Tropical Animal Health and Production (2026) 58:99
https://doi.org/10.1007/s11250-026-04907-z

REGULAR ARTICLES

Exploring genetic variability and its association with growth traits in Rohilkhandi goats

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Received: 22 July 2025 / Accepted: 27 January 2026 / Published online: 12 February 2026

Abstract

Goats offer unique social, economic and biological benefits compared to other livestock species and are often referred to as the "poor man's cow". This study aimed to examine the polymorphism of six gene loci, namely *IGF1*, *GH1*, *GH2*, *MSTN*, *IGFBP3* and *GDF9*, in Rohilkhandi goats using PCR-RFLP and assessed their association with various growth traits like body weight measured from birth to 48 months of age. Polymorphism was detected only in *IGF1*, *GH1* and *MSTN*, whereas *GH2*, *IGFBP3* and *GDF9* were monomorphic in this population. Body weight, as an indicator of growth traits, increased significantly from 2.17 ± 0.04 kg at birth to 28.55 ± 0.32 kg at 48 months of age. Association analysis showed that *IGF1* genotypes were related to body weight across all age points ($p<0.05$, $p<0.01$ and $p<0.001$), with the BB genotype exhibiting higher mean body weights. *GH1* genotypes showed effects only at 9 and 15 months and *MSTN* exhibited genotype-body weight associations at multiple ages across the growth period ($p<0.05$, $p<0.01$ and $p<0.001$), with the AA genotype consistently showed higher body weight than BB. Although the study was based on a modest sample of female goats from a single herd, the consistent trends observed across age points provide a valuable initial indication of genotype-growth relationships in Rohilkhandi goats. The patterns detected for *IGF1* and *MSTN* highlight their potential biological relevance for growth performance. Building on this foundation, future multi-herd studies with larger and more diverse populations will be important for confirming these promising signals and refining their usefulness for genetic improvement programs.

Keywords Body weight · *GH1* · Growth traits · *IGF1* · *MSTN* · PCR-RFLP · Polymorphism

Abbreviations

BW	Body weight
BW (0 to 48)	Body weight at birth to 48 months of age
DMRT	Duncan's Multiple Range Test
GDF9	Growth Differentiation Factor 9
GH	Growth Hormone
GLM	General Linear Model
ICAR	Indian Council of Agricultural Research
IGF1	Insulin Like Growth Factor 1
IGFBP3	Insulin Like Growth Factor Binding Protein 3
LPM	Livestock Production Management
MAS	Marker Assisted Selection
MSTN	Myostatin
NBAGR	National Bureau of Animal Genetic Resources

Introduction

In developing countries, agriculture has undergone significant transformations due to industrial integration, globalization and technological advancements. Within this evolving landscape, goats play a pivotal role in resource-poor communities, contributing significantly to household income and nutritional security (Ronda-Borzone et al. 2022). Globally, the goat population is estimated at approximately 1.1 billion, with 57.7% concentrated in Asia. India accounts

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for about 148.88 million goats, encompassing 41 registered breeds, which represent 27.8% of the nation's total livestock population (Kerven 2024; Basic Animal Husbandry Statistics Book, 2024). Among these, the Rohilkhandi goat was officially recognized by the National Bureau of Animal Genetic Resources (NBAGR), Karnal, India in 2018. Native to the Rohilkhand region of Uttar Pradesh, this medium-sized breed is characterized by a rectangular black body, small greyish-black backward-curving horns, a bunchy tail and absence of beard or wattles. Adult body weights typically range from 25 to 36 kg in males and 21–31 kg in females (Singh et al. 2019).

Growth in animals is influenced by a combination of genetic, environmental and nutritional factors. It is commonly assessed using parameters such as body length, body width, chest girth and body weight (Ofori et al. 2021). These growth traits are complex and polygenic, regulated by several candidate genes including *IGF1*, *IGFBP3*, *GH1-2*, *MSTN* and *GDF9* which play key roles in muscle development, skeletal growth and various physiological processes. Notably, specific mutations within these genes have been associated with diverse phenotypic variations across goat breeds (Chalbi et al. 2023; Hosseinzadeh Shirzeyli et al. 2023; Sun et al. 2023; Buranakarl et al. 2024). Earlier molecular investigations have consistently shown that polymorphisms in gene loci such as *IGF1*, *GH1*, *GH2*, *MSTN*, *IGFBP3* and *GDF9* are associated with variation in growth-related traits across several goat breeds, indicating their potential functional relevance in growth regulation (Lan et al. 2007; Li et al. 2008; Singh et al. 2015; Zhao et al. 2016; Naicy et al. 2017; Shareef et al. 2018; Bi et al. 2020; Gitanjli et al. 2020; Buranakarl et al. 2024). These findings underline the biological relevance of these genes and justify their continued evaluation in indigenous populations such as Rohilkhandi goats.

Genomic selection such as marker-assisted selection (MAS) enables the identification and application of genetic markers associated with economically important traits, thereby expediting genetic improvement in livestock populations. Among the various molecular tools available, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is widely recognized as a reliable, cost-effective and reproducible method for detecting single nucleotide polymorphisms (SNPs) (Hashim and Al-Shuhaib 2019; Sharma et al. 2024). Recent studies in various goat breeds have identified several genetic markers significantly associated with superior growth traits, reinforcing the utility of molecular techniques in genetic improvement programs. Despite these advances, genomic studies on Indian breeds remain limited, creating gaps in population-specific information that is essential for sustainable utilization and conservation of native germplasm. The Rohilkhandi breed, being recently recognized and relatively understudied at the molecular level, presents an important opportunity to investigate the genetic underpinnings of growth performance. Although growth assessment includes several morphometric traits such as body length, body width and chest girth, only body-weight data were available as long-term, consistently recorded measurements for this population. Therefore, the present study focuses on body weight as the primary growth parameter, while acknowledging that the absence of other traits represents a limitation in fully characterizing growth biology in this breed. The present study aims to evaluate the impact of gene polymorphisms in Rohilkhandi goats by identifying specific candidate genes and mutations that influence growth and physiological performance. By investigating polymorphisms in *IGF1*, *GH1*, *GH2*, *MSTN*, *IGFBP3* and *GDF9*, and assessing their association with body weight from birth to 48 months, this study provides foundational data that may guide future marker-assisted selection strategies and contribute to the genetic enhancement of indigenous breed.

Materials and methods

Experimental animals

A total of fifty Rohilkhandi goats (female), each aged four years or older, were selected from the Sheep and Goat Farm, Livestock Production and Management Section, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh (243122), India.

Experimental methods

Sample collection

Blood collection was carried out under sterile conditions and with prior approval from the Institute Animal Ethics Committee (IAEC), ICAR-Indian Veterinary Research Institute, India. Approximately 3 mL of blood was aseptically drawn from the jugular vein of each animal using sterile disposable syringes and collected into 5 mL BD Vacutainer® tubes containing EDTA as an anticoagulant. The tubes were immediately sealed and gently inverted several times to ensure thorough mixing of the anticoagulant. Subsequently, samples were transported to the laboratory in an icebox and stored at −20 °C until further analysis.

Genomic DNA extraction

Genomic DNA was extracted from the blood samples using the QIAamp® DNA Blood Mini Kit (QIAGEN, Germany),

Table 1 Details of the gene loci with their primers, amplicon size and optimized annealing temperature

S. No.	Gene	Sequence (5'-3')	Amplicon size (bp)	Annealing temperature	References
1.	IGF1	F: CACAGCGTATTATCCCAC *R*: GACACTATGAGCCAGAAG	363	53 °C	(Surya et al. 2021)
2.	*GH1*	F: CTCTGCCTGCCCTGGACT R: GGAGAAGCAGAAGGCAACC	422	65 °C	(Hua et al. 2009)
3.	*GH2*	F: TCAGCAGAGTCTTCACCAAC R: CAACAACGCCATCCTCAC	116	54 °C	(Hua et al. 2009)
4.	*MSTN*	F: TGGCGTTACTCAAAAGCAAA R: AACAGCAGTCAGCAGAGTCG	497	56 °C	(Li et al. 2008)
5.	*IGFBP3*	F: GAA ATG GCA GTG AGT CGG R: TGG GCT CTT GAG TAA TGG TG	316	58 °C	(Lan et al. 2007)
6.	*GDF9*	F: CCTGTTACATATGGCATTACTGTTGGAAT R: TTATCACCAGGTTGCATATAC	187	56 °C	(Shokrollahi and Moram-mazi 2018)

Table 2 Restriction enzyme used for each gene fragment (with fragment ID), their incubation temperature and restriction site

S. No.	Gene	Fragment ID	Restriction Enzymes	Incubation Temperature	Restriction site
1.	IGF1	A	HaeIII	37 °C	5' GG↓CC 3'
2.	*GH1*	B	*Hae*III	37 °C	5' GG↓CC 3'
3.	*GH2*	C	*Hae*III	37 °C	5' GG↓CC 3'
4.	*MSTN*	D	*Dra*I	37 °C	5' TTT↓AAA 3'
5.	*IGFBP3*	E	*Hae*III	37 °C	5' GG↓CC 3'
6.	*GDF9*	F	*Ssp*I	37 °C	5' AAT↓ATT 3'

according to the manufacturer's instructions. The quality, purity and concentration of the extracted DNA were assessed using 1.0% agarose gel electrophoresis and a spectrophotometer (Eppendorf BioSpectrometer®, Eppendorf AG, Germany). Only samples meeting the required quality criteria (i.e., intact DNA bands, A260/280 ratio between 1.8 and 2.0 and a concentration greater than 30 ng/µL) were selected for downstream molecular analyses.

Selection of gene loci and their PCR primers

Genes associated with the growth traits under investigation, including *IGF1*, *GH1*, *GH2*, *MSTN*, *IGFBP3* and *GDF9* were selected through a comprehensive review of relevant literature. The corresponding primer sequences used for amplification are listed in Table 1. A total of six primer pairs were procured from GCC Biotech (India). To prepare stock solutions, the primers were diluted with nuclease-free water to a concentration of 100 pmoL/µL. Subsequently, working solutions were prepared by performing a 10-fold dilution of the stock, resulting in a final concentration of 10 pmoL/µL.

PCR amplification and detection

Polymerase chain reaction (PCR) was performed in a 25 µL reaction volume using a thermal cycler (Agilent Technologies, USA). Each reaction contained 2 µL of genomic DNA, 1 µL each of forward and reverse primers, 12.5 µL of 2X DreamTaq™ PCR Master Mix (Thermo Scientific™, USA) and 8.5 µL of nuclease-free water. The amplification protocol included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at temperatures ranging from 53.0 to 65.0 °C for 45 s, and extension at 72 °C for 60 s. A final extension was carried out at 72 °C for 10 min, and the amplified products were held at 4 °C until further analysis.

PCR amplification products were analysed by agarose gel electrophoresis using a 1.5% agarose gel prepared in 1X TAE buffer. A 5 µL aliquot of each PCR product was loaded and electrophoresed at 100 V for 60 min. DNA fragments were visualized under UV illumination using a gel documentation system (Bio-Rad Laboratories, USA).

Restriction enzyme digestion

Restriction enzyme (RE) digestion was carried out in 0.2 mL microcentrifuge tubes in a total reaction volume of 23 µL. Each reaction mixture contained 1 unit of restriction enzyme, 2.3 µL of 10X reaction buffer, 0.6 µL of nuclease-free water, and 20 µL of PCR product. The mixtures were incubated overnight at 37 °C in a digital water bath (Labman, India), following the manufacturer's instructions to ensure complete digestion. The enzymes used included *Dra*I for *MSTN*; *Hae*III for *IGF1*, *GH1*, *GH2* and *IGFBP3*; and *Ssp*I for *GDF9*, as detailed in Table 2. The digested products were stored −20 °C for further analysis.

PCR-RFLP genotyping

Genotyping of polymorphisms in the six gene loci was conducted using the PCR-RFLP technique. The digested PCR products were resolved by 3% agarose gel electrophoresis at 100 V for 90 min. Following electrophoresis, the gels

were visualized under UV illumination using a gel documentation system (Bio-Rad Laboratories, USA) and images were captured for genotyping analysis. Allelic patterns and fragment sizes were analysed using Quantity One® software (Bio-Rad Laboratories, USA).

Statistical analysis

Genotypic and allelic diversity analysis

Genotypic and allelic frequencies for the *IGF1*, *GH1* and *MSTN* loci were estimated from PCR-RFLP patterns and confirmed sequence data. Population-genetic parameters, including the observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), Nei's gene diversity, inbreeding coefficient (Fis) and polymorphic information content (PIC), were computed using PopGen 1.32. Departure from Hardy-Weinberg equilibrium (HWE) was tested using Pearson's chi-square (χ^2) and the likelihood-ratio G-test (df = 1) implemented in PopGen 1.32.

Association analysis

To assess associations between candidate-gene genotypes and body weight traits, a mixed-model analysis was performed using the MIXED procedure in IBM SPSS Statistics, Version 29.0 (IBM Corp.). Season of birth and dam parity were included as fixed non-genetic effects. The statistical model used for each gene was:

$$Y_{ijk} = \mu + G_i + S_j + P_k + e_{ijk}$$

Where:

Y_{ijk}= observation of the trait for the k^{th} animal of the i^{th} genotype in the j^{th} season, μ = overall mean, G_i= fixed effect of genotype (*IGF1*, *GH1* or *MSTN*), S_j= fixed effect of season (1 = summer; 2 = autumn), P_k= fixed effect of dam parity (1 = ≤3 parities; 2 = >3parities) and e_{ijk}= random residual error.

Estimated marginal means were compared using Bonferroni-adjusted post-hoc tests to control for Type I error across multiple pairwise contrasts.

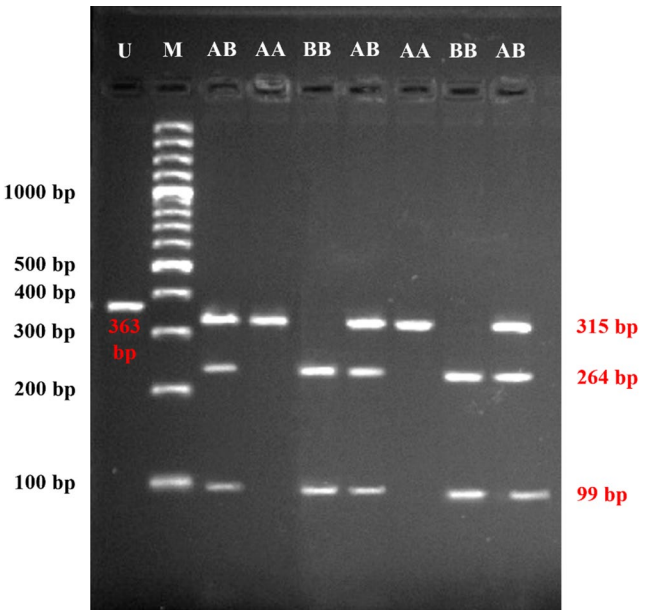

Fig. 1 PCR RFLP Agarose Gel Image of 363bp Fragment A (corresponding to *IGF1* gene) using *Hae*III Restriction Enzyme

Results

PCR-RFLP

Genomic DNA was extracted from blood samples, followed by PCR amplification of the target gene fragments. he PCR products were analysed using 1.5% agarose gel electrophoresis, which confirmed that the amplified bands corresponded to the expected product sizes, indicating high amplification specificity. The amplicons were subsequently digested with their respective restriction enzymes to identify allelic variants. The restriction enzyme *Hae*III cleaved gene fragment C (116 bp) corresponding to *GH2* gene into two fragments of 88 bp and 28 bp; and gene fragment E (316 bp) corresponding to *IGFBP3* gene into two fragments of 264 bp and 52 bp. However, both loci were found to be monomorphic in the Rohilkhandi goat population under study.

DNA fragment A, corresponding to the *IGF1* gene, was digested with *Hae*III, resulting in three genotypes: AA (315 bp), AB (315, 264 and 99 bp) and BB (264 and 99 bp), with genotype frequencies of 0.40, 0.44 and 0.16, respectively. The corresponding allele frequencies for A and B were 0.62 and 0.38. For DNA fragment B, corresponding to the *GH1* gene, was digested with *Hae*III, resulting in two genotypes: AB (422 and 366 bp) and BB (366 bp), with genotype frequencies of 0.54 and 0.46, respectively. The allele frequencies for A and B were 0.27 and 0.73, respectively. For DNA fragment D, corresponding to the *MSTN* gene, digested with *Dra*I, resulting in two genotypes: AA (497 bp) and BB (427 and 70 bp), with respective genotype frequencies of 0.78 and 0.22. The corresponding allele frequencies were 0.78 for A and 0.22 for B. The genotypic banding patterns for *IGF1*, *GH1* and *MSTN* genes are illustrated in Figs. 1, 2 and 3, respectively. DNA fragments below 70 bp tend to diffuse or migrate off 3% agarose gels, these very small products (<70 bp) were not evaluated through direct visualization. Genotype identification relied on the presence

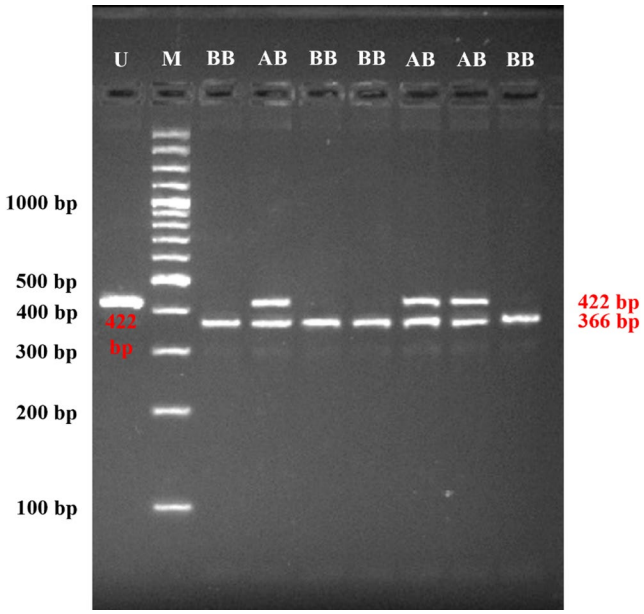

Fig. 2 PCR RFLP Agarose Gel Image of 422 bp Fragment B (corresponding to *GH1* gene) using *Hae*III Restriction Enzyme

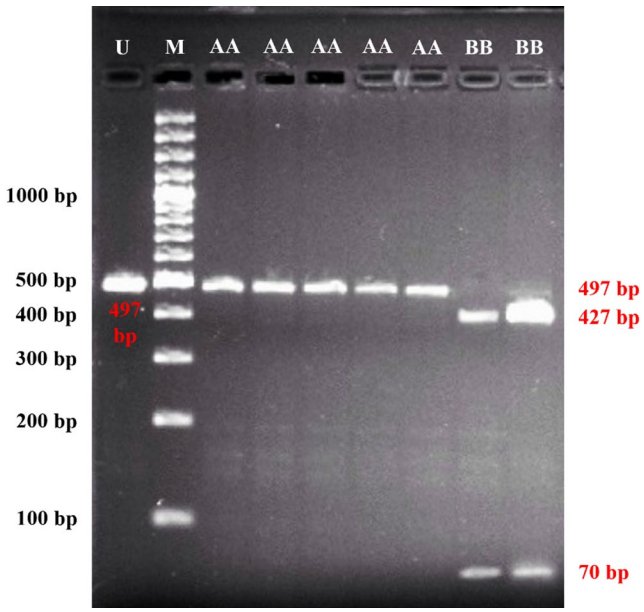

Fig. 3 PCR RFLP Agarose Gel Image of 497 bp Fragment D (corresponding to *MSTN* gene) using *Dra*I restriction enzyme. (U: Undigested PCR Product, M: 100 bp ladder, Bands in right side lanes are digested PCR product) *

of clearly resolved diagnostic bands together with the expected restriction-enzyme digestion patterns.

All genetic diversity estimates, including allele frequencies, heterozygosity measures, inbreeding coefficients and Hardy-Weinberg equilibrium tests for the *IGF1*, *GH1* and *MSTN* gene loci, are summarized in Table 3.

Table 3 Summary of allelic diversity, heterozygosity and Hardy-Weinberg equilibrium statistics for *IGF1*, *GH1* and *MSTN* gene loci in the studied goat population

S. No.	Parameter	IGF1 Gene	GH1 Gene	MSTN Gene
1.	Total individuals (*N*)	50	50	50
2.	Observed number of alleles (Na)	2.00	2.00	2.00
3.	Effective number of alleles (Ne)	1.89	1.65	1.52
4.	Allele A frequency	0.62	0.27	0.78
5.	Allele B frequency	0.38	0.73	0.22
6.	Observed heterozygosity (Ho)	0.44	0.54	0.00
7.	Expected heterozygosity (He)	0.47	0.39	0.34
8.	Nei's gene diversity (He)	0.47	0.39	0.34
9.	Inbreeding coefficient (Fis)	0.07	-0.37	1.00
10.	Polymorphic Information Content (PIC)	0.36	0.32	0.28
11.	Chi-square (χ^2) statistic	0.22	6.84**	50.00***
12.	Chi-square P-value	0.64	0.009	0.000
13.	G-test (G^2) statistic	0.22	10.23**	52.69***
14.	G-test P-value	0.64	0.001	0.000

Note: Significance levels, *$P<0.05$, **$P<0.01$ and ***$P<0.001$

Data on growth traits

Growth trait data from birth up to 48 months of age were obtained from historical records maintained at the Sheep and Goat Farm, Livestock Production and Management (LPM) Section, ICAR–IVRI, Izatnagar, Uttar Pradesh, India. For the estimation of growth traits, body weight was recorded at regular intervals. The average body weight at birth was 2.17 ± 0.04 kg and the corresponding mean body weights (± standard error) at subsequent ages were as follows: at 3 months, 4.64 ± 0.06 kg; at 6 months, 7.26 ± 0.10 kg; at 9 months, 9.84 ± 0.16 kg; at 12 months, 12.85 ± 0.22 kg; at 15 months, 14.29 ± 0.22 kg; at 18 months, 15.80 ± 0.23 kg; at 21 months, 17.33 ± 0.23 kg; at 24 months, 18.59 ± 0.22 kg; at 27 months, 19.80 ± 0.21 kg; at 30 months, 21.20 ± 0.24 kg; at 33 months, 22.59 ± 0.28 kg; at 36 months, 23.97 ± 0.30 kg; at 39 months, 25.42 ± 0.32 kg; at 42 months, 26.62 ± 0.31 kg; at 45 months, 27.56 ± 0.32 kg; and at 48 months, 28.55 ± 0.32 kg, also presented in Table 4.

Association between genetic variants and growth traits

In the Rohilkhandi goat, individuals were genotyped for *IGF1*, *GH1* and *MSTN* gene loci, and corresponding phenotypic data on growth traits were analysed. The GLM was used to evaluate the association between genotypes and body weight traits. The corresponding p-values are presented in Table 5. Estimated marginal means (± standard error) were estimated for the three polymorphic loci (*IGF1*, *GH1* and *MSTN*) in relation to the following body weight traits: BW0,

Table 4 Mean (with standard Error) of different growth traits in Rohilkhandi goats

S. No.	Trait Name	Abbreviations	Means ± SE (*n*)
1.	Body Weight at Birth (kg)	BW0	2.17 ± 0.04
2.	Body Weight at 3 Months (kg)	BW3	4.64 ± 0.06
3.	Body Weight at 6 Months(kg)	BW6	7.26 ± 0.10
4.	Body Weight at 9 Months(kg)	BW9	9.84 ± 0.16
5.	Body Weight at 12 Months(kg)	BW12	12.85 ± 0.22
6.	Body Weight at 15 Months (kg)	BW15	14.29 ± 0.22
7.	Body Weight at 18 Months(kg)	BW18	15.80 ± 0.23
8.	Body Weight at 21 Months(kg)	BW21	17.33 ± 0.23
9.	Body Weight at 24 Months(kg)	BW24	18.59 ± 0.22
10.	Body Weight at 27 Months(kg)	BW27	19.80 ± 0.21
11.	Body Weight at 30 Months(kg)	BW30	21.20 ± 0.24
12.	Body Weight at 33 Months(kg)	BW33	22.59 ± 0.28
13.	Body Weight of 36 Months(kg)	BW36	23.97 ± 0.30
14.	Body Weight at 39 Months(kg)	BW39	25.42 ± 0.32
15.	Body Weight at 42 Months(kg)	BW42	26.62 ± 0.31
16.	Body Weight at 45 Months (kg)	BW45	27.56 ± 0.32
17.	Body Weight at 48 Months(kg)	BW48	28.55 ± 0.32

Table 5 General linear model (GLM) p-values for the effects of *IGF1*, *GH1* and *MSTN* genotypes on body weight traits in Rohilkhandi goats

S. No.	Trait Name	IGF1	GH1	MSTN
1.	Body Weight at Birth (kg)	0.027*	0.188	0.000***
2.	Body Weight at 3 Months (kg)	0.000***	0.355	0.021*
3.	Body Weight at 6 Months (kg)	0.001**	0.521	0.007**
4.	Body Weight at 9 Months (kg)	0.000***	0.007**	0.009**
5.	Body Weight at 12 Months (kg)	0.011*	0.051	0.007**
6.	Body Weight at 15 Months (kg)	0.001**	0.033*	0.001***
7.	Body Weight at 18 Months (kg)	0.006**	0.123	0.004**
8.	Body Weight at 21 Months (kg)	0.011*	0.147	0.014*
9.	Body Weight at 24 Months (kg)	0.002**	0.123	0.005**
10.	Body Weight at 27 Months (kg)	0.005*	0.352	0.008**
11.	Body Weight at 30 Months (kg)	0.012*	0.252	0.005**
12.	Body Weight at 33 Months (kg)	0.026*	0.567	0.022*
13.	Body Weight of 36 Months (kg)	0.022*	0.278	0.019*
14.	Body Weight at 39 Months (kg)	0.023*	0.259	0.018*
15.	Body Weight at 42 Months (kg)	0.010*	0.232	0.008**
16.	Body Weight at 45 Months (kg)	0.004**	0.214	0.003**
17.	Body Weight at 48 Months (kg)	0.001**	0.194	0.001***

Note: Significance levels, *$p<0.05$, **$p<0.01$ and ***$p<0.001$

BW3, BW6, BW9, BW12, BW15, BW18, BW21, BW24, BW27, BW30, BW33, BW36, BW39, BW42, BW45 and BW48; presented in Table 6.

Discussion

Growth traits are polygenic, influenced by the combined effects of multiple genes. Genetic variation within genes associated with body weight is considered valuable for marker-assisted selection in livestock improvement programs. Among such variations, SNPs represent the most common and informative type of genetic marker and are widely used in association studies between genetic variants and phenotypic trait (Zonaed Siddiki et al. 2020). In the present study, six candidate genes (*IGF1*, *GH1*, *GH2*, *MSTN*, *IGFBP3* and *GDF9*) were selected based on their known roles in growth regulation and metabolic processes. These genes were investigated to identify genotypic variants in the Rohilkhandi goats, with the aim of assessing their potential associations with growth performance.

In the present study, the *GH2* gene fragment exhibited a monomorphic pattern, consistent with identical RFLP patterns previously reported in Surti and Mehsani goats (Bayan et al. 2018), and Beetal goats (Shareef et al. 2018). Similarly, for the IGFBP3 gene, monomorphic PCR-RFLP patterns were observed in Rohilkhandi goats, aligns with earlier findings in Guizhou White, Leizhou and Shaannan White goats (Lan et al. 2007), as well as in Mongolian Cashmere goats (Liu et al. 2012). In contrast, a study on Liaoning Cashmere goats revealed polymorphism at the *IGFBP3* locus (Sun et al. 2023). The *GDF9* fragment examined in this study was

Table 6 Estimated marginal means with standard error, number of observations and bonferroni post-hoc tests for body weight at different time points in Rohilkhandi goats for *IGF1*, *GH1* and *MSTN* genotypes

Body Weight	IGF-1			GH1		MSTN	
	AA (20)	AB (22)	BB (8)	AB (27)	BB (23)	AA (39)	BB (11)
BW0	2.08 ± 0.06^{a}	2.21 ± 0.04^{ab}	2.37 ± 0.08^{b}	2.15 ± 0.04	2.23 ± 0.04	2.25 ± 0.03^{a}	1.96 ± 0.06^{b}
BW3	4.36 ± 0.11^{a}	4.61 ± 0.07^{a}	5.16 ± 0.14^{b}	4.57 ± 0.09	4.69 ± 0.09	4.69 ± 0.07^{a}	4.31 ± 0.14^{b}
BW6	6.88 ± 0.16^{a}	7.39 ± 0.11^{b}	7.97 ± 0.22^{c}	7.18 ± 0.15	7.31 ± 0.14	7.41 ± 0.10^{a}	6.75 ± 0.21^{b}
BW9	9.09 ± 0.26^{a}	9.82 ± 0.18^{a}	11.02 ± 0.35^{b}	9.39 ± 0.22^{a}	10.27 ± 0.22^{b}	10.05 ± 0.17^{a}	8.93 ± 0.37^{b}
BW12	11.84 ± 0.38^{a}	12.94 ± 0.27^{ab}	13.79 ± 0.52^{b}	12.41 ± 0.30	13.26 ± 0.30	13.08 ± 0.23^{a}	11.54 ± 0.49^{b}
BW15	13.13 ± 0.40^{a}	14.36 ± 0.28^{a}	15.78 ± 0.54^{b}	13.82 ± 0.32^{a}	14.80 ± 0.31^{b}	14.61 ± 0.23^{a}	12.70 ± 0.51^{b}
BW18	14.59 ± 0.43^{a}	15.97 ± 0.30^{ab}	16.89 ± 0.57^{b}	15.46 ± 0.34	16.22 ± 0.34	16.12 ± 0.25^{a}	14.32 ± 0.55^{b}
BW21	16.23 ± 0.41^{a}	17.38 ± 0.29^{a}	18.34 ± 0.56^{b}	17.01 ± 0.34	17.71 ± 0.34	17.59 ± 0.26^{a}	16.03 ± 0.55^{b}
BW24	17.38 ± 0.35^{a}	18.65 ± 0.24^{a}	19.53 ± 0.47^{b}	18.23 ± 0.33	18.97 ± 0.33	18.88 ± 0.24^{a}	17.18 ± 0.53^{b}
BW27	18.72 ± 0.38^{a}	19.98 ± 0.26^{b}	20.79 ± 0.51^{c}	19.61 ± 0.31	20.02 ± 0.31	20.03 ± 0.23^{a}	18.52 ± 0.50^{b}
BW30	20.03 ± 0.47^{a}	21.50 ± 0.33^{ab}	22.26 ± 0.63^{b}	20.89 ± 0.36	21.47 ± 0.35	21.45 ± 0.26^{a}	19.66 ± 0.56^{b}
BW33	21.36 ± 0.55^{a}	22.99 ± 0.38^{ab}	23.59 ± 0.73^{b}	22.35 ± 0.43	22.70 ± 0.43	22.85 ± 0.31^{a}	21.09 ± 0.67^{b}
BW36	22.54 ± 0.62^{a}	24.48 ± 0.43^{b}	25.04 ± 0.83^{b}	23.52 ± 0.47	24.25 ± 0.47	24.22 ± 0.35^{a}	22.21 ± 0.75^{b}
BW39	24.00 ± 0.62^{a}	25.92 ± 0.43^{b}	26.55 ± 0.84^{b}	24.91 ± 0.49	25.70 ± 0.49	25.66 ± 0.36^{a}	23.55 ± 0.78^{b}
BW42	25.11 ± 0.59^{a}	27.22 ± 0.41^{b}	27.68 ± 0.80^{b}	26.11 ± 0.47	26.92 ± 0.47	26.90 ± 0.34^{a}	24.61 ± 0.74^{b}
BW45	25.86 ± 0.60^{a}	28.22 ± 0.42^{b}	28.78 ± 0.81^{b}	27.06 ± 0.48	27.91 ± 0.47	27.91 ± 0.34^{a}	25.30 ± 0.74^{b}
BW48	26.72 ± 0.56^{a}	29.24 ± 0.39^{b}	29.79 ± 0.75^{b}	28.00 ± 0.48	28.90 ± 0.48	28.96 ± 0.33^{a}	26.09 ± 0.71^{b}

Note: Superscripts (a, b, c) indicate homogeneous subsets identified through Bonferroni-adjusted post-hoc comparisons. Values sharing the same superscript do not differ, whereas those with different superscripts differ at $p<0.05$. Association analysis results showed that variation at the *IGF1* locus was consistently related to body weight from birth to 48 months ($p<0.05$, $p<0.01$ and $p<0.001$). Bonferroni-adjusted comparisons indicated that goats with BB genotype exhibited higher mean body weight at nearly all time points, whereas AA and AB genotypes generally clustered together, reflecting comparable growth trajectories. For *GH1*, genotype differences emerged only at 9 ($p<0.01$) and 15 months ($p<0.05$), where animals with the AB genotype tended to have the higher-weight subset relative to BB individuals. The *MSTN* locus also showed consistent genotype effects on body weight across multiple age intervals from birth to 48 months ($p<0.05$, $p<0.01$ and $p<0.001$). In almost every age interval, animals with the AA genotype fell into the higher-weight subset, while those with the BB genotype consistently belonged to the lower-weight group. All Bonferroni-adjusted post-hoc comparisons corresponding to these genotype effects are presented in Table 5

monomorphic, consistent with earlier reports documenting similar PCR-RFLP patterns in Beetal and Jhakrana goats (Ahlawat et al. 2016). However, polymorphic variants at this locus have been reported in several other breeds, including Cashmere goats (Zhao et al. 2016; Song et al. 2023) and Beetal and Teddy goats (Islam et al. 2019), indicating that *GDF9* variability may be population-specific.

Furthermore, in the current study, polymorphisms were identified in three gene fragments: B, C and E, corresponding to the *IGF1*, *GH1* and *MSTN* genes, respectively. Among them, *IGF1* exhibited the highest allelic diversity, *GH1* showed a pronounced excess of heterozygotes and *MSTN* displayed low diversity with no heterozygous genotypes detected. The significant departures from Hardy-Weinberg equilibrium observed at the *GH1* and *MSTN* loci likely reflect the small sample size, sampling from a single herd, or potential selective pressures acting on these genes. These loci were subsequently evaluated for their associations with growth traits, specifically body weight measured from birth up to 48 months of age at regular intervals.

The *IGF1* gene, a critical mediator of growth hormone (GH) activity, plays a central role in regulating somatic growth and development by promoting cell proliferation and differentiation. These biological processes are essential for muscle accretion, skeletal development and overall body size. Previous studies in Attappady Black goats (Naicy et al. 2017), Beetal goats (Shareef et al. 2018) and Etawah Grade Goats (Surya et al. 2021) have reported polymorphisms in the *IGF1* gene, with the BB genotype being consistently associated with higher body weight compared to other genotypes. Consistent with these findings, the present study also identified three genotypes (AA, AB and BB) at the *IGF1* locus in Rohilkhandi goats, with individuals carrying the BB genotype exhibiting higher mean body weights from birth up to 48 months of age (BW0 to BW48).

The growth hormone (*GH*) gene plays a key role in regulating overall growth, metabolism and development in goats by stimulating the production of *IGF1*, which promotes tissue growth. Previous studies have reported polymorphic patterns in the *GH1* gene across various goat breeds, including Sirohi, Barbari breeds (Singh et al. 2015) and Gaddi goats (Gitanjli et al. 2020). Similar genotypic variants were also identified in the present study on Rohilkhandi goats. Association analysis revealed genotype effect of *GH1* genotypes on body weight at 9 and 15 months of age (BW9 and BW15). No significant associations were found for other

body weight traits, which is aligns with previous findings in Sirohi and Barbari goats (Singh et al. 2015).

In the *MSTN* gene, we observed polymorphic patterns similar to those previously reported in Boer goats (Li et al. 2008) and in four other goat breeds (Boer, Matou, Haimen and Nubian) (Zhang et al. 2012). Previous studies on Chinese goat breeds (Li et al. 2008) and Shaanbei White Cashmere goats (Bi et al. 2020) indicated that the AA genotype was associated with superior growth traits, including body weight, body height and chest girth index, compared to other genotypes. Consistent with these findings, our study on the Rohilkhandi goats demonstrated that individuals with the AA genotype exhibited higher mean body weights from birth up to body weight at 48 months of age (BW0 to BW48).

This study analysed the association between polymorphic loci within three candidate genes and growth traits from birth to four years of age in Rohilkhandi goats. As a pioneer investigation of genotype-growth relationships in this indigenous breed, it offers initial molecular insight into variation at the *IGF1*, *GH1* and *MSTN* loci. The study was based on fifty female goats from a single well-managed herd, which ensured consistent records but limited genetic diversity, resulted in some low-frequency genotypes, such as *IGF1* BB and *MSTN* BB. Because only body weight data were available across all ages and multiple genotype-trait comparisons were performed, the associations detected here are best viewed as preliminary trends rather than definitive effects. Nevertheless, the observed patterns, particularly for *IGF1* and *MSTN*, suggest potential relevance for marker-assisted selection. The work establishes an important foundation for future research involving larger and more diverse populations, both sexes, additional growth traits and a broader panel of candidate genes to strengthen the precision and reliability of genotype-phenotype relationships in Rohilkhandi goats.

Conclusion

This study offers a pioneering molecular assessment of growth-related genetic variation in Rohilkhandi goats and helps fill an important knowledge gap for this indigenous breed. Among the six candidate genes examined, *IGF1*, *GH1* and *MSTN* showed polymorphisms that allowed us to explore their potential influence on body weight across different ages. Animals carrying the *IGF1* BB genotype and the *MSTN* AA genotype tended to exhibit higher body weights within this dataset, suggesting possible roles for these loci in shaping growth performance. These observations should be viewed as preliminary, as the study was based on a small sample size, unbalanced genotype groups and exploratory statistical comparisons without formal multiple-testing correction. Because the animals originated from a single herd with long-term, reliable records, the dataset provided a uniform and well-controlled environment for detecting trends, but it also limits generalizability. Validation in larger, multi-herd and multi-trait studies that include both sexes will be essential to confirm and quantify the effects reported here. Future research should involve larger sample sizes, more diverse farms and breeds, and an expanded set of candidate genes to improve the precision and reliability of genotype–phenotype association estimates.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11250-026-04907-z.

Acknowledgements This study was supported by the ICAR-Indian Veterinary Research Institute with the necessary facilities. The authors would also wish to acknowledge the Director and Joint Director of Academic and Research, Indian Veterinary Research Institute for their kind support to carry out this work. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Author contributions Nitish Gaitri: Data Curation, Formal analysis, Writing- Original draft, Investigation. Subodh Kumar: Conceptualization, Supervision, Resources, Review & editing. Pushpendra Kumar, Amit Kumar & Hari Om Pandey: Supervision, Conceptualization, Review & editing. Subodh Kumar: Data Curation and Validation.

Data availability The datasets generated and analysed during the current study will be made available alongside the published article to support transparency and reproducibility. Additional information can be obtained from the corresponding author upon reasonable request.

Declarations

Ethical approval The animal study was reviewed and approved by the Institute Animal Ethics Committee, Indian Veterinary Research Institute, India (Approval No. 26−1/2024-25/JD(R)/IAEC) dated 12/07/2024.

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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