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Dziesevi Clement Vizo
West Bengal University of Animal and Fishery Sciences
Manoranjan Roy
West Bengal University of Animal and Fishery Sciences
Ananta Kumar Das
West Bengal University of Animal and Fishery Sciences
Sanjoy Datta
West Bengal University of Animal and Fishery Sciences
Uttam Sarkar
West Bengal University of Animal and Fishery Sciences
Santanu Bera
West Bengal University of Animal and Fishery Sciences
Hemanta Maity
West Bengal University of Animal and Fishery Sciences
Sharadindu Shil
dr.sharadindu@gmail.com

Genomics and Molecular Biology Laboratory, PBGSBS, Govt of West Bengal https://orcid.org/0000-0002-6167-2244

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Sequence variation at BMP15 exon 2 and GLUD1 exon 8 is not associated with reproductive traits in Black Bengal goats

Dziesevi Clement Vizo[1], Manoranjan Roy[1]*, *Ananta Kumar Das[1], Sanjoy Datta[1], Uttam Sarkar[1], Santanu Bera[2], Hemanta Maity[3], Sharadindu Shil[4]**

[1] Department of Animal Genetics and Breeding, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata 700 037, West Bengal, India

[2] Department of Livestock Production and Management, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata 700 037, West Bengal, India

[3] Department of Avian Sciences, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata 700 037, West Bengal, India

[4] CLART Genomics and Molecular Biology Laboratory (GMBL), Paschimbanga Go Sampad Bikash Sanstha (PBGSBS), Animal Resources Development Department, Government of West Bengal, Kalyani, Nadia, West Bengal, India

*Corresponding authors: Sharadindu Shil (dr.sharadindu@gmail.com (https://orcid.org/0000-0002-6167-2244) and Manoranjan Roy drmanoranjanroy@gmail.com (https://orcid.org/0000-0001-7500-467X)

Abstract

Bone morphogenetic protein 15 (BMP15) and glutamate dehydrogenase 1 (GLUD1) are candidate loci for prolificacy in small ruminants, but they have not been tested in Black Bengal goats across contrasting agro-climatic zones. We screened BMP15 exon 2 and GLUD1 exon 8 by Sanger sequencing in does from the Nadia (alluvial) and Sunderban (coastal saline) zones of West Bengal, India. A single synonymous C>T site in BMP15 exon 2 (K62K) separated 29 does into a C group (n = 21) and a T group (n = 8). Linear models including zone and parity showed no association between BMP15 group and litter size, inter-kidding interval, days open, services per conception or age at first kidding (all $P > 0.65$); only parity predicted litter size ($P < 0.001$). A 90 bp GLUD1 exon 8 interval shared by 24 does was invariant, so no association test was possible. Given the small cohort and the 21:8 imbalance, these data show only that no association was detected at these sites; they do not support either locus as a marker of reproductive performance in this cohort.

Keywords: Black Bengal goat; BMP15; GLUD1; candidate gene; litter size; Sanger sequencing

Introduction

Mutations in the oocyte-derived growth factor gene BMP15 raise ovulation rate in heterozygous ewes and cause sterility in homozygotes (Galloway et al. 2000; Hanrahan et al. 2004), which makes BMP15 exon 2 an obvious target in prolific breeds. In goats, however, associations with litter size are locus- and population-specific: exon 2 variants were associated with litter size in Bangladeshi Black Bengal goats (Das et al. 2021), whereas meta-analyses and other populations show null or site-dependent results (Zergani et al. 2022; Gholizadeh and Mohammadnezhad 2024; Shaha et al. 2022). GLUD1, a mitochondrial enzyme linking amino acid and energy metabolism, has been preliminarily associated with kidding number in Jining Grey goats (Wang et al. 2023). Neither gene has been examined in Indian Black Bengal goats reared in different agro-climatic zones. We therefore screened BMP15 exon 2 and GLUD1 exon 8 and tested association with reproductive traits.

Materials and methods

Records were available for 33 adult Black Bengal does kept under field conditions in Nadia (alluvial zone; n = 13) and South 24 Parganas/Sunderban (coastal saline zone; n = 20), West Bengal. For each doe we recorded parity, litter size, age at first kidding (AFK), inter-kidding interval (IKI), services per conception (SPC) and days open; missing values (e.g. IKI and days open at parity 1) were excluded pairwise. In September 2025, licensed veterinarians collected about 5 mL jugular blood into EDTA during routine farm visits, with the owners' consent. Genomic DNA was extracted with the PureLink Genomic DNA Mini Kit (Invitrogen). A BMP15 exon 2 fragment (reference FJ665809.1) and a GLUD1 exon 8 fragment (reference XM_005699318.3) were amplified with Takara Taq, purified with ExoSAP-IT, cycle-sequenced in the forward direction (single-pass reads) with BigDye Terminator v3.1 and run

on a SeqStudio 8-Flex analyser (Applied Biosystems); primers and cycling conditions are in Online Resource 1. No template controls were included in the PCR reactions.

Chromatograms were trimmed and edited. BMP15 consensus sequences were mapped to NM_001285588.1, and does were assigned to a C or T sequence group at edited-amplicon position 425. Because single-pass consensus sequences cannot separate heterozygotes from homozygotes, diploid genotypes were not called; group assignment was cross-checked against previously assigned AA/BB labels (concordance 29/29). GLUD1 reads were Phred-filtered (reads with fewer than five usable bases were failures), mapped to XM_005699318.3, and variants were sought only in the interval covered by all retained reads.

Each trait was analysed with a linear model (type III sums of squares; car package, R 4.3): BMP15 group, zone and parity for litter size, IKI, days open and SPC, and group and zone for AFK. Least-squares means were obtained with emmeans. Sensitivity analyses for litter size were Poisson and negative-binomial models and a leave-one-out reanalysis. Significance was set at $\alpha = 0.05$.

Results

Thirty does had BMP15 sequence and 30 had GLUD1 chromatograms. For BMP15, one doe (N9) lacked a group assignment and three phenotyped does (S5, S13, S18) had no molecular data, leaving a 29-doe cohort (12 Nadia, 17 Sunderban). Position 425 was C in 21 does (72.4%) and T in 8 (27.6%); this is the synonymous substitution K62K (AAA>AAG, codon 62). For GLUD1, three chromatograms (S10, S17, S20) failed and three (N9, N12, S7) were excluded for quality or coverage, leaving 24 does (11 Nadia, 13 Sunderban) that shared a 90 bp interval (XM_005699318.3:1394–1483) with no variable position.

BMP15 group was not associated with any trait (Table 1). Zone was also non-significant for every trait ($P \geq 0.103$). Parity was the only significant predictor, for litter size ($F_{1,25} = 30.05$, $P < 0.001$). Adjusted litter size was nearly identical in the T group (2.23 kids, 95% CI 1.82–2.63) and C group (2.20, 1.94–2.45). Poisson modelling agreed (incidence-rate ratio 0.98, $P = 0.942$), and no leave-one-out iteration made the group effect significant (P 0.65–0.97; Online Resource 3). Residuals departed from normality for IKI (Shapiro–Wilk $P = 0.028$) and SPC ($P < 0.001$), so those models should be read cautiously.

Table 1 Association of BMP15 exon 2 sequence group with reproductive traits in Black Bengal does

Trait	n (T/C)	F (df)	P
Litter size	29 (8/21)	0.02 (1, 25)	0.896
Inter-kidding interval	24 (6/18)	0.20 (1, 20)	0.659
Days open	24 (6/18)	0.08 (1, 20)	0.785

Services per conception	29 (8/21)	0.21 (1, 25)	0.654
Age at first kidding	18 (–)	0.06 (1, 15)	0.804

Type III tests of BMP15 group; models also contained zone and parity (age at first kidding: zone only). T, T group; C, C group.

Discussion

In 29 Black Bengal does, neither a synonymous BMP15 exon 2 site nor a 90 bp GLUD1 exon 8 interval was associated with reproductive performance. The BMP15 site (K62K) is synonymous with no demonstrated splice or regulatory effect, so it differs in kind from the non-synonymous FecX mutations of sheep, and this result neither tests nor refutes FecX-type biology. Variation in the Bangladeshi Black Bengal population was reported at other exon 2 positions (Das et al. 2021), and the wider literature shows that BMP15 effects on litter size depend on the locus and population (Zergani et al. 2022; Gholizadeh and Mohammadnezhad 2024; Faris et al. 2025). A null result at one site is thus consistent with, and not contrary to, a role for BMP15 elsewhere in the gene.

The GLUD1 finding must be limited to the 90 bp window examined; it does not show that GLUD1 is invariant, and coding variation elsewhere in the gene has been linked to kidding number in another breed (Wang et al. 2023). Parity, a known determinant of litter size, was detected, which suggests that the models can recover a real effect when one is present.

The main limitations are the small cohort, the 21:8 group imbalance (so only large effects could be detected), trait-specific sets as small as 18 does, one exon per gene, and reliance on single-pass, forward-strand consensus sequences without diploid genotype calls, so Hardy–Weinberg proportions were not tested. We conclude that these two loci cannot be recommended as markers of reproductive performance in this cohort. Confirmation or exclusion will require full-gene resequencing, phased genotype calls and larger, balanced samples across both zones.

Statements and Declarations

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: The study was exempted from ethical review by the Institutional Animal Ethics Committee (IAEC), Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India. The exemption was documented under Certificate No. 763/GO/Re/SL/03/CPCSEA/22/2021–22, dated 15 July 2022, for the project entitled *"Genetic analysis of survival potential and estimation of genetic trends of productivity indicators of Black Bengal Goat (Capra hircus bengalensis) at farmers field."* Blood (about 0.4 mL per animal) was collected by a

registered veterinarian by single jugular venipuncture, with owner consent and following standard veterinary practice and the Prevention of Cruelty to Animals Act, 1960. No experimental intervention was performed, ethics committee approval was not sought, and no animals were sacrificed.

Consent: Owners of the animals consented to sampling in written.

Use of AI tools: Quillbot and Gemini were used in drafting and figure generation.

Data availability: Consensus sequences are deposited in GenBank under accessions PZ136072–PZ136100 (BMP15) and PZ150612.1–PZ150636.1 (GLUD1). Per-doe phenotype and sequence-group data are provided in Online Resource 2.

Author contributions: Conceptualization, supervision and formal analysis: Manoranjan Roy, Sharadindu Shil. Investigation, formal analysis and original draft: Dziesevi Clement Vizo. Supervision and investigation: Ananta Kumar Das, Sanjoy Datta, Uttam Sarkar, Santanu Bera, Hemanta Maity. All authors read and approved the final manuscript.

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Figures

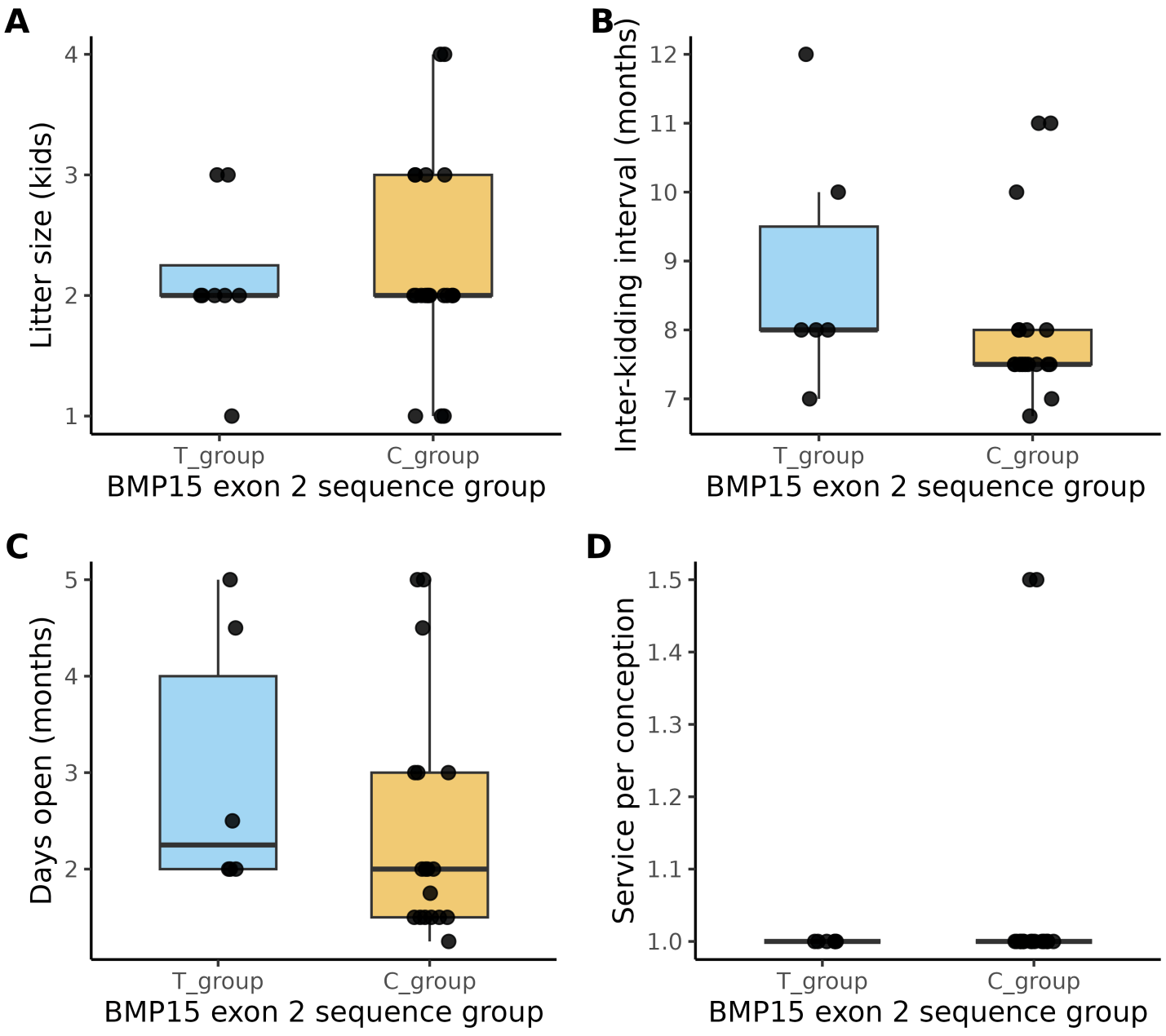


Figure 1

Figure legend not provided with this version

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- OnlineResource1extendedmethods.docx
- OnlineResource2BMP15cohortdata.csv

- OnlineResource3littersizeleaveoneout.csv
- OnlineResource5Tables.docx