



Association of PROP1 Gene Polymorphism (g.1860C>T) with Growth Traits in Assam Hill Goats

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ABSTRACT

Background: The PROP1 (Prophet of Pit-1) gene encodes a critical transcription factor involved in pituitary development and the regulation of growth-related pathways in livestock. Understanding the genetic variation in PROP1 and its association with phenotypic traits could support marker-assisted selection (MAS) strategies in goats.

Methods: A total of 147 Assam Hill goats were phenotypically assessed and blood samples were collected for DNA extraction. The PROP1 gene (exon 2) was amplified and a g.1860C>T SNP was genotyped using PCR-RFLP with *StuI*. Genetic diversity was analyzed using PopGene and protein structure predictions were performed using SOPMA and SWISS-MODEL. Association analysis between genotypes and growth traits was conducted using GLM in SPSS v18.

Result: Three genotypes (CC, CT, TT) were detected at the PROP1 g.1860C>T locus, with CT most common and C as the major allele. The SNP deviated from Hardy-Weinberg equilibrium and showed moderate polymorphism (PIC=0.32). Growth traits differed significantly ($p<0.05$) among genotypes: CC goats had higher early-age body weight and measurements, TT showed better growth at later stages and CT showed intermediate performance. Structural modeling indicated no major protein changes and phylogenetic analysis showed close similarity to sheep PROP1 sequences.

Key words: Assam hill goat, Growth traits, Polymorphism, PROP1 gene.

INTRODUCTION

Goat farming remains a vital contributor to the livelihoods of millions of small-scale and marginal farmers worldwide (Jaber *et al.*, 2025). Particularly in resource-limited and ecologically vulnerable areas, goats have emerged as a reliable source of nutrition, income and social security (Aslam *et al.*, 2023). Their capacity to adapt to diverse agro-climatic conditions, low feed requirements and multi-purpose utility for meat, milk, manure and hides make them indispensable to integrated farming systems (Singh *et al.*, 2023; Meza-Herrera *et al.*, 2024). In the context of climate-resilient agriculture, goat production is increasingly recognized as a vital component of sustainable livestock development (Koluman, 2023; Kerven, 2024). According to the 20th Livestock Census (BAHS, 2019), the India is home to approximately 148.88 million goats, representing approximately 27.8% of the total livestock population and recording a 10.14% growth since the previous census. These animals contribute approximately 13.53% of the total meat and 3.0% of the milk production in the country, thereby significantly supporting the animal protein supply chain (Singh *et al.*, 2023). Beyond economics, goats also serve essential cultural and ritualistic functions in many Indian communities, particularly among tribal populations.

Among India's diverse goat breeds, the Assam Hill goat is particularly important in the northeastern Hill ecosystem. Known for its adaptability to mountainous terrain, early maturity, higher litter size and good meat quality, it serves as a critical genetic resource for low-input production systems (Gawat *et al.*, 2023; Sarma *et al.*, 2025).

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However, productivity under traditional systems remains suboptimal, with both genetic and non-genetic factors significantly influencing post-weaning growth and morphometric traits in Assam Hill goats (Sarma *et al.*, 2019). Genetic improvement in goats is essential for enhancing growth, reproduction and adaptability. While phenotypic selection has been widely used, its progress is slow and influenced by environmental factors (Siddiki *et al.*, 2020). Marker-assisted selection (MAS) relies on the identification of functional gene polymorphisms associated with economically important traits and has been

successfully applied in goats using candidate genes such as IGF-1 and POU1F1 (Singh *et al.*, 2023; Dige *et al.*, 2020). MAS relies on the identification of functional gene polymorphisms associated with economically important traits. This approach has been successfully used in several goat improvement programs both globally and within India.

One of the most promising candidate genes for growth and reproductive traits is the PROP1 (Prophet of Pit-1) gene, a transcription factor crucial for the development of the anterior pituitary and the regulation of multiple hormones, including Growth Hormone (GH), Prolactin (PRL) and Thyroid Stimulating Hormone (TSH). Genetic variations in PROP1 and its downstream pituitary transcription factors, including POU1F1, are associated with growth and production traits in goats, influencing body weight, milk yield and overall productivity (Liu *et al.*, 2015; Zhou *et al.*, 2016; Dorjay and Abraham, 2021). In goats, polymorphisms in PROP1 have been associated with bodyweight, average daily gain, weaning weight and litter size, which are critical productivity indicators (Ma *et al.*, 2017; Jafari *et al.*, 2014). Although genomic tools have been widely explored in several goat breeds (Ahlawat *et al.*, 2015; Gipson, 2019), there remains a clear knowledge gap regarding the genetic architecture and functional polymorphisms of the PROP1 gene in the Assam Hill goat. Despite its importance as a hardy, underutilized genetic resource, limited or no systematic studies have investigated the association of PROP1 variants with growth and reproductive traits in this breed. Addressing this gap is essential for designing region-specific genomic selection strategies. Integrating PROP1 gene markers into breeding programs supports the early identification of genetically superior individuals and accelerates genetic gains. Such programs can enhance the productivity, profitability and sustainability of goat farming, particularly for marginal farmers in the northeastern and eastern regions of India, where goat rearing is a significant source of livelihood. Ultimately, this approach will contribute not only to the conservation and improvement of indigenous goat breeds but also to the resilience of livestock production systems in climate-sensitive regions.

MATERIALS AND METHODS

Study location and sampling

The study involved 147 Assam Hill goats reared in their native breeding tracts of Meghalaya and Assam. Animals were selected based on defined criteria, including good health, absence of close genetic relatedness and strict conformity to established breed characteristics. For accurate morphometric assessment, each animal was positioned on a flat, levelled surface to ensure proper vertical measurements. To evaluate growth performance and morphological development, key biometric traits, including body weight (BW), body length (BL), withers height (WH) and chest girth (CG), were measured at 3, 6, 9 and 12 months of age. These measurements were carried out

following the standardized phenotypic characterization guidelines recommended by the FAO (2012) for the assessment of animal genetic resources.

Blood collection and DNA extraction

Approximately 3-4 mL of blood was collected aseptically from the jugular vein into sterile EDTA-coated tubes. Samples were immediately cooled and stored at -20°C until DNA extraction was performed. Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit and the DNA integrity was confirmed via 1.3% agarose gel electrophoresis. DNA samples were then prepared at 50 ng/μL for polymerase chain reaction (PCR) analysis.

Primer design and PCR amplification

The complete PROP1 gene sequence (*Capra hircus*) was retrieved from GenBank (NC_030814.1). Primers targeting exon 2 were designed using Primer 3 (v0.4.0) and synthesized by Eurofins Genomics (India). PCR amplification comprised a 25/ μL reaction containing 50 ng genomic DNA, 0.5 μM of each primer, 9.5 μL nuclease-free water and 12.5 μL Dream Taq Green PCR Master Mix. The thermocycler program was as follows: initial denaturation at 94°C for 3 min; 34 cycles of 94°C for denaturation, 60.3°C for annealing (45 s) and 72°C for extension (1 min), followed by a final extension at 72°C for 7 min. PCR products were resolved on 2% agarose gel in 1× TAE buffer (Fig 1). Selected amplicons were sequenced and analyzed using Chromas software to identify sequence polymorphisms.

Genotyping via PCR-RFLP

SNPs identified from the sequence analysis were screened with NEB Cutter v2.0, identifying a g.1860C>T variant with a *Stu*I restriction site. For genotyping, the PCR products were digested with 2 U of *Stu*I at 37°C for 12 hours. Digestion products were separated on a 3.2% agarose gel stained with ethidium bromide to assess genotype patterns (Fig 2).

Genetic diversity and bioinformatics analysis

Genetic parameters, including allele and genotype frequencies, Shannon's information index, observed and expected heterozygosity and polymorphic information content (PIC), were calculated using PopGene v1.32. Deviation from Hardy-Weinberg equilibrium (HWE) was assessed using χ^2 testing at $P \leq 0.05$. To explore the structural impact of missense mutations, secondary structures were predicted using SOPMA and tertiary models of both wild-type and variant PROP1 proteins were generated with SWISS-MODEL. Phylogenetic relationships were inferred using the Neighbor-Joining algorithm with pairwise genetic distances in MEGA v11.2 and the resulting tree was visualized using the Interactive Tree of Life (iTOL).

Association analysis of growth traits

Data on growth traits across genotypes were analyzed using the General Linear Model (GLM) procedure in SPSS v18. Initially, potential effects of farm, sex and season of

birth were tested and subsequently excluded due to non-significance. The final model applied was:

$$Y_{ijk} = \mu + A_i + G_j + e_{ijk}$$

Where,

Y_{ijk} = The observed body measurement.

μ = The overall mean.

A_i = The fixed effect of age.

G_j = The fixed effect of PROP1 genotype.

e_{ijk} = The residual error.

The model demonstrated that both age and genotype had a significant impact on trait variation. The least squares mean differences among genotypes were statistically meaningful, as indicated by p-values below 0.05.

RESULTS AND DISCUSSION

Characterization of PROP1 SNPs and allele frequencies

The PROP1 gene was genotyped for a polymorphic locus, namely g.1860C>T. The SNP g.1860C>T was analyzed using the restriction enzyme *StuI*, which allowed the identification of CC, CT and TT genotypes in the examined goat breed. The heterozygote CT genotype showed the highest frequency and allele C was the most frequent in the resource population.

Population genetic parameters and Hardy Weinberg equilibrium (HWE)

At the SNP locus g.1860C>T, three genotypes (both homozygous and heterozygous) were identified. The frequency of allele T was recorded at a minimum of 0.41 and allele C at a maximum of 0.59 (Table 1). This study

observed significant deviations ($P < 0.05$) from HWE at the analyzed SNP locus in the examined populations. These deviations may be attributed to a combination of positive selection, genetic drift and the limited sample size, which potentially influences allelic and genotypic distributions at the examined loci.

Heterozygosity and genic variation statistics

The number of alleles for polymorphic markers was two, similar to the case of biallelic markers. The SNP g.1860C>T showed a PIC (polymorphism information content) value of 0.32 (Table 2). PIC is a statistical measure used to evaluate the informativeness of a genetic marker. The effective number of alleles (n_e) for g.1860C>T is 1.94. These values align with those observed for biallelic markers. Effective alleles represent the number of rare variants needed to reach a level of heterozygosity comparable to that of reference populations. Notably, the SNPs under study displayed medium effective allele counts, indicating their potential suitability for selection and their potential impact on goat breeding. The average heterozygosity estimate, reflecting Nei's genetic differences, is 0.48 in the locus in the examined breed. Shannon's Information Index at the g.1860C>T is 0.67, assessing within-population genetic diversity in the resource populations. The mean observed homozygosity was 0.79 and the observed heterozygosity value was 0.21 in the examined population. The fixation index is 0.55 in the examined goat population (Table 2). The genotypic distribution and measures of genetic variation suggest a heterozygote deficiency in the examined populations.

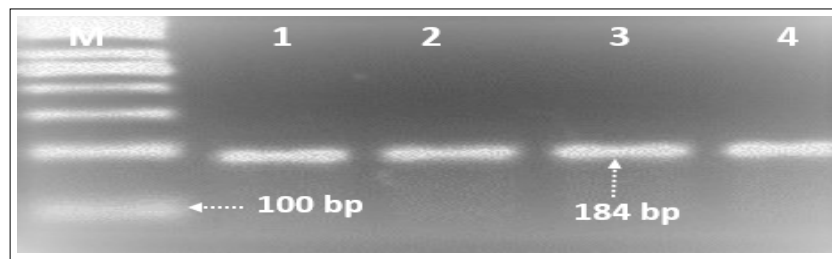


Fig 1: PCR products of the PROP1 gene (M: Marker; 1-4: PCR products of the PROP1 gene).

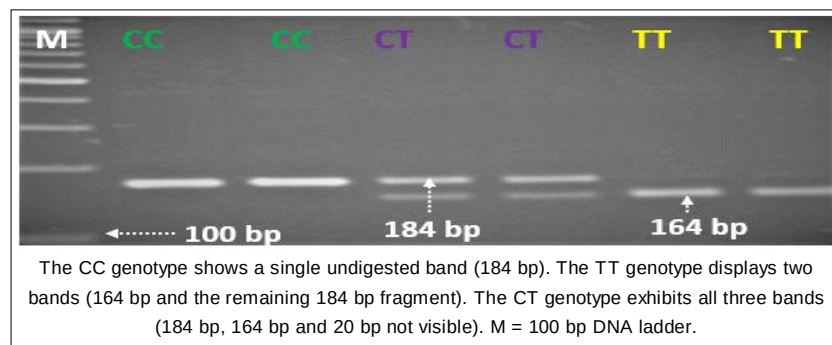


Fig 2: Electrophoresis pattern of the PROP1 gene digested with *StuI*.

Prediction of protein structure and genetic divergence analysis

This study detected a missense mutation at the rs1860 site of the PROP1 gene, resulting in an amino acid substitution from Alanine (Ala) to Valine (Val). According to SOPMA predictions, the secondary structure of proteins (2-D), the wild-type PROP1 protein at the rs1860 locus, was composed of approximately 33.33% extended strand and 66.67 % random coil. The mutant protein at the rs1860 site of the PROP1 gene showed similar compositions of all parameters. The predicted two-dimensional structures for the SNP locus of PROP1 protein are shown in Fig.3a. The predicted three-dimensional (3-D) structures and QMEAN values for both the wild-type and mutant PROP1 (rs1860) proteins are shown in Fig 3b. No alterations in the overall 3-D structure were detected in either the wild-type or mutant proteins.

Phylogenetic analysis of the PROP1 gene

Phylogenetic analysis was conducted to investigate the evolutionary position of the PROP1 gene in the studied goat breed. PROP1 gene sequences from several species were obtained through the NCBI BLAST tool. The phylogenetic tree was constructed using these sequences to visualize evolutionary relationships (Fig 4). The tree revealed a close clustering of the goat PROP1 gene with that of *Ovis aries*, suggesting significant genetic similarity. Additionally, pairwise genetic distances between the goat breed and other species were computed to quantify evolutionary divergence, with results summarized in Table 3. These distances align with the clustering observed in the

phylogenetic tree, further confirming the inferred evolutionary relationships.

Association of PROP1 genotypes with growth traits

Significant variation ($p < 0.05$) was observed in growth traits among PROP1 genotypes (g.1860C>T) in Assam Hill goats (Table 4). Animals with the CC genotype exhibited consistently higher body weights at 3 months (5.88 ± 0.24 kg), 6 months (9.47 ± 0.75 kg) and 12 months (14.09 ± 0.20 kg) compared to CT and TT genotypes. At 9 months, TT animals showed a marked increase in body weight (11.93 ± 0.42 kg), exceeding both CC (11.28 ± 0.60 kg) and CT (10.97 ± 0.84 kg), although the differences were not statistically significant ($p > 0.05$). A highly significant difference ($p < 0.01$) in body length was observed at 6 months, where CC animals (47.16 ± 0.24 cm) outperformed than CT (45.07 ± 0.83 cm) and TT (45.69 ± 0.72 cm) animals. Withers height at 3 months also differed significantly ($p < 0.05$), with CC goats (25.78 ± 0.09 cm) measuring taller than CT (23.17 ± 0.42 cm) and TT (24.14 ± 0.79 cm). However, no significant differences in withers height were recorded at 6, 9, or 12 months ($p > 0.05$). Chest girth was highest in CC animals at 3 months (24.82 ± 0.76 cm), though not statistically significant and converged across genotypes by 6 and 9 months. At 12 months, TT animals exhibited a marginal advantage in chest girth (55.14 ± 0.88 cm), compared to CC (54.02 ± 0.52 cm) and CT (54.92 ± 0.47 cm), with no significant genotype effect ($p > 0.05$). These results indicate that the CC genotype is positively associated with superior early growth traits, while TT individuals demonstrate compensatory growth in later stages. The CT genotype

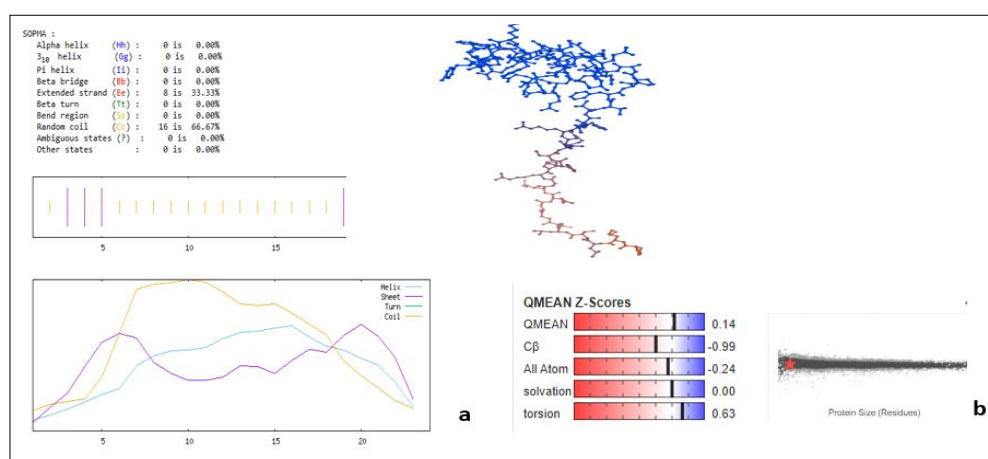


Fig 3: Predicted 2D (Fig 3a) and 3D structure with QMEAN score (Fig 3b) of the caprine PROP1 gene carrying the g.1860C>T.

Table 1: Primer pairs, amplicon sizes and amplified sites for PROP1 gene in goats.

Primers' code	Primer's sequence (5' - 3')	Amplified region	Amplicon length (bp)	Annealing temperature Ta (°C)
PROP1-F	GACCAAAGCTTTCCCTGCAA	Exon 2	184	60.3
PROP1-R	TGGATTCTGGCCTCACTGAG			

In the current investigation, SNP within PROP1, such as g.1860C>T, were genotyped and analyzed for associations with growth traits in Assam hill goats. The observed association of the PROP1 g.1860C>T polymorphism with growth traits in Assam Hill goats aligns with previous findings in cattle and sheep, where PROP1 variants were linked to somatic growth performance (Pan *et al.*, 2013; Ekegbu *et al.*, 2019). The superior early growth

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in CC genotypes and later compensatory growth in TT animals suggest genotype-specific growth patterns consistent with PROP1's regulatory role in pituitary development and growth hormone expression. These findings support the potential utility of the gene in caprine genetic improvement programs. Genetic diversity parameters further support the utility of this SNPs in breeding programs. The PIC values are 0.32, indicating moderate polymorphism and sufficient allelic variation for effective selection (Botstein *et al.*, 1980). The observed heterozygosity levels in the present study suggest a balanced genetic structure essential for maintaining allelic diversity while allowing for the selection of desirable traits (Tosser-Klopp *et al.*, 2016; Kichamu *et al.*, 2025). These findings emphasize the importance of combining unlinked loci in breeding programs to enhance trait predictability and selection efficiency.

An Alanine-to-Valine substitution at the rs1860 position of the PROP1 gene may subtly alter its DNA-binding affinity and transcriptional activity despite preserving overall structural conformation. Even minor side-chain substitutions, such as Ala!Val, are known to influence transcription factor specificity and binding strength (Aditham *et al.*, 2021). This suggests that the g.1860C>T variant in goats may impact growth traits by modulating PROP1's regulatory function. Phylogenetic analyses further demonstrate that PROP1 is highly conserved among small ruminants, particularly goats and sheep. This underscores its crucial regulatory role in growth and pituitary development, suggesting its potential utility in cross-species genetic improvement strategies. Incorporating PROP1 SNPs into marker-assisted selection (MAS) can significantly enhance early selection by reducing dependence on late-appearing phenotypic traits (Deniskova and Barbato, 2022; Khan *et al.*, 2023). However, growth

Table 3: Evolutionary distance and genetic variation of the PROP1 gene in different species.

	1	2	3	4	5	6	7	8	9	10
1										
2	0.005									
3	0.022	0.016								
4	0.022	0.016	0.000							
5	0.022	0.016	0.000	0.000						
6	0.022	0.016	0.010	0.010	0.010					
7	0.022	0.016	0.000	0.000	0.000	0.010				
8	0.022	0.016	0.000	0.000	0.000	0.010	0.000			
9	0.039	0.033	0.027	0.027	0.027	0.016	0.027	0.027		
10	0.105	0.099	0.093	0.093	0.093	0.080	0.093	0.093	0.099	0.000

(The symbols stand for the following terms: 1.*Capra hircus* (>EU327393.1:114-297); 2. *Ovis aries*(>EU340144.1:117-300); 3. *Bos taurus* (>LC706455.1:1717-1900); 4. *Bos mutus* (>XM_070374187.1:188-371); 5. *Bos taurus* × *Bos indicus* (Karan fries) (>KT362137.1:1714-1897); 6. *Bubalus bubalis* (>KT259321.1:763-946); 7. *Bison bison* (>XM_010846491.1:329-512); 8. *Bos indicus* (>XM_019964797.2:580-763); 9. *Cervus elaphus* (>XM_043913961.1:546-729); 10. *Sus scrofa* (>AB187272.1:201-384).

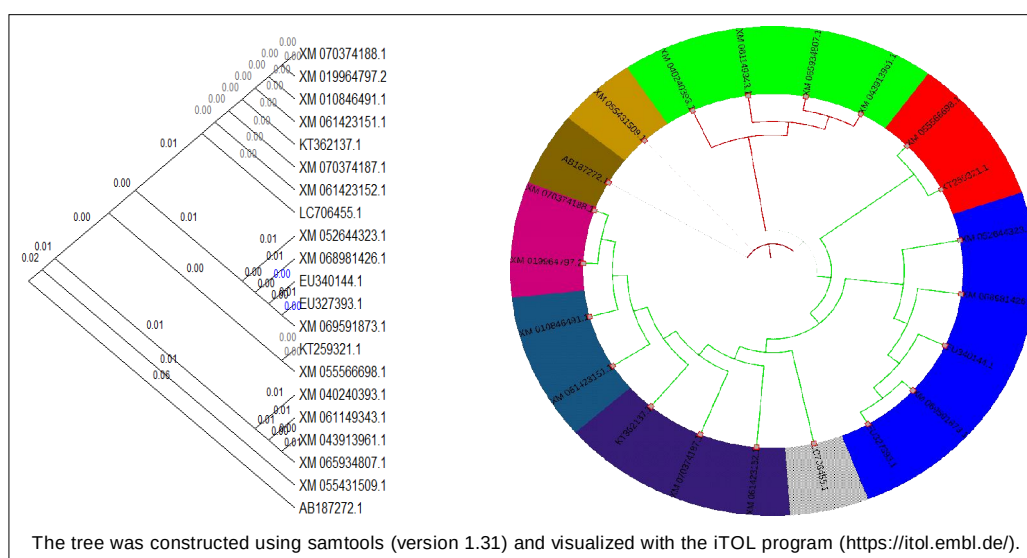


Fig 4: Phylogenetic tree depicting the evolutionary relationships among different species.

Table 4: Genotypic influence of POU1F1 (g.1860C>T) on growth traits (Mean±SE) in Assam hill goats.

Traits	Genotypes at g. 1860 C>T		
	CC	CT	TT
Body weight (kg)			
3-month BW	5.88±0.24	5.74 ± 0.20	5.19 ± 0.70
6-month BW	9.47±0.75	9.17±0.50	8.79±0.66
9-month BW	11.28±0.60	10.97±0.84	11.93±0.42
12-month BW	14.09±0.20	13.18±0.14	13.85±0.61
Body length (cm)			
3-month BL	31.08±0.62	30.10±0.28	31.01±0.10
6-month BL	47.16±0.24 ^a	45.07±0.83 ^b	45.69±0.72 ^b
9-month BL	52.15±0.37	53.12±0.80	54.13±0.92
12-month BL	59.51±0.73	59.16±0.82	60.11±0.48
Wither height (cm)			
3-month WH	25.78±0.09 ^a	23.17±0.42 ^b	24.14±0.79 ^{ab}
6-month WH	40.06±0.38	39.32±0.29	39.06±0.17
9-month WH	46.11±0.56	45.98±0.86	45.06±0.37
12-month WH	49.19±0.36	49.69±0.73	50.18±0.03
Chest girth (cm)			
3-month CG	28.82±0.76	28.99±0.77	29.08±0.14
6-month CG	42.23±0.60	42.10±0.13	43.11±0.82
9-month CG	48.40±0.71	49.18±0.63	49.05±0.11
12-month CG	54.02±0.52	54.92±0.47	55.14±0.88

characteristics are inherently polygenic and influenced by environmental factors, making genomic selection (GS) based on high-density SNP arrays a more robust strategy for accurately predicting breeding values (Deniskova and Barbato, 2022; Ncube *et al.*, 2025).

Considering these results, the study holds significant relevance for practical breeding programs. The superior early growth performance observed in goats carrying the CC genotype suggests its potential as an effective early selection marker for improving body weight and linear body measurements in meat-oriented production systems. Integrating the PROP1 g.1860C>T variant into marker-assisted selection (MAS) schemes could therefore accelerate genetic progress by facilitating more precise and timely identification of superior kids. Given the crucial role of Assam Hill goats in supporting the livelihoods of tribal communities across Northeast India, the identification of functional polymorphisms such as PROP1 offers opportunities to develop more structured, sustainable breeding strategies while maintaining the breed's adaptation to local environments.

CONCLUSION

This study underscores the genetic and functional relevance of the PROP1 g.1860C>T SNP in Assam Hill goats. The allele frequencies, significant deviation from the Hardy-Weinberg equilibrium and moderate polymorphism suggest the presence of underlying selective forces and constrained genetic diversity. Although structural modeling of the Ala'→Val substitution revealed no

significant changes, potential effects on transcriptional regulation remain to be explored. Phylogenetic analysis confirmed the evolutionary conservation of PROP1 among small ruminants. Notably, the CC genotype was consistently associated with superior early growth traits, supporting its utility as a candidate marker in selection programs. However, further studies are required in larger and more diverse caprine populations to validate these findings and enhance their applicability in genetic improvement programs.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the Institute of Animal ethics Committee.

Conflict of interest

All authors declare that they have no conflict of interest.

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