;35(6):1547-1549.

21. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJJJomb. Basic local alignment search tool. *J Mol Biol*. 1990;215(3):403-410.

22. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*. 1994;22(22):4673-4680.

23. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res*. 2019. Jul 2;47(W1):W256-W259

24. Li Z, Zhang Z, He Z, et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis. bio-x. cn). *Cell Res*. 2009;19(4):519.

25. Yue C, Bai WL, Zheng YY, et al. Correlation analysis of candidate gene SNP for high-yield in Liaoning cashmere goats with litter size and cashmere performance. *Anim Biotechnol*. 2019;19:1-8.

26. Gorlov IF, Kolosov YA, Shirokova NV, et al. GDF9 gene polymorphism and its association with litter size in two Russian sheep breeds. *Rendiconti Lincei. Scienze Fisiche e Naturali*. 2018;29(1):61-66.

27. Mather K, Jinks JL. Introduction to biometrical genetics: Chapman and Hall London; 1977.

28. Chen N, Juric I, Cosgrove EJ, et al. Allele frequency dynamics in a pedigreed natural population. *PNAS*. 2019;116(6):2158-2164.

29. Chu M, Wu Z, Feng T, et al. Polymorphism of GDF9 gene and its association with litter size in goats. *Vet Res Commun*. 2011;35(6):329-336.

30. Loewe L. Genetic mutation. *Nature education*. 2008;1(1):113.

31. Van Hoof A, Frischmeyer PA, Dietz HC, Parker R. Exosome-mediated recognition and degradation of mRNAs lacking a termination codon. *Science*. 2002;295(5563):2262-2264.

32. Scofield DG, Hong X, Lynch M. Position of the final intron in full-length transcripts: determined by NMD? *Mol Biol Evol*. 2007;24(4):896-899.

33. Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends Bioch Sci*. 1998;23(6):198-199.

34. Hu J, Ng PC. Predicting the effects of frameshifting indels. *Genome Biol*. 2012;13(2):R9.

35. Ran X-q, Lin J-B, Du Z-y, Qing C, Wang J-f. Diversity of Bmp15 and Gdf9 genes in White goat of Guizhou province and evolution of the encoded proteins. *Zool Res*. 2009;30(6):593-602.

36. Zwart MP, Schenk MF, Hwang S, et al. Unraveling the causes of adaptive benefits of synonymous mutations in TEM-1 β-lactamase. *Heredity*. 2018;121(5):406.

37. Feng T, Chu M-X, CAO G-L, et al. Screening for S32G mutation of BMP15 gene in 18 goat breeds. *Turkish J Vet Anim Sci*. 2014;38(5):463-468.

38. Davis GH. Major genes affecting ovulation rate in sheep. presented at: Genetics Selection Evolution 2005.

39. Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF□9 and BMP□15 in ovarian function. *Mol Reprod Dev*. 2011;78(1):9-21.

Table 1. List of primer used to amplify specific segment of *GDF9* and *BMP15* gene.

Gene	Region	Oligonucleotide sequence (5'-3')	Amplicon size (bp)
BMP15	Exon2	F: CACTGTCTTCTTGTTACTGTATTTCAATGAC	141
		R: GATGCAATACTGCCTGCTTG	
		F: TCCCTAAAGGCCTGAAAGAGT	575
		R: GCTGAAGGCAAGGAATAGAATC	
GDF9	Exon1	F: GAAGACTGGTATGGGGAAATG	462
		R: CCAATCTGCTCCTACACACCT	
	Exon2	F: CCACACAAATACAACCCTCGATAC	183
		R: AGGCTCGATGGCCAAAACACT	

Table 2. Identified polymorphisms in *GDF9* and *BMP15* genes in Bangladeshi Black Bengal goat breed.

Gene	Region	Mutation	Amino acid substitution	Type of mutation
BMP15	Exon2	g.735G>A	-	Synonymous
		g.743C>A	p.Pro248His	Non-synonymous
		g.754G>T	p. Gly252Cys	Non-synonymous
		g.781C>A	p. Pro261Thr	Non-synonymous
		g.808C>G	p.Gln270Glu	Non-synonymous
		g.1061C>T	p.Ala354Val	Non-synonymous
GDF9	Exon1	g.118C>T	-	Synonymous
		g.302_303insT	p.Gly101Glyfs2X	Frameshift
	Exon2	g.1173_1174insA	p.Arg392Glnfs16X	Frameshift

Table 3. Genotypic and allelic frequencies and population parameters for mutations detected in *GDF9* and *BMP15* genes in Bangladeshi Black Bengal Goat breed (N=40).

Gene	Mutation	Genotypic frequencies			Allelic frequencies		PIC	He	Ne	H-W test ($\chi 2$)	p value
		PP	Pq	qq	P	q					
BMP15	g.735G>A	0.625	0.275	0.100	0.762	0.237	0.237	0.361	1.567	2.317	0.127
	g.743C>A	0.300	0.700	0	0.650	0.350	0.351	0.455	1.834	11.597	0.000
	g.754G>T	0.700	0.300	0	0.85	0.15	0.222	0.255	1.342	1.245	0.264
	g.781C>A	0.450	0.550	0	0.725	0.275	0.319	0.398	1.663	5.755	0.016
	g.808C>G	0	1.000	0	0.500	0.500	0.375	0.500	2.000	40.000	0.000
	g.1061C>T	0	0	1.000	0	1.000	0	0	1.000	-	-
GDF9	g.118C>T	0	0	1.000	0	1.000	0	0	1.000	-	-
	g.302_303insT	0.200	0.800	0	0.600	0.400	0.364	0.480	1.923	17.778	0.000
	g.1173_1174insA	0.300	-	0.700	0.300	0.700	0.331	0.420	1.724	39.999	0.000

PP, homozygous reference allele, Pq, heterozygous mutant allele; qq, homozygous mutant allele; *PIC*, polymorphism information content; *He*, effective number of heterozygosities; *Ne*, effective number of alleles.

Table 4. Genotype wise least squares mean ± standard error of litter size in Bangladeshi Black Bengal goat.

Gene	Locus	Genotype	N	Litter size	Significance
BMP15	G735A	GG	25	3.00^{a}±0.33	*
		GA	11	2.52^{ab}±0.13	
		AA	4	2.27^{b}±0.20	
	C743A	CC	12	2.42±0.20	NS
		CA	28	2.53+0.13	
		AA	0	-	
	G754T	GG	28	2.39±0.14	NS
		GT	12	2.75±0.13	
		TT	0	-	
	C781A	CC	18	1.83^{a}±0.09	***
		CA	22	3.04^{b}±0.45	
GDF9	302_303insT	GG	8	2.47±0.12	NS
		GGT	32	2.63±0.24	
	1173_1174insA	TT	7	2.86±0.69	NS
		TTA	28	2.42±0.66	

*, p value <0.05 ***, p value <0.01; rows with different superscripts differed significantly.

Figure Legends

Figure 1. Sequencing chromatograms of the detected single nucleotide polymorphisms (SNPs) in exon 2 of the *BMP15* gene in Black Bengal goat breed. a. g.735G>A (heterozygous); b. g.743C>A (heterozygous); c. g.754G>T (heterozygous); d. g.781C>A (heterozygous); d. g.808C>G (heterozygous) and f. g.1061C>T (homozygous TT). Positions of the mutations are based on the full sequences of the *BMP15* gene (Gene ID: 100861233).

Figure 2. Sequencing chromatograms of the detected polymorphisms in the *GDF9* gene in Black Bengal goat breed. a. g.118C>T (homologous TT) and b. 302_303insT in the exon 1 of *GDF9* gene; c. g.1173_1174insA in the exon 2 of *GDF9* gene. Positions of the mutations are based on the full sequences of the *GDF9* gene (Gene ID: 100861233).

Figure 3. Predicted effects (using Ployphen2) of five single nucleotide polymorphisms (SNPs). a, b, c, d and e represent the of effect of g.743C>A, g.754G>T, g.781C>A, g.808C>G and g.1061C>T on the functional BMP15 protein, respectively.

Figure 4. Phylogenetic tree of *BMP15* gene.

Figure 5. Phylogenetic tree of *GDF9* gene.

Supplementary Figure S1. Multiple alignment for g.1061C>T of *BMP15* gene (indicated by box arrow). *BMP15* gene sequences from this study aligned with BMP15 mRNA sequence from the NCBI database using MEGA version 10.0.5.[20]

Supplementary Figure S2. Multiple alignment for 302_303insT of *GDF9* gene (Condon changes are indicated by box arrow). *GDF9* gene sequences from this study aligned with GDF9 mRNA sequence from the NCBI database using MEGA version 10.0.5.[20]

Supplementary Figure S2. Multiple alignment for 302_303insT of *GDF9* gene (Condon changes are indicated by box arrow). *GDF9* gene sequences from this study aligned with GDF9 mRNA sequence from the NCBI database using MEGA version 10.0.5.[20]

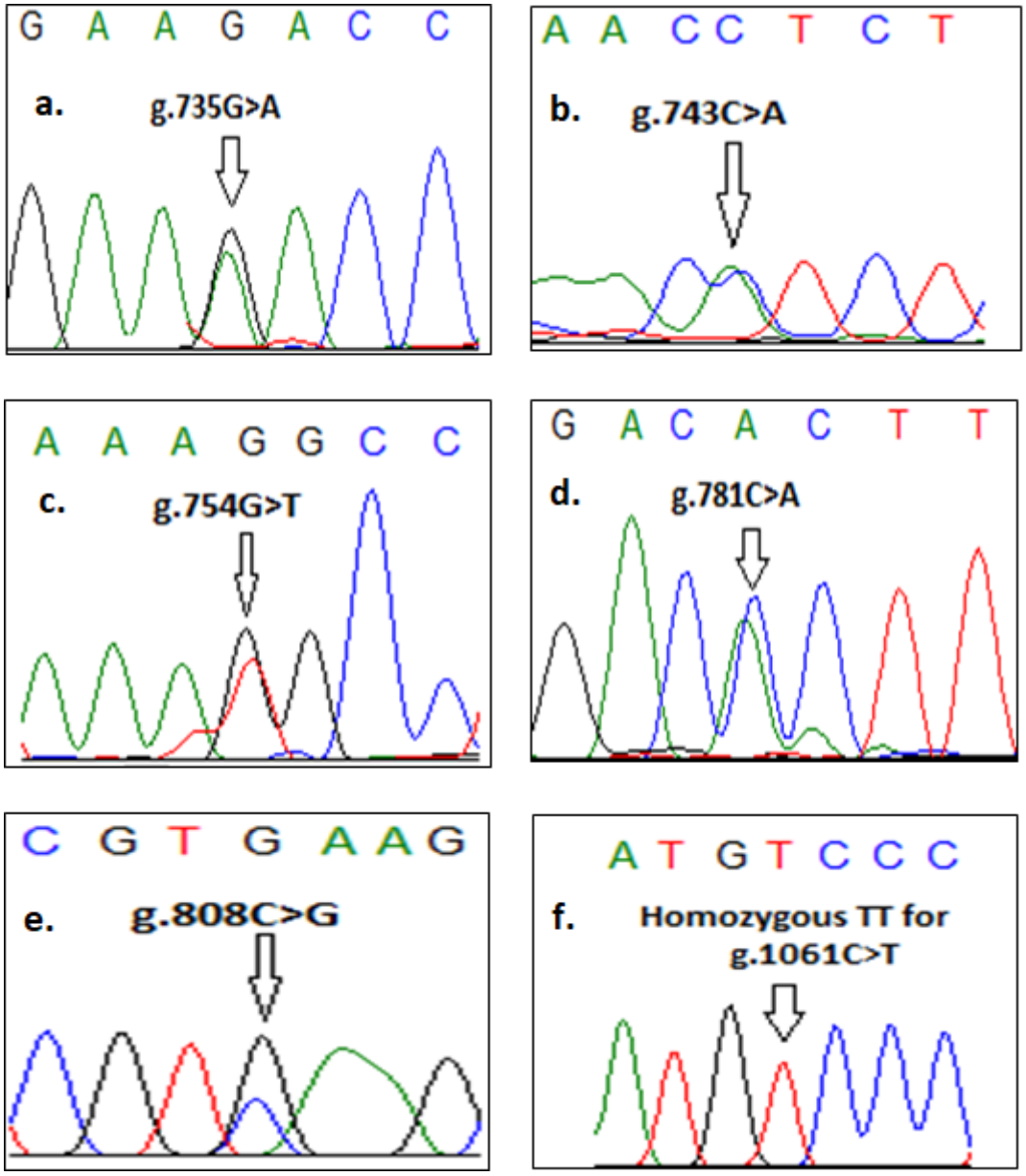

Figure 1. Sequencing chromatograms of the detected single nucleotide polymorphisms (SNPs) in exon 2 of the *BMP15* gene in Black Bengal goat breed. a. g.735G>A (heterozygous); b. g.743C>A (heterozygous); c. g.754G>T (heterozygous); d. g.781C>A (heterozygous); d. g.808C>G (heterozygous) and f. g.1061C>T (homozygous TT). Positions of the mutations are based on the full sequences of the *BMP15* gene (Gene ID: 100861233).

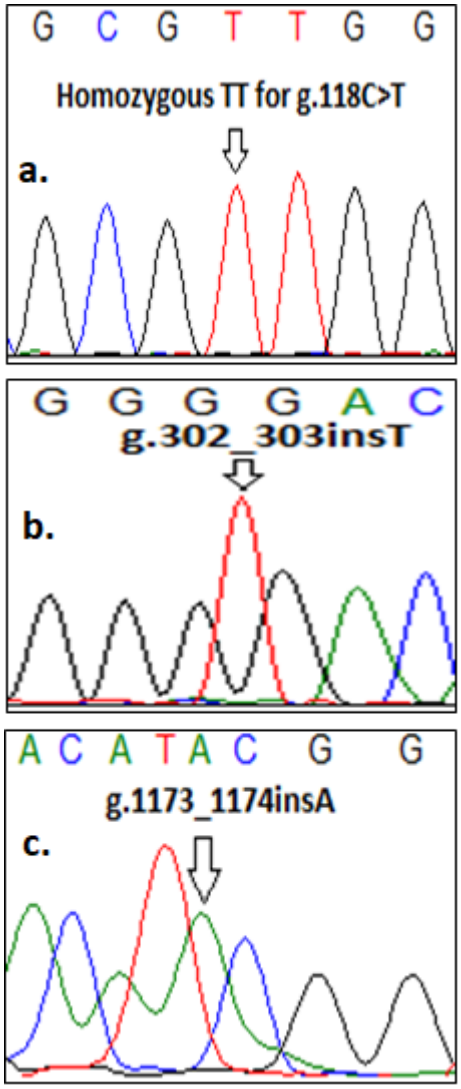

Figure 2. Sequencing chromatograms of the detected polymorphisms in the *GDF9* gene in Black Bengal goat breed. a. g.118C>T (homologous TT) and b. 302_303insT in the exon 1 of *GDF9* gene; c. g.1173_1174insA in the exon 2 of *GDF9* gene. Positions of the mutations are based on the full sequences of the *GDF9* gene (Gene ID: 100861233).

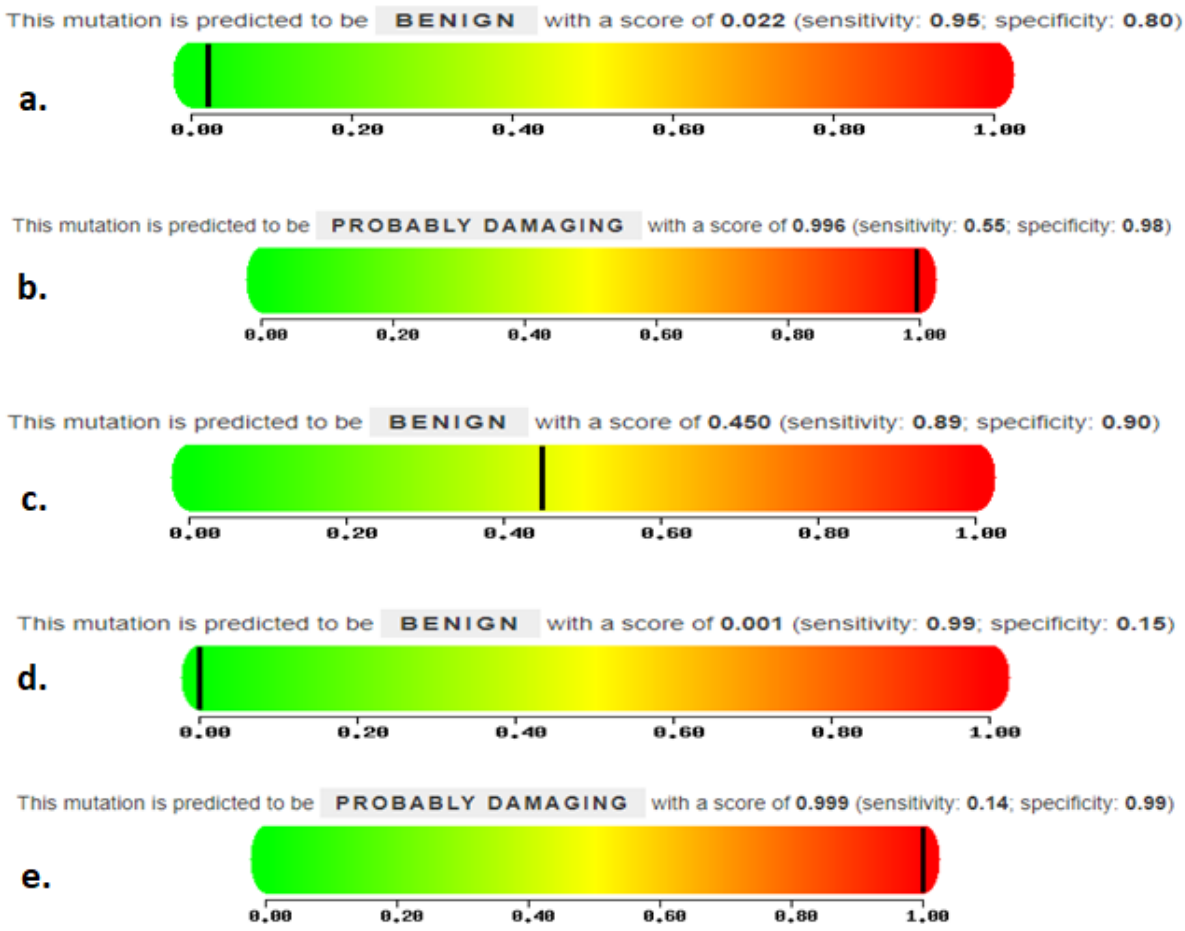

Figure 3. Predicted effects (using Ployphen2) of five single nucleotide polymorphisms (SNPs). a, b, c, d and e represent the of effect of g.743C>A, g.754G>T, g.781C>A, g.808C>G and g.1061C>T on the functional BMP15 protein, respectively.

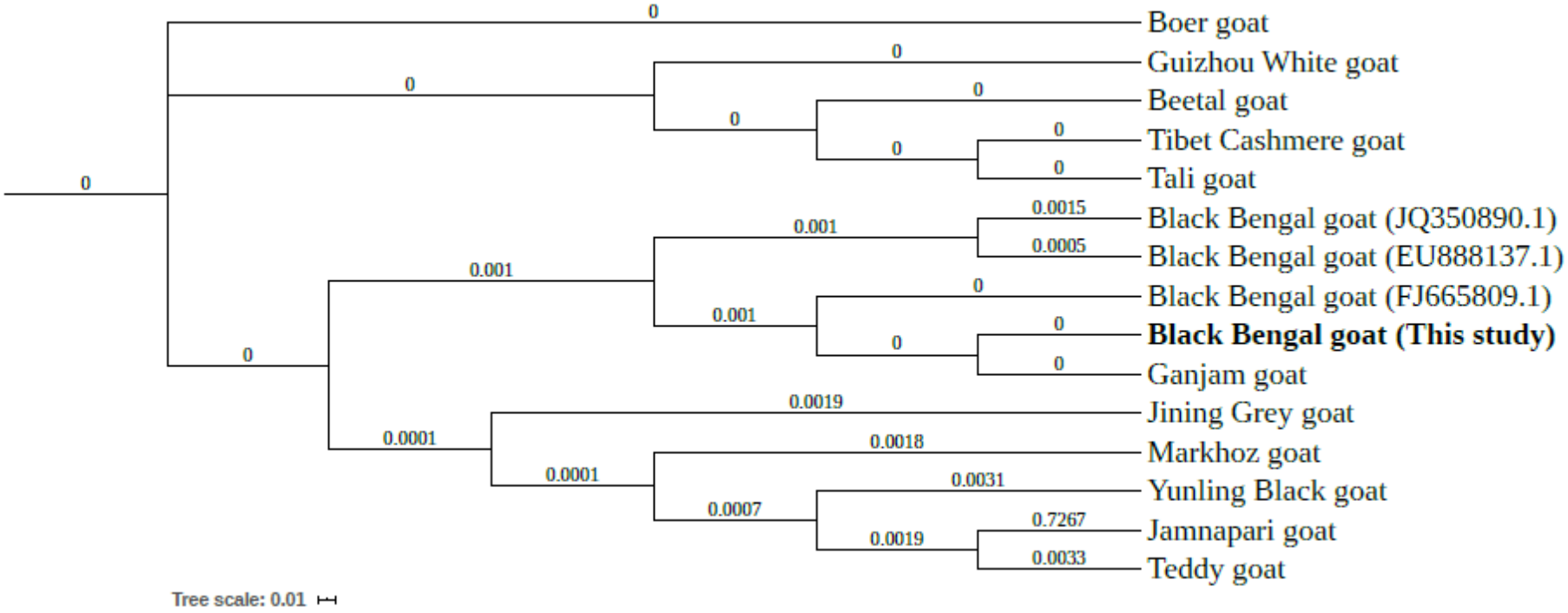

Figure 4. Phylogenetic tree of *BMP15* gene.

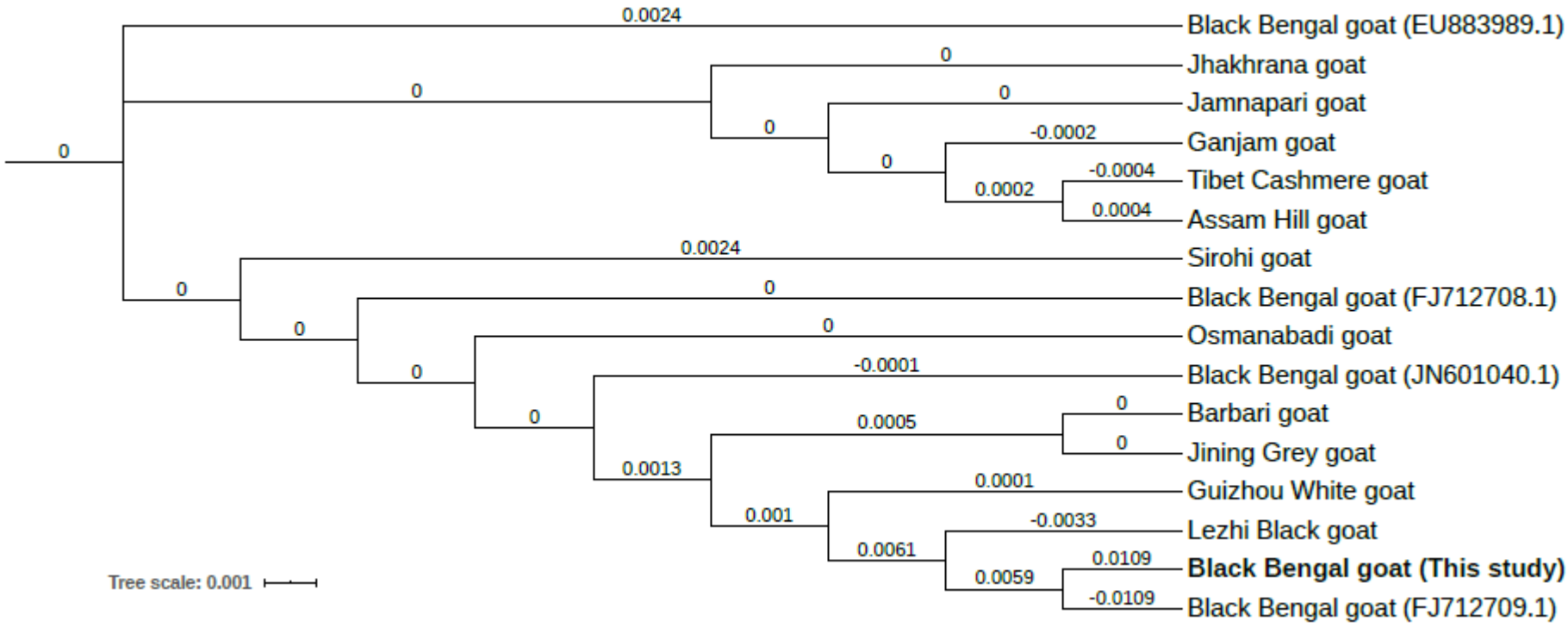

Figure 5. Phylogenetic tree of *GDF9* gene.