Identification of polymorphisms in *GDF9* and *BMP15* genes in Jamunapari and crossbred goats in Bangladesh

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**Abstract**

Polymorphisms in growth differentiation factor 9 (*GDF9*) and bone morphogenetic protein 15 (*BMP15*) genes have been found to be associated with litter size in goats across the globe. Our previous study detected single-nucleotide polymorphisms (SNPs) in *GDF9* and *BMP15* genes associated with litter size in Black Bengal, Bangladesh’s primary native goat breed. However, Jamunapari and crossbred goats in Bangladesh are yet to be investigated for litter size–associated polymorphisms. In this study, we screened Jamunapari and crossbred (50% Black Bengal × 50% Jamunapari) goats to identify polymorphisms in the *GDF9* and *BMP15* genes and to assess the association between identified SNPs and litter size. The genomic DNA from 100 female goats (50 Jamunapari and 50 crossbred) was used in polymerase chain reactions (PCRs) to amplify exon 2 of the *GDF9* and exon 2 of the *BMP15* genes. PCR products were sequenced employing the BigDye Terminator cycle sequencing protocol to identify SNPs. We used a generalized linear model to perform the association analysis for identified SNPs and litter size. Seven SNPs were identified, of which four, C818CT, G1073A, G1189A, and G1330T, were in the *GDF9* gene and three, G616T, G735A, and G811A, were in the *BMP15* gene. G735A was a synonymous SNP, whereas the remaining were non-synonymous SNPs. Identified SNP loci in *GDF9* were low polymorphic (PIC < 0.25), while loci in *BMP15* were moderately polymorphic (PIC ≥ 0.25). The genotypes at the G1330T locus had a significant (*p* < 0.05) difference in litter size in Jamunapari goats, but no significant difference was observed for all genotypes at other loci. Therefore, the G1330T loci could be useful as a marker in marker-assisted selection for litter size traits in goats of Bangladesh.

**Keywords** Goat · Fecundity genes · SNPs · Litter size

**Introduction**

Goat is one of the most prolific domestic food animals with a remarkable ability to adapt under harsh tropical conditions. In Bangladesh, the goat population represented the country’s third largest livestock species (DLS 2020) and was primarily reared by rural landless and small-scale farmers. The Black Bengal goats make up more than 90% of the Bangladeshi’s goat population, and the remaining are Jamunapari and their crosses (Siddiky 2017). Black Bengal goats are well known for their fertility, prolificacy, delicate meat, and skin quality (Miah et al. 2016). Jamunapari is a dual-purpose goat. This breed sometimes exhibits a larger litter size (Bhuiyan 2014). In comparison with the Black Bengal breed, crossbred goats have a smaller litter size (Hassan et al. 2007).

Moreover, the study of breeds using molecular techniques is very important and useful for their characterization (Mohammad et al. 2009; Ahsani, et al. 2011; Mohammadabadi et al. 2017). Conservation of genetic diversity in animal species requires the proper performance of conservation superiorities and sustainable handling plans that should be based on universal information on population structures, including genetic diversity resources among and between breeds (Mohammadabadi et al. 2010a; Norouzy et al. 2005). Genetic diversity is an essential element for genetic improvement, preserving populations, evolution, and adapting to variable environmental situations (Mohammadabadi et al. 2010b; Ahsani, et al. 2010). In contrast, determination of
gene polymorphism is important in farm animal breeding (Koopaei et al. 2012; Gooki et al. 2019) in order to define genotypes of animals and their associations with productive, reproductive, and economic traits (Nassiry et al. 2005; Ebrahimi et al. 2015).

Litter size in goats has a high economic value in breeding and improving reproductive traits since a slight increase in litter size may equate to substantial gains in profit. However, the low heritability and sex-limited nature make it difficult to improve by conventional selective breeding (Ahlawat et al. 2015a). Moreover, another constraint is the absence of information on genes controlling the trait and the likely gene inter-linkage. However, advancement in molecular genetics can overcome this impediment by providing an appliance to investigate genetic variation precisely at the nucleotide level with the likelihood of recognizing the individual gene affecting the litter size trait.

Polymorphisms of fecundity genes with substantial effects on litter size have been detected worldwide in sheep and goat breeds (Mishra et al. 2017; Gootwine 2020; Plakkot et al. 2020). Most of those polymorphisms are in genes related to the TGFβ superfamily. These genes are key regulators of intra-ovarian processes for follicular growth and maturation (Drouilhet et al. 2013; McNatty et al. 2017) and pituitary functions associated with high prolificacy (Zheng et al. 2019).

Bone Morphogenetic Protein Receptor Type 1B (BMPR1B), GDF9, and BMP15 are three major genes for fecundity belonging to the TGFβ superfamily and have been extensively investigated in the goat (Abadi and Zaki-zadeh 2010; Shokrollahi et al. 2014; Ahlawat et al. 2015a; Moghadaszadeh et al. 2015; Mishra et al. 2017; Wang et al. 2019b; Mohammadabadi and Soflaei 2020). However, an association of BMPR1B mutation and designated FecB mutation with high prolificacy in goats has yet to be established in several global goat breeds (Ahlawat et al. 2015a; Sasi et al. 2020). Therefore, mutations in GDF9 and BMP15 genes remain meaningful markers to investigate high prolificacy in the goat.

Studies on the detection of genetic polymorphisms for litter size in Bangladeshi goats are scanty. In our previous study (Das et al. 2021), we detected litter size–associated polymorphic loci in the Black Bengal goat of Bangladesh, which led us to screen genetic markers in litter size–associated genes in Bangladeshi Jamunapari and crossbred goats. In this study, two major fecundity genes, GDF9 and BMP15, were investigated to identify litter size–associated polymorphisms.

Materials and methods

Experimental animal selection and DNA isolation

Animals were selected from three Upazila (a sub-district level area) under the Chattogram district in Bangladesh. A total of 100 (50 Jamunapari and 50 crossbred (50% Black Bengal × 50% Jamunapari)) does of third parity were utilized in this study. Selected animals were allowed to kid in the autumn season of 2016 and 2017. The animals had no selection history for litter size and other fertility-related traits. The animals had a previous history of twining. The average age of the selected animals was 34.4 ± 82 months. The mean litter size was 1.64 ± 33.

Approximately 2 mL of blood was collected from the jugular vein from each animal and kept in a sterile vacutainer coated interiorly with spray-dried K2-EDTA. All the blood samples were shipped to the Poultry Research and Training Centre (PRTC) laboratory at Chattogram Veterinary and Animal Sciences University (CVASU) using an icebox. Genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) according to the manufacturer’s guidelines. The quality and purity of the extracted DNA were assessed using agarose gel electrophoresis (0.8%) at a constant voltage of 80–90 V for 40 min in 1 × TAE buffer.

Primer designing and PCR amplification

Two pairs of primers were designed using the online Primer-BLAST software tool from NCBI to amplify exon 2 of the GDF9 gene (Gene ID: 100,860,859) and exon 2 of the BMP15 gene (Gene ID: 100,861,233). The primer sequence, annealing temperature, and amplicon sizes are shown in Table 1.

| Table 1 | Nucleotide sequences of the primers used to amplify GDF9 and BMP15 genes. |
|---------|--------------------------------------------------------------------------------|
| Gene    | Region of the gene | Size of the PCR product (bp) | Primer sequence (5ʹ–3ʹ) | Annealing temperature (°C) |
| GDF9    | Exon 2              | 799                         | F: GATTGATGTGACGGCTCTCTCT   | 61.2                     |
|         |                     |                             | R: CTCCCAAAGGGCATAGACAGGA   |                          |
| BMP15   | Exon 2              | 527                         | F: ACGCTTTGTCTTGTCTCC       | 60                       |
|         |                     |                             | R: AATACTGCGCTGGACGA        |                          |
PCR reactions were accomplished on a thermocycler (2720 Applied Biosystems; Thermo Fisher Scientific, Singapore) by setting the program as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min; annealing at 61.2 °C for GDF9 and 60 °C for BMP15 for 45 s; extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. The PCR products were visualized in 1.5% ethidium bromide (Sigma Aldrich Inc., Missouri, USA)–stained agarose gel. A Gel Documentation System photographed the fragment specific bands (BDA Digital; Biometra GmbH, Germany) and their sizes were estimated using a 1 kb Plus DNA ladder (GeneRuler, 1 kb Plus; Thermo Scientific Fermentas International Inc., USA) (Fig. 1).

Sequencing and analysis

The purified PCR products for 100 samples (one for each animal) were bi-directionally Sanger-sequenced using Big-Dye Terminator v. 3.1 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) cycle sequencing protocol by Macrogen Co., Korea. Raw sequences were edited by Chromas version 2.6.6 (http://technelysium.com.au/wp/chromas).

MEGA version 7.0.26 (Kumar et al. 2016) was used to perform multiple sequence alignment to identify SNPs. The online BLAST algorithm was used to compare the identified SNPs with the reference Capra hircus nucleotide sequences in NCBI GenBank (http://www.ncbi.nlm.nih.gov).

Statistical analysis

Allele frequencies, heterozygosity (He), polymorphism information content (PIC), and \( \chi^2 \) values for the Hardy–Weinberg equilibrium (HWE) test were computed using p,q CHWE: PolyPiCker (https://www.genecalculators.net/pq-chwe-polypicker.html). For PIC, (1) low polymorphism if PIC value < 0.25, (2) moderate polymorphism if PIC value \( \geq 0.25 \) to \( \leq 0.50 \), and (3) high polymorphism if PIC 0.50.

A generalized linear model was employed to estimate the effects of different genotypes on litter size using SPSS 25 statistical software (SPSS Inc., Chicago, Illinois, USA). The least-squares mean was used for litter size among different genotypes:

\[
Y_{ij} = \mu + G_i + e_{ij}
\]

where \( Y_{ij} \) is the phenotypic value (litter size), \( \mu \) is the mean of litter size, \( G_i \) is the fixed effect of the genotype, and \( e_{ij} \) is the random residual effect of individual observation.

Results

Sequence analysis and identification of polymorphisms

The sequenced nucleotide covered 688–1362 bp and 340–813 bp of coding sequences (CDS) of the GDF9 and BMP15 genes, respectively. The consensus of assembled sequences for Jamunapari and crossbred goats was deposited in the NCBI-GenBank database under the accession numbers MN629928 and MN629929 for exon 2 of GDF9 and MN629925 and MN629926 for exon 2 of BMP15, respectively. Sequence analysis unveiled four SNPs (C818T, G1073A, G1189A, and G1330T) in exon 2 of GDF9 (Fig. 2) and three SNPs (G616T, G735A, and G811A) in exon 2 of the BMP15 gene (Fig. 3). Six of these polymorphisms predicted to result in amino acid changes in the resulting polypeptide (Table 2).

Population parameters for the identified polymorphic loci

Population parameters for all loci in Jamunapari and crossbred goats of Bangladesh are presented in Table 3. Except for G735A locus of BMP15, homozygous mutant genotypes were missing for all polymorphic loci in the

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**Fig. 1** Results of PCR assay for two fecundity genes: GDF9 and BMP15. **A** 799-bp amplicon of exon 2 of the GDF9 gene: lane M, 1 kb Plus DNA ladder; lane P, positive control; lane N, negative control; lanes L1–L5, positive for GDF9 amplicon. **B** 527-bp amplicon of exon 2 of the BMP15 gene: lane M, 1 kb Plus DNA ladder; lane P, positive control; lane N, negative control; lanes L1–L5, positive for BMP15 amplicon.
studied goat population. The G735A locus of *BMP15* was observed with all three possible genotypes in Jamunapari goats while in crossbred goat it was missing the heterozygous genotype. G811A was only polymorphic in Jamunapari goat. The population was in Hardy–Weinberg equilibrium (*p* > 0.05) at all the identified loci in *GDF9* whereas loci in *BMP15* gene were not in equilibrium (*p* < 0.05) except G616T in Jamunapari goats. All loci in *GDF9* were low polymorphic (PIC < 0.25) while *BMP15* loci were moderately polymorphic (PIC ≥ 0.25).
Association between SNP loci and litter size

The least-squares means and standard errors for litter size of different genotypes of seven loci are shown in Table 4.

Results of association analysis indicated Jamunapari goat with GG genotype at G1330T in GDF9 gene recorded with significantly ($p < 0.05$) higher litter size (2.50 ± 0.36) than those of GT genotype (1.50 ± 0.39). However, the crossbred goat with different genotypes of G1330T had a similar litter size. On the other hand, different genotypes for BMP15 loci did not show a significant association with litter size.

Discussion

This study identified seven SNPs in coding exons of GDF9 and BMP15 in Jamunapari and crossbred goats of Bangladesh. The population carries G1189A (also known as p.Val397Ile/V397I), one of the two most well-known SNPs in the GDF9 gene associated with different prolificacy in goats across the world (Wang et al. 2019b). Apart from G1189A, the study population carries C818T (p.Ala273Val),
a known SNP in the GDF9 gene. This mutation is also segregating in Bangladeshi Black Bengal goats (Shaha 2019). The C818T has only been reported in goat breeds of the Indian subcontinent so far (Wang et al. 2019b), and the mutant allele frequencies are stable in different breeds, indicating that it has application value for breed-specific goat breeding.

In a recent study, the Bangladeshi Black Bengal goat was reported to carry five SNPs within 686 to 813 bp of exon 2 of BMP15 (Das et al. 2021). Jamunapari and crossbred goats in the present study showed two SNPs (G735A and G811A) within the same region of the BMP15 gene. The G811A locus was found to be novel by searching the literature for polymorphisms in fecundity genes. However, the G735A locus was recorded in the present and previous studies. The G735A locus was also reported in different goat breeds in India (Ahlawat et al. 2013, 2015b; Maitra et al. 2016). These previous studies indicated that G was the major allele for G735A; however, our research results showed a higher frequency for the A allele in Jamunapari goats. These disparities in allele frequencies may be attributed to population stratification, random genetic drift, or local adaptation. Furthermore, the dominant genotype for a particular mutation could differ in different goat breeds (Wang et al. 2019b).

Associations of polymorphisms in GDF9 and BMP15 genes with litter size in goats were well known (Mishra et al. 2017). However, no apparent associations were observed in the present study except for the G1330T locus in the GDF9 gene. Jamunapari goats with a heterozygous GT genotype had significantly smaller litter size than the GG genotype at the G1330T locus. Novel and rare non-synonymous SNP in the GDF9 gene had application value for breed-specific goat breeding. This study found that two fecundity-associated genes, GDF9 and BMP15, were polymorphic in a selected population of Jamunapari and crossbred goats in Bangladesh. Of the four novel SNPs identified in this study, one SNP (G1330T) significantly affected the litter size. G1330T can be used as a marker in marker-assisted selection for economic traits in Bangladeshi goat population. The SNPs detected in this study can also be used for the caprine SNP chip design in the future.

Conclusion

This study found that two fecundity-associated genes, GDF9 and BMP15, were polymorphic in a selected population of Jamunapari and crossbred goats in Bangladesh. Of the four novel SNPs identified in this study, one SNP (G1330T) significantly affected the litter size. G1330T can be used as a marker in marker-assisted selection for economic traits in Bangladeshi goat population. The SNPs detected in this study can also be used for the caprine SNP chip design in the future.

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Author contribution

M.S.: data collection, investigation, methodology, funding acquisition, and writing—review. G.M.: supervision and editing—original draft. A.L.: investigation and methodology. O.F.M.: investigation and writing—review. M.D.G.: data curation, editing and finalizing draft. A.D.: conceptualization, methodology, data curation, formal analysis, funding acquisition, project administration, supervision, writing—original draft, editing and finalizing draft.

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Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Declarations

Ethics approval

All the techniques employed on the experimental animals were approved by the Animal Experimentation Ethics Committee (CVASU/Dir(R&E)/EC/2020/169/8) at Chattogram Veterinary and Animal Sciences University.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no competing interests.

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