Identification of Polymorphisms in GDF9 and BMP15 Genes in Jamunapari and Crossbred Goats in Bangladesh

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

Running title: Polymorphisms in fecundity genes in goat

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Abstract

Growth differentiation factor 9 (*GDF9*) and bone morphogenetic protein 15 (*BMP15*) are two crucial fecundity genes associated with litter size traits in the goat. Our previous study on *GDF9* and *BMP15* genes detected single nucleotide polymorphisms (SNPs) associated with litter size in Bangladeshi Black Bengal goats. In this study, Jamunapari and crossbred goats of Bangladesh were screened to identify polymorphisms in the *GDF9* and B*MP15* genes and to assess the association between identified SNPs and litter size. The genomic DNA from 100 goats (50 Jamunapari and 50 crossbred) was used in Polymerase Chain Reaction (PCR) to amplify the exon 2 of the *GDF9* and exon 2 of the *BMP15* gene. PCR products were sequenced employing the BigDye Terminator cycle sequencing protocol, to identify SNPs. A generalized linear model was utilized 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, 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 goat, but no significant difference was observed for all genotypes at other loci. This study provides additional molecular markers that would be useful for future research on the litter size trait 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 goat 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).

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. The low heritability and sex-limited nature make it difficult to improve litter size 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, the 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 (Ahlawat et al. 2015a; 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 (Ahlawat et al. 2015a; Mishra et al. 2017; Wang et al. 2019b). However, an association of *BMPR1B* mutation, designated *FecB* mutation with high prolificacy in goat 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 goat is 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 viz. *GDF9* and *BMP15* were investigated to identify litter size-associated polymorphisms.

Materials and Methods

Experimental animal selection and DNA isolation

In the present experimental study, 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) does were utilized in this study. Selected animals were allowed to kid between 2016 and 2017. The animal has no history of selection for litter size and other fertility-related traits. 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 for 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 71 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 minutes in 1X TAE buffer.

Primers 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: 100860859) and exon 2 of the *BMP15* gene (Gene ID: 100861233). The primers sequence, annealing temperature and amplicon sizes are shown in Table 1.

PCR amplification was performed in a final reaction volume of 25 µL consisted of 12.5 µl DreamTaq PCR Master Mix 2X (Thermo Scientific), 10 pM of each primer (OD:2), 50-100 ng of DNA template and nuclease free water. PCR reactions were accomplished on a thermocycler (2720 Applied Biosystem, Thermo Fisher scientific, Singapore) by setting program as follows: initial denaturation at 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 1 min; annealing at 61.2ºC for GDF9 and 60ºC for BMP15 for 45 s; extension at 72ºC for 45 s, and a final extension at 72ºC for 5 min. The PCR products were visualized in 1.5% ethidium bromide (Sigma Aldrich Inc., Missouri, USA) stained agarose gel. The fragment specific bands were photographed by a Gel Documentation System (BDA digital, Biometra GmbH, Germany) and their sizes were estimated using a 1kb plus DNA ladder (GeneRuler, 1 kb Plus, Thermo Scientific Fermentas International Inc., USA).

Sequencing and analysis

The purified PCR products for 100 samples (one for each animal) were bi-directionally Sanger- sequenced using BigDye Terminator v. 3.1 (ThermoFisher 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 Hardy–Weinberg equilibrium (HWE) test were computed using p,q CHWE: PolyPICker (https://www.genecalculators.net/pq-chwe-polypicker.html).For PIC, 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 employed to estimate the effects of different genotypes on liter size using SPSS 25 statistical software, (SPSS Inc., Chicago, Illinois, USA). The least-squares mean was used for litter size among different genotypes:

$$Y_i = \mu + G_j + e_i$$

where, Y_i is the phenotypic value (litter size), μ is the mean of litter size, $G_i$is the fixed effect of the genotype and $e_i$is the random residual effect of each observation.

Ethical considerations

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.

Results

Sequence analysis and Identification of polymorphisms

The sequenced nucleotide covered 688-1362 bp and 340-813 bp of coding sequence (CDS) of the *GDF9* and *BMP15* genes, respectively. The consensus of assembled sequences for Jamunapari and crossbred goats was deposited in NCBI-Genbank database under the accession no. of MN629928 and MN629929 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 the exon 2 of GDF9 and three SNPs (G616T, G735A and G811A) in the exon 2 of the *BMP15* gene (Figure 1). Six of these polymorphisms will result in amino acid changes in the resulting

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,* all polymorphic loci are missing the homozygous mutant genotypes in the studied goat population. The G735A locus of *BMP15* was observed with all three possible genotypes in Jamunapari goat while in crossbred goat it is missing the heterozygous genotype. G811A was only polymorphic in Jamunapari goat. The study population was in Hardy–Weinberg equilibrium ($p>0.05$) at all the identified loci in *GDF9* whereas loci in *BMP15* gene were not in an equilibrium ($p<0.05$). 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

Results of association analysis indicated Jamunapari goat with GG genotype at G1330T in *GDF9* gene recorded with a 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 shows a similar litter size. Different genotypes for *BMP15* loci did not show a significant association with litter size. Although not statistically significant, AA genotyped Jamunapari goat had a lower litter size than GG and GA genotypes at the G735A locus of *BMP15* gene. The least-square means and standard error for litter size of different genotypes of seven loci are shown in Table 4.

Discussion

This study identified seven SNPs in coding exons of *GDF9* and *BMP15* in Jamunapari and crossbred goats of Bangladesh. The present study 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). The mutated allele frequencies for the G1189A were 0.06 and 0.15 in Jamunapari and crossbred goats, respectively (Table 3), indicating it is stable in the population. We did not observe the remainder of well-known SNP A959C (also known as p.Gln320Pro/Q320P) in *GDF9* in studied Jamunapari and crossbred population, which could be attributed to small population size or genetic drift as they are sampled from a particular region of the country. Apart from G1189A, the present study population carry 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 it has application value for breed-specific goat breeding.

In a recent study, the Bangladeshi Black Bengal goat was reported carried five SNPs within 686 to 813 bp of the exon 2 of *BMP15* (Das et al. 2021). Jamunapari and crossbred goat 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 both present and previous studies. The G735A locus was also reported in different goat breeds in India (Ahlawat et al. 2013; Ahlawat et al. 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 goat. 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. However, no apparent associations were observed in the present study except for the G1330T locus in the *GDF9* gene. Jamunapari goats with heterozygous GT genotype had a significantly smaller litter size than the GG genotype at the G1330T locus. Novel and rare missense SNP in the *GDF9* gene have been reported considerably associated with litter size in the Shaanbei White Cashmere goat (Bi et al. 2020). Previous studies recorded both positive (Feng et al. 2011; An et al. 2013; Wang et al. 2013) and negative (Wang et al. 2019a) effects of the mutant allele of the G1089A(V397I) locus on the litter size trait in goats. In agreement with our observation, other studies also reported no association of this SNP with litter size in goats (Hadizadeh et al. 2014; Ahlawat et al. 2015b; Ahlawat et al. 2016; Shokrollahi and Morammazi, 2018). In our previous research (Das et al. 2021), the effect of different genotypes at the G735A locus in the *BMP15* gene on the litter size in the Black Bengal goats of Bangladesh was significant. Though not statically significant, Jamunapari goats with AA genotype in the present study had a smaller litter size than GG and GA genotypes at the G735A locus of the *BMP15* gene. As of the present study, some other studies also had less statistical power to validate positive or negative effects of variants on litter size because of small sample sizes. A deep screening with large sample sizes is required to address this problem.

Conclusion

In conclusion, this study found two fecundity genes- *GDF9* and *BMP15* in Jamunapari and crossbred goat-polymorphic. This study uncovered four SNPs in exon 2 of *GDF9* and three SNPs in exon 2 of the *BMP15* genes. We only observed an association between the different genotypes at the G1330T locus and litter size. Other SNPs did not

show association with litter size. The identified SNPs need to be investigated in a larger goat population for their association with litter size traits. This study enriches SNP data for fecundity genes, hence promoting molecular goat breeding in Bangladesh.

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Data availability

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

Authors' Contribution

M. S.; Data collection, investigation, methodology, funding acquisition and writing-review; G. M.; Supervision and editing original draft. A. L.; Investigation and methodology; A. D.; Conceptualization, methodology; data curation, formal analysis, funding acquisition, project administration, supervision, writing-original draft, editing and finalizing draft.

Statement of animal rights

The manuscript does not contain clinical studies or patient data.

Competing Interests

The authors declare that they have no competing interests.

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Figures

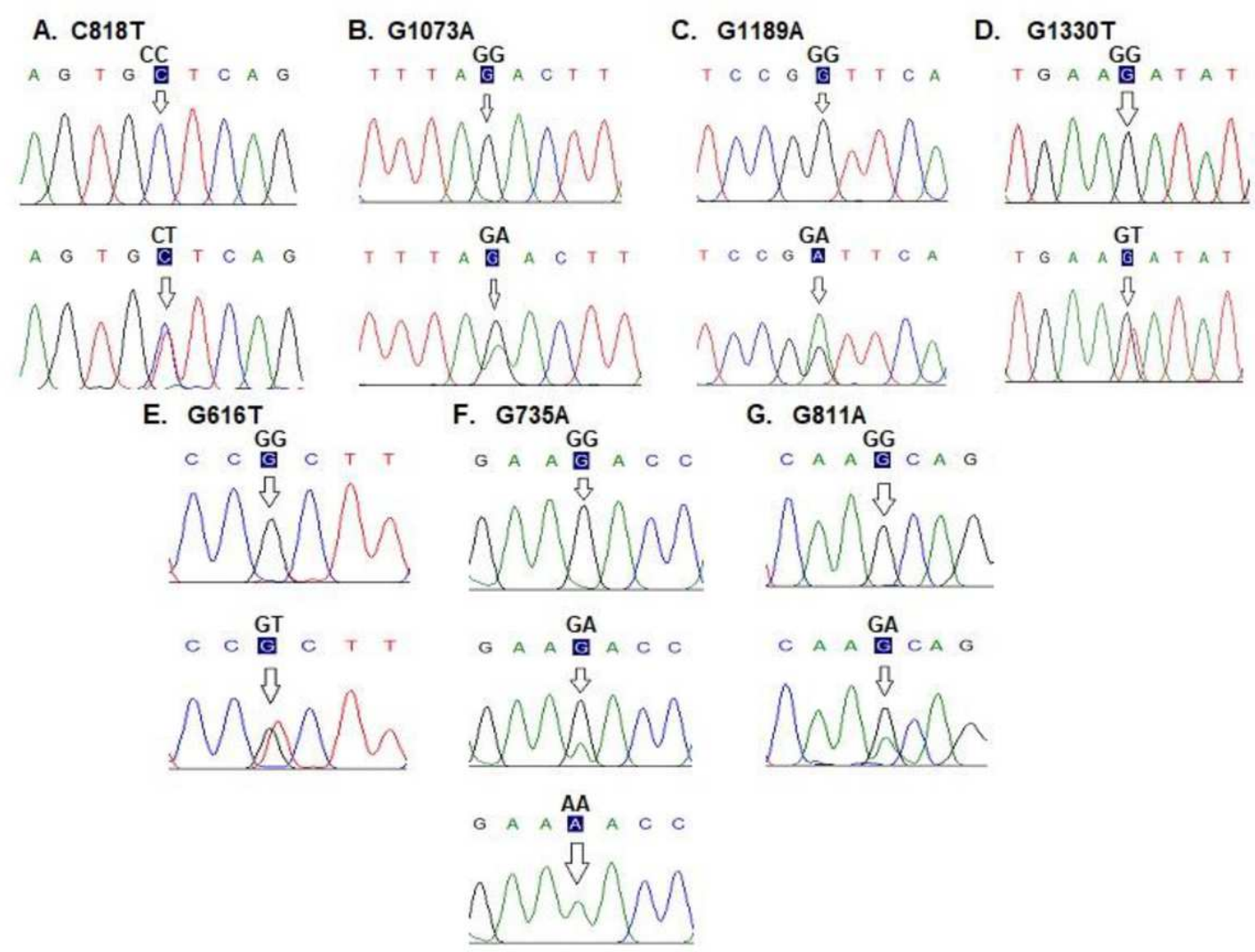

Figure 1

Sequence chromatograms of seven SNPs in GDF9 and BMP15 genes. A. C818T, B. G1073A, C.G1189A, D. G1330T in exon 2 of the GDF9 gene, E. G616T, F. G735A, G. G811A in exon 2 of the BMP15 gene. Positions of the mutations are determined using the full sequences of the GDF9 and BMP15 gene (Gene IDs:100860859 and 100861233, respectively).

Supplementary Files

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