Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.045

RESEARCH ARTICLE

# Assessment of Myf5 and MSTN Genetic Variants as Predictors of Growth Traits in Saanen and Alpine Goats

M. Durmuş[1*]

[1]Department of Animal Science, Faculty of Agriculture, Cukurova University, 01330 Adana, Turkey
*Corresponding author: durmusm@cu.edu.tr

ARTICLE HISTORY (26-117)

Received: February 12, 2026
Revised: March 08, 2026
Accepted: March 13, 2026
Published online: March 15, 2026

**Key words:**
Genotyping
*in-silico*
Molecular docking
MSTN
Mtf5
SNP

**ABSTRACT**

This study assessed exon 1 sequence variants in both genes and investigated their associations with growth traits in Saanen and Alpine goats. Sequencing identified an MSTN exon 1 c.163C>G polymorphism causing a missense substitution (Leu→Val) at the 15th position of the protein (p.Leu15Val). In contrast, Myf5 exon 1 exhibited a c.232T>C variant representing a synonymous (silent) change of the Phe residue at the 32nd position of myogenic factor 5 (p.Phe32=). In Alpine goats, the Myf5 p.Phe32= SNP was significantly associated with chest girth (P=0.011), with the TC genotype exhibiting the highest mean value. In Saanen goats, the same Myf5 silent SNP was significantly associated with chest width (P=0.028), again with TC showing the highest mean. For the MSTN p.Leu15Val SNP, Alpine goats displayed a significant association with body weight (P=0.042), with the CC genotype having higher body weight than CG and GG. In Saanen goats, the MSTN missense variant was significantly associated with body length (P=0.031), with the CC genotype showing the highest means. While *in-silico* tools collectively suggested non-deleterious effects of p.Leu15Val on protein structure, function, and stability, molecular docking of MSTN with its receptor indicated greater contributions of residues in the altered protein compared with the wild-type form. Given the negative effect of MSTN on muscle cell development, the computational analyses indicated that this missense mutation further supports binding with the receptor in the altered genotypes. Due to this more compact interaction, increased negative regulation of muscle cell development is expected in individuals with the altered genotypes.

**To Cite This Article:** Durmuş M, 2026. Assessment of Myf5 and MSTN genetic variants as predictors of growth traits in saanen and alpine goats. Pak Vet J, 46(3): 564-574. http://dx.doi.org/10.29261/pakvetj/2026.045

## INTRODUCTION

Goat production contributes substantially to food security and rural livelihoods by providing milk and meat across diverse production systems, from intensive commercial dairies to smallholder farms (Bayraktar *et al*., 2022; Bayraktar *et al*., 2024). In developing livestock systems, goats are particularly important due to their adaptability and low input requirements, and genetic improvement has been emphasized as a key strategy to enhance productivity and sustainability (Hyder *et al*., 2002a; Hussain *et al*., 2013). Within dairy-oriented systems, breed choice and genetic improvement remain central to profitability and sustainability, because animals that combine high production with robust growth and functional conformation typically show improved lifetime performance and management efficiency (Hyder *et al*., 2002b; Bayraktar and Shoshin 2021a; Bayraktar and Shoshin 2021b). Among specialized dairy breeds, Saanen and Alpine goats are widely used in organized breeding programs and commercial production due to their strong milking potential and well-described breed characteristics (Ayele *et al*., 2024). Although milk yield is the principal selection goal in dairy goats, growth-related traits such as body weight and linear body measurements (e.g., withers height, body length, chest and rump dimensions) are also economically relevant because they reflect skeletal development, maturation rate, and overall capacity, and can influence rearing costs and reproductive readiness (Hyder *et al*., 2002c; Cetin *et al*., 2025). Consequently, identifying genetic variants that contribute to measurable growth and body conformation phenotypes can support marker-informed selection strategies as a complement to traditional evaluation methods, particularly when phenotyping is costly or requires long recording periods (Mrode *et al*., 2018).

Candidate-gene approaches remain useful in goats when the biological pathways underlying target traits are

well established. Two biologically plausible candidates for growth-related phenotypes are myostatin (MSTN; also known as GDF8 or GDF-8) and myogenic factor 5 (MYF5). Myostatin is a transforming growth factor-β (TGF-β) superfamily member that acts as a key negative regulator of skeletal muscle mass; disruption or functional variation in MSTN has repeatedly been linked to altered musculature and growth in multiple species (McPherron *et al*., 1997; Fadhıl and Zülkadir, 2017; Fadhıl and Zülkadir, 2021; Lee, 2023).

In goats, several studies have documented polymorphisms within MSTN and reported associations with growth traits, including body weight, average daily gain, body height, body length, and chest-related measurements, although effect sizes and trait significance may vary by population, production type, and variant class (An *et al*., 2011; Zhang *et al*., 2013; Khani *et al*., 2017; Bi *et al*., 2020). These inconsistencies are not unexpected because quantitative growth traits are polygenic and environmentally sensitive; nevertheless, the repeated implication of MSTN across goat populations supports its continued evaluation as a candidate locus in breed-specific contexts (An *et al*., 2011; Khani *et al*., 2017).

MYF5 is an early-acting myogenic regulatory factor (MRF) that contributes to the determination and progression of the skeletal muscle lineage. Foundational functional genetics demonstrates that MYF5 and MYOD have partially redundant but essential roles in myogenesis, with combined loss resulting in profound impairment of skeletal muscle formation (Rudnicki *et al*., 1993; Bayraktar, 2022). More broadly, contemporary syntheses of MRF biology highlight MYF5 as a central regulator of muscle development, identity, and regeneration, functioning within coordinated transcriptional networks that shape myofiber formation and growth potential (Hernández-Hernández *et al*., 2017). Despite this compelling biological rationale, MYF5 polymorphisms have been comparatively less studied in goats than MSTN, and available evidence across livestock suggests that MYF5 variation can be linked to body size or muscling-related traits in certain populations, warranting targeted investigation in specialized dairy breeds (Wang *et al*., 2017).

Myf5 gene (NCBI Gene ID: 100861252) is positioned in the chromosome 5, and it is made of only three coding exons in goats (GenBank NC_030812.1). Among these three exons, exon 1 represents the largest coding fragment because it is made of higher number of amino acid residues compared with exons 3 and 2, respectively. In this respect, it is quite reasonable to focus on this fragment, which might exhibit a considerable connection with the phenotypic variation of the caprine groups that are being studied. Similarly, the MSTN gene (NCBI gene ID: 10086087) on chromosome 2 also contains 3 exons (Genbank NC_030812.1) and thus can provide a reasonable genomic model on comparative and population based genetic analysis.

Coding sequences have especial interest in genotyping studies since the single nucleotide polymorphisms (SNPs) that occur in these sequences, in particular, missense mutations, directly change the amino acid composition of the proteins they encode (Peka and Balatsky, 2024). This direct effect is reflected in altering the primary structure of the protein and has the potential to change higher-order folding, stability, and biological activity (Cheng *et al*., 2023). These changes can influence the most important physicochemical characteristics, such as the distribution of charges, hydrophobicity, and the flexibility of the conformation, which are all that define the properties of proteins and their interactions with other biomolecules. Growth-related proteins are particularly susceptible to such changes in the context of livestock production because even small structural alterations may cause quantifiable differences in muscle development (Baneh *et al*., 2025), carcass traits (Lee *et al*., 2023), growth traits (Aljubouri and Al-Shuhaib, 2023), and productivity (Huang *et al*., 2025). Accordingly, the description of genetic variation in coding regions will give essential information on the molecular surfaces underlying phenotypic variation and allow the recognition of functionally pertinent marks to be used in genetic enhancement and selective breeding courses.

Nevertheless, the functional interpretation of coding-region variants should not be restricted to aminoacid altering substitutions alone. Missense mutations may directly change protein structure or activity, whereas synonymous (silent) substitutions although not altering the encoded amino acid can still influence phenotype via effects on mRNA stability, splicing, translation kinetics, or co-translational folding (Chamary *et al*., 2006; Kimchi-Sarfaty *et al*., 2007). Based on the above considerations, the primary goal of the current research was to characterize and genotype the selected coding exons of the MSTN and MYF5 genes of Saanen and Alpine goats and to assess their possible connections with growth- and body conformation-related characteristics. In particular, the objective of the study was to determine both missense and synonymous sequence change in the major sections of these biologically important candidate genes and to determine their distribution among and between breeds. This study attempts to present a possible mechanism on how these functionally important coding-region polymorphisms could contribute to phenotypic variation in specialized dairy goats to substantiate the identification of informative genetic markers that can be utilized in marker-assisted selection and breed improvement efforts.

## MATERIALS AND METHODS

**Ethical statement:** This study was approved by the Local Ethics Committee for Animal Experiments of Cukurova University (approval number: 2024/11).

**Study area, animal and feed material:** The present study was conducted on Alpine (n = 150) and Saanen (n = 150) kids kept at the Dairy Goat and Sheep Breeding Research and Application Unit of Cukurova University, Faculty of Agriculture, Adana, Turkey. The animals were reared under semi-intensive conditions. While this region is hot and dry in summer, it is mild and humid in winter. The relative average humidity is 66%, and the prevailing wind direction is north-south. It has been confirmed that the kids born on the farm received colostrum in the first three days after birth. The kids stayed with their mother until they were two months old and subsequently started eating roughage (alfalfa hay, corn silage) and concentrates in the

second week. The kids were then weaned at the age of two months. The nutrient contents of the feed used to feed the animals in the study are listed in Table 1.

Body weight (BW) and body measurements (Fig. 1) such as withers height (WH), rump height (RH), rump width (RW), chest width (CW), chest depth (CD), chest girth (CG) and body length (BL) were measured at birth, 1, 2 and 3 months of age. A measuring tape was used to determine the chest girth, whereas the other measurements were taken with a measuring stick.

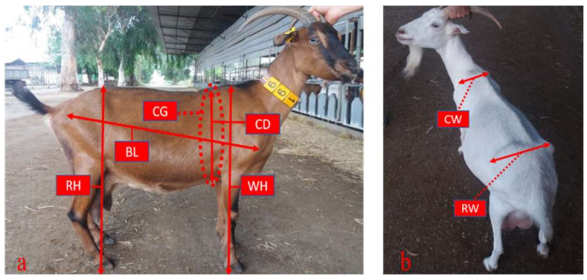


**Fig. 1:** A typical morphological traits for A) Alpine breed, and B) Saanen breed.

**Table 1:** Nutrient content of feeds used in feeding program in the studied populations

| Nutrient Content (%) | Concentrated Feed | Alfalfa | Silage |
|---|---|---|---|
| Dry Matter | 90.32 | 89.41 | 34.30 |
| Crude protein | 18.53 | 14.03 | 6.05 |
| Ether Extract | 4.90 | 2.22 | 3.27 |
| Cellulose | 9.94 | 36.82 | 19.53 |
| Ash | 7.00 | 7.46 | 13.61 |
| ADF | 18.78 | 46.13 | 24.47 |
| NDF | 28.87 | 56.34 | 39.93 |

ADF (Acid Detergent Fiber), NDF (Neutral Detergent Fiber)

**Molecular analysis:** Blood samples (approx. 10 ml) were taken from the jugular vein of each kid in vacutainer tubes with EDTA as anticoagulant. Care was taken during peripheral blood collection to minimize stress and disturbance to the animals. Genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's instructions. The concentration and purity of the extracted DNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and the DNA samples were then stored at −20°C until further analysis.

**Genotyping protocols:** Target regions of the Myf5 and MSTN genes were amplified by polymerase chain reaction (PCR) and subsequently genotyped by Sanger sequencing. By employing the online NCBI-Primer Blast tool (Ye *et al*., 2012), four specific of PCR primer pairs were designed using the *Capra hircus* reference sequences for MYF5 that are deposited in NCBI database (chromosome 5; RefSeq/GenBank accession NC_030812.1) and MSTN (chromosome 2; RefSeq/GenBank accession NC_030809.1). For Myf5 genotyping, two amplicons were designed to amplify the entire sequences of the exon 1 (assinged Myf5 A amplicon), which is extended to include the maority of the intron 1 (assigned Myf5 B amplicon). For MSTN genotyping, two amplicons were also designed to amplify the entire sequences of the exon 1 (assinged MSTN A amplicon), which is extended to include the maority of the intron 1 (assigned MSTN B amplicon). PCRs were carried out in a final volume of 25 µL containing approximately 100 ng genomic DNA, 12.5 µL of 2× PCR Master Mix (Thermo Scientific, USA), 0.4 µM of each primer (forward and reverse) and nuclease-free water to the required volume. Amplification was performed using the following thermal profile: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, primer-specific annealing for 30 s (Table 2), and extension at 72°C (with extension time adjusted according to amplicon length), followed by a final extension at 72°C for 5 min. PCR products were verified on 1.5% agarose gels that are pre-stained with ethidium bromide and visualized under UV illumination unit.

**Table 2:** The nucleic acid sequences of the designed primers of the Myf5 and MSTN genes alongside their optimal annealing temperature, lengths, and specific locations

| Primer Name | Primer Sequences (5'–3') | Annealing (°C) | Length (bp) | Location |
|---|---|---|---|---|
| Myf5 A | F:ATGGACATGATGGACGGCTGCCA R:CATGCCATCAGAGCAACTTGAGG | 55 | 501 | Exon I |
| Myf5 B | F: CTGGTGGATAGTTGGGTATTGGG R:CAGCAGTGTACACTGGGTTAACC | 54 | 540 | Intron I |
| MSTN A | F: AAAGCAAAAGAAAAGTAAAA R: GTACAAGCCAGCAGCTTGTT | 45 | 402 | Exon I |
| MSTN B | F: GCTGAACACTTAGAATGACT R: ACTGGGACGGCCTTTTAAAG | 47 | 840 | Intron I |

For genotyping and SNP discovery, PCR amplicons were purified and sequenced bidirectionally using the same primers employed for amplification. Raw electropherograms were inspected and contigs were assembled, and aligned to the corresponding reference sequences (MYF5: NC_030812.1; MSTN: NC_030809.1). The aligned sequences were screened for nucleotide variation using the standard sequence analysis software SnapGene viewer (https://www.snapgene.com/) and MEGA 11 tool (Tamura *et al*., 2021). Subsequently, the potential novelty of the SNPs among the caprine populations were assessed using the variant table script that is accessed in January – 2026 within the ensembl genome browser 115 (https://www.ensembl.org/index.html) (Howe *et al*., 2021). Detected polymorphisms of the identified SNPs within the amplified regions were then used to assign individual genotypes for downstream population-genetic and association analyses.

**Virtual protein modeling:** Before predicting the impact of the identified SNP on its corresponding position in the protein of interest, the 3D structure of this protein was generated. Due to the absence of any crystallized caprine structure for the growth differentiation factor 8 (GDF8) (the product of MSTN gene) in the protein data bank server (https://www.rcsb.org/) (Gore *et al*., 2017), it was computationally predicted using Swiss model server (https://swissmodel.expasy.org/) (Bienert *et al*., 2017). The physiochemical characteristics of the generated PDB structure was evaluated by the means of Ramachandran plot that is already incorporated in the Swiss model-based structure assessment script. ProSA-Web server is used to assess the overall quality and reliability of virtually generated protein model with those obtained through experimental methods such as X-ray crystallography and NMR spectroscopy (Wiederstein and Sippl, 2007).

***In-silico* prediction tools:** After verifying the accuracy of the generated PDB structure of the protein, various *in-silico* tools were utilized to assess the possible impact of the identified missense SNP on its structural and functional performance. SIFT (Sorting Intolerant From Tolerant) tool was used to assess the functional impact of the missense SNP by evaluating the degree of its evolutionary conservation on the protein function (https://sift.bii.a-star.edu.sg/) (Ng and Henikoff, 2003). PolyPhen-2 (Polymorphism Phenotyping v2) tool was utilized to evaluate the potential impact of the missense SNP on the protein structure and function (http://genetics.bwh.harvard.edu/pph2/) (Adzhubei *et al*., 2013). PhD-SNP (Predictor of human Deleterious Single Nucleotide Polymorphisms) was used to assess whether the missense SNP IS likely to be neutral or deleterious based on its impact on protein function (https://snps.biofold.org/phd-snp/) (Capriotti and Fariselli, 2023). Likewise, SNPs & GO was also employed to assess the functional impact of the nonsynonymous SNP by integrating protein sequence information with functional annotations derived from Gene Ontology (GO) terms (https://snps.biofold.org/snps-and-go/snps-and-go.html).
The overall accuracy of the conducted prediction is further supported by the evaluation provided by meta-SNP tool (Capriotti *et al*., 2013). This tool is designed to assess the functional impact of a SNP by combing the outputs of multiple established *in-silico* tools, including PANTHER (Protein ANalysis THrough Evolutionary Relationships) and SNAP (Screening for Non-Acceptable Polymorphisms) to improve the robustness of the machine-learning prediction (https://snps.biofold.org/meta-snp/). After predicting the effect of the missense SNP on protein structure and function, several tools were employed to evaluate its effect on protein stability upon mutation by estimating the change in Gibbs free energy (ΔΔG) associated with an amino acid substitution. INSP-3D (Impact of Non-synonymous mutations on Protein Stability – 3D) tool was used to assess effect of the missense SNP on protein stability by integrating sequence information with 3d structural features (https://inpsmd.biocomp.unibo.it/submitstruct/)
(Savojardo *et al*., 2016). DynaMut2 is an *in-silico* tool designed to predict the impact of missense SNP on protein stability and dynamics by integrating structural and vibrational information. It combines normal mode analysis with graph-based signatures to estimate ΔΔG and to assess how a substitution affects the flexibility and conformational dynamics of a protein (https://biosig.lab.uq.edu.au/dynamut2/) (Rodrigues *et al*., 2021). Further confirmation for effect of the SNP on protein stability was came from SDM (Site-Directed Mutator). This computational tool is developed to predict the effect of missense SNP on protein stability by incorporating solvent accessibility and secondary structure to provides insight into whether a given SNP is likely to stabilize or destabilize the protein (https://compbio.medschl.cam.ac.uk/sdm2/) (Pandurangan *et al*., 2017).

**Molecular docking:** After predicting the impact of the identified missense SNP on protein structure, function, and stability, further assessments were conducted to assess its effect on the GDF8 with its receptor. Due to the binding of GDF8 with activin receptor type-2A (AVR2A) to negatively regulate the mammalian muscular growth development (Gonzalez Trotter *et al*., 2025), this study exploited molecular docking predictions to assess the effect of the identified missense SNP on the binding of GDF8 with its cognate receptor before and after mutation. As in the case of the GDF8, the 3D structure of AVR2A was generated and validated using Swiss Model suit. Subsequently, two separate molecular docking were conducted to assess the effect of the identified SNP on GDF8 interaction with the receptor. In the first one, the wild-type GDF8 interacted with AVR2A using the residues located in the extracellular domain of AVR2A with GDF8 residues. Using the same setting, a second molecular docking was conducted between the altered-GDF8 with the same receptor. HADDOCK tool (https://rascar.science.uu.nl/haddock2.4/), a well-known molecular docking tool that is mainly used to assess protein – protein interaction (Honorato *et al*., 2024), was employed in both docking reactions to evaluate the extent of the detected SNP on such interaction before and after mutation. In both conducted docking reactions, the best pose was retrieved and visualized by means of PyMol suit (https://www.pymol.org/) (Schrödinger *et al*., 2020). Several crucial output scores were compared between both conditions, including HADODCK, cluster size, RMSD (Root-Mean-Square Deviation), Van der Waals energy, electrostatic energy, buried size area, and Z-score. Further prioritization was provided by comparing the GDF8–AVR2A interactions using PDBSum tool (https://www.ebi.ac.uk/thornton-srv/software/PDBsum1/) (Laskowski *et al*., 2018).

**Statistical analysis:** The allele and genotype frequencies of the identified SNPs were calculated with the Genepop package in the R software. Hardy-Weinberg equilibrium (HWE) was assessed for each SNP locus to determine whether the observed genotype frequencies deviated from those expected under equilibrium conditions. Growth traits data were analyzed using the PROC MIXED procedure in SAS software. The following linear mixed model was used to evaluate the effects of Myf5 and MSTN genotypes, age and sex on growth traits:

$$Y_{ijk}=\mu+G_i+A_j+S_k+e_{ijk}$$

Where:
$Y_{ijk}$ = observed value of the growth trait
$\mu$ = overall mean
$G_i$ = fixed effect of the genotypes for Myf5 and MSTN gene
$A_j$ = fixed effect of the age (birth, 1 month, 2 months, 3 months)
$S_k$ = fixed effect of sex (male, female)
$e_{ijk}$ = *random error*

## RESULTS

**SNPs identification and annotation:** While no SNP was detected in the MSTN B amplicons, direct sequencing of exon 1 of the MSTN gene revealed the identification of one SNP at c.163C>G. Multiple sequences alignment with the reference gene sequences indicated that this SNP is located in the 103$^{th}$ position of the MSTN A amplicon. After

identifying the genomic location of the c.163C>G SNP (NC_030809.1;g.130232728), it was confirmed that this substitution corresponds to leucine residue at position 15 of the growth differentiation factor 8. Using the Expasy translate tool, the missense (nonsynonymous) effect of the c.163C>G was determined, resulting in the substitution of leucine (Leu) with valine (Val) at position 15 of the protein (p.Leu15Val). Further annotation for this SNP was performed to assess its potential novelty among the deposited variants of the caprine MSTN gene in the ensembl database (ensembl gene ID; ENSCHIG00000022964). By reviewing the cataloged variant table of the MSTN gene database, no previous position of the detected p.Leu15Val SNP is found and its novelty is therefore confirmed.

Based on the three-zygosity states observed by DNA electropherograms (Fig. 2), genotype distributions for this locus were classified as TT, TC, and CC in both breeds. In Alpine goats, genotype frequencies were 0.45 (TT), 0.20 (TC), and 0.35 (CC), yielding allele frequencies of 0.55 (T) and 0.45 (C). In Saanen goats, genotype frequencies were 0.55 (TT), 0.10 (TC), and 0.35 (CC), with corresponding allele frequencies of 0.60 (T) and 0.40 (C). The T allele was therefore the more prevalent in both Alpine and Saanen populations. For this polymorphism, the observed genotype distributions in both breeds were consistent with HWE (P>0.05; Table 3).

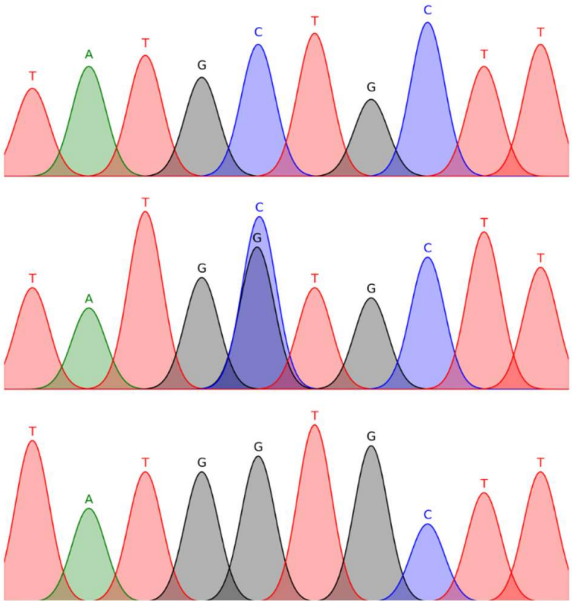

**Fig. 2:** The electropherogram of the investigated ovine MSTN gene, showing the three typical zygosity states in the identified c.163C>G SNP.

**Table 3:** Genotype and allele frequencies of the *MSTN* c.163C>G polymorphism in Alpine and Saanen goats

| Breed | TT frequency | TC frequency | CC frequency | T frequency | C frequency | HWE |
|---|---|---|---|---|---|---|
| Alpine | 0.45 (45) | 0.20 | 0.35 | 0.55 | 0.45 | P>0.05 |
| Saanen | 0.55 (55) | 0.10 | 0.35 | 0.60 | 0.40 | P>0.05 |

For the Myf5 gene, exon 1 sequencing identified a polymorphism at c.232T>C, corresponding to position 10070843 as indicated by multiple alignment sequences with the reference genomic sequences of the Myf5 gene (NC_030812.1;g.10070843T>C). This position is located in the third codon of the amino acid residue phenylalanine (Phe) at position 32 of the caprine myogenic factor 5 (NCBI Reference Sequence NP_001273966.1). Due to the wobble hypothesis in the third position of the codon, the encoded Phe residue unchanged and the final consequence of this SNP was characterized as a silent (synonymous) mutation, namely p.32Phe=. As in the case of the detected SNP in the investigated Myf5 gene, additional annotation for this silent SNP was also conducted to assess its potential novelty among the deposited SNPs of the caprine Myf5 gene in the ensembl database (ensembl gene ID; ENSCHIG00000015029). By reviewing the cataloged variant table of the Myf5 gene database, no previous position of the detected p.32Phe= SNP is found and its novelty is also confirmed.

As per the three-zygosity states revealed by DNA electropherograms (Fig. 3), genotypes at this locus were accordingly grouped as CC, CG, and GG. In Alpine goats, genotype frequencies were 0.56 (CC), 0.15 (CG), and 0.29 (GG), producing allele frequencies of 0.64 (C) and 0.36 (G). In Saanen goats, genotype frequencies were 0.66 (CC), 0.11 (CG), and 0.23 (GG), with allele frequencies of 0.73 (C) and 0.27 (G). Across both breeds, the C allele represented the major allele, with a higher frequency in Saanen than Alpine goats. As observed for MSTN, genotype distributions for the Myf5 polymorphism did not deviate from HWE in either breed (P>0.05; Table 4).

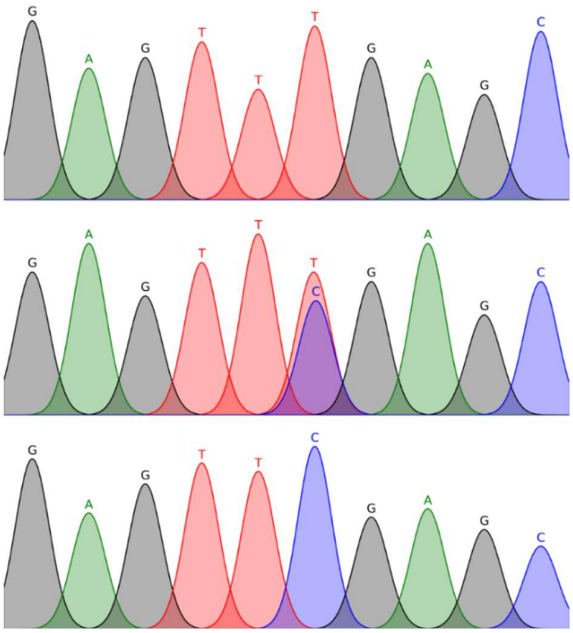

**Fig. 3:** The electropherogram of the investigated ovine Myf5 gene, showing the three zygosity states in the identified c.232T>C SNP.

**Table 4:** Genotype and allele frequencies of the *Myf5* c.232T>C polymorphism in Alpine and Saanen goats

| Breed | TT frequency | TC frequency | CC frequency | T frequency | C frequency | HWE |
|---|---|---|---|---|---|---|
| Alpine | 0.56 | 0.15 | 0.29 | 0.64 | 0.36 | P>0.05 |
| Saanen | 0.66 | 0.11 | 0.23 | 0.73 | 0.27 | P>0.05 |

**Association of Myf5 SNP with growth traits:** In the Alpine population, the Myf5 c.232T>C polymorphism showed no significant association with body weight, withers height, rump height, rump width, chest width, chest depth, or body length (P>0.05). Nevertheless, numerical

variation among genotypes was observed across several traits. For example, body weight ranged from 9.074 ± 0.197 kg (CC) to 10.238±0.188kg (TC), and withers height varied between 46.139±0.277cm (CC) and 47.628±0.238cm (TC); however, these differences did not reach statistical significance. In contrast, a significant genotype effect was detected for chest girth (P = 0.011; P<0.05), indicating that this trait was influenced by the Myf5 genotype in Alpine goats. The TC genotype exhibited the highest chest girth (48.590±0.250cm) and differed significantly from TT (46.351±0.290cm). The CC genotype showed an intermediate value (47.205±0.289cm) and was statistically comparable to TC, suggesting that the heterozygous genotype was associated with a favorable chest girth phenotype relative to TT in this breed (Table 5). In Saanen goats, the Myf5 c.232T>C polymorphism was likewise not associated with body weight, withers height, rump height, rump width, chest depth, chest girth, or body length (P>0.05). Although not significant, the TC group tended to show higher mean values for several measures (e.g., body length 46.798±0.382cm in TC vs. 44.237±0.424cm in TT), suggesting a potential trend that did not reach the conventional significance threshold. Importantly, a significant association was observed for chest width (P=0.028; P<0.05), demonstrating that genotype at this locus contributed to variation in this trait in the Saanen breed. The TC genotype presented the greatest chest width (12.021±0.123cm) and was significantly higher than TT (9.639±0.139cm). The CC genotype (10.993±0.132cm) was intermediate and did not differ significantly from either TT or TC (shared superscript "ab"), indicating a partial separation among genotypic classes with the strongest contrast between heterozygotes and the TT genotype (Table 5).

**Table 5:** Association of the Myf5 c.232T>C polymorphism with growth traits in Alpine and Saanen goats. Bolded p-values refer to the significant effect among the detected genotypes

| Breed/Trait | TT | TC | CC | p-value |
|---|---|---|---|---|
| Alpin | | | | |
| Body weight | 9.970±0.197 | 10.238±0.188 | 9.074±0.197 | 0.111 |
| Withers height | 46.318±0.277 | 47.628±0.238 | 46.139±0.277 | 0.435 |
| Rump height | 45.900±0.277 | 47.277±0.239 | 46.342±0.277 | 0.372 |
| Rump width | 9.803±0.0986 | 11.028±0.0818 | 10.049±0.0984 | 0.113 |
| Chest width | 9.890±0.108 | 11.108±0.0909 | 10.071±0.108 | 0.234 |
| Chest depth | 17.092±0.130 | 18.150±0.108 | 17.276±0.130 | 0.56 |
| Chest girth | 46.351±0.290$^{b}$ | 48.590±0.250$^{a}$ | 47.205±0.289$^{a}$ | 0.011* |
| Body length | 41.181±0.293 | 42.737±0.258 | 41.575±0.292 | 0.482 |
| Saanen | | | | |
| Body weight | 12.155±0.326 | 13.346±0.306 | 12.061±0.317 | 0.506 |
| Withers height | 46.997±0.404 | 49.448±0.349 | 47.704±0.380 | 0.121 |
| Rump height | 46.387±0.388 | 47.786±0.334 | 46.225±0.364 | 0.381 |
| Rump width | 9.827±0.120 | 11.095±0.104 | 9.971±0.113 | 0.153 |
| Chest width | 9.639±0.139$^{b}$ | 12.021±0.123$^{a}$ | 10.993±0.132$^{ab}$ | 0.028* |
| Chest depth | 18.359±0.286 | 20.998±0.270 | 18.656±0.279 | 0.321 |
| Chest girth | 49.213±0.475 | 50.612±0.415 | 49.159±0.449 | 0.354 |
| Body length | 44.237±0.424 | 46.798±0.382 | 44.680±0.406 | 0.410 |

*(P<0.05)

**Association of MSTN gene SNP with growth traits:** In Alpine goats, a significant association was observed between MSTN c.163C>G genotype and body weight (P=0.042; P<0.05). The CC genotype showed the highest body weight (10.650±0.252) and was significantly greater than both CG (10.100±0.190) and GG (9.550±0.320), as indicated by different superscript letters. In contrast, the MSTN c.163C>G polymorphism was not significantly associated with withers height, rump height, rump width, chest width, chest depth, chest girth, or body length in Alpine goats (P>0.05; Table 6). In the Saanen population, the MSTN c.163C>G polymorphism showed no significant association with body weight, withers height, rump height, rump width, chest width, chest depth, or chest girth (P>0.05). Notably, a significant genotype effect was detected for body length (P=0.031; P<0.05). The CC genotype exhibited the greatest body length (45.200±0.420) and differed significantly from GG (44.800±0.406), while CG (42.900±0.383) presented an intermediate value and did not differ significantly from either homozygous group (Table 6).

**Table 6:** Association of the MSTN c.163C>G polymorphism with growth traits in Alpine and Saanen goats. Bolded p-values refer to the significant effect among the detected genotypes

| Breed/Trait | CC | CG | GG | p-value |
|---|---|---|---|---|
| Alpin | | | | |
| Body weight | 10.650±0.252$^{a}$ | 10.100±0.1900$^{b}$ | 9.550±0.320$^{b}$ | 0.042* |
| Withers height | 48.200±0.281 | 47.100±0.2441 | 46.300±0.252 | 0.180 |
| Rump height | 47.900±0.271 | 46.950±0.3254 | 46.100±0.314 | 0.300 |
| Rump width | 11.200±0.112 | 10.600±0.1542 | 10.050±0.201 | 0.201 |
| Chest width | 11.350±0.110 | 10.700±0.0910 | 10.100±0.109 | 0.115 |
| Chest depth | 18.450±0.134 | 17.900±0.2752 | 17.250±0.195 | 0.910 |
| Chest girth | 49.100±0.412 | 47.800±0.3574 | 46.950±0.321 | 0.401 |
| Body length | 43.100±0.520 | 42.200±0.4125 | 41.500±0.362 | 0.281 |
| Saanen | | | | |
| Body weight | 14.100±0.330 | 13.200±0.310 | 12.400±0.320 | 0.331 |
| Withers height | 50.200±0.400 | 49.000±0.350 | 48.100±0.380 | 0.404 |
| Rump height | 48.500±0.390 | 47.600±0.335 | 46.700±0.365 | 0.349 |
| Rump width | 11.300±0.120 | 10.700±0.105 | 10.050±0.113 | 0.124 |
| Chest width | 12.400±0.140 | 11.600±0.124 | 10.900±0.133 | 0.340 |
| Chest depth | 21.400±0.290 | 20.100±0.270 | 18.900±0.280 | 0.170 |
| Chest girth | 51.800±0.480 | 50.300±0.415 | 49.100±0.449 | 0.601 |
| Body length | 45.200±0.420$^{a}$ | 42.900±0.383$^{ab}$ | 44.800±0.406$^{b}$ | 0.031* |

*(P<0.05)

***In-silico* prediction analysis:** Due to the straightforward effect of the missense SNP on a wide range of structural, functional, and stability performance of the protein, various *in-silico* tools have been used to predict this possible impact. Despite the differences in the algorithms of the utilized prediction tools, all of them predicted a non-deleterious and neutral effect of the identified p.Leu15Val on the GDF8 structure and function. While SIFT tool showed that the identified SNP has a tolerable effect of the SNP on the GDF8 protein, PolyPhen-2 algorithm showed that this tolerable effect causes benign consequences on the protein. Further affirmations for these non-deleterious effects came from the PhD SNP tool, which predicted a neutral impact of the p.Leu32Val on the GDF8. In accordance with this prediction, the SNPs&Go tool added further verification for this neutral impact, showing non-damaging effects on GDF8 caused by the SNP. Likewise, the collective computational prediction provided by the meta-SNP tool added a further layer of confirmation for the non-deleterious impact of the identified SNP on the protein upon mutation. The combination of several tools, including PANTHER and SNAP, that exert different algorithms of predictions within the meta-SNP server, were found to be in consensus in terms of the neutral effect of p.Leu32Val on the GDF8 structure and function. In addition to the collective computationally predicted neutral effect of the p.Leu32Val SNP on the structure and function of the protein, several *in-silico* tools were in agreement about the stabilizing effects of this SNP on the GDF8. These

predicted effects came from the utilization of the INSP-3D, Dynamut2, and SDM tools. These tools predicted ΔΔG values did not show any destabilizing effect for the SNP on the GDF8. Accordingly, the neutral and stabilizing effects of the p.Leu35Val SNP on the GDF8 structure, function, and stability are demonstrated (Fig. 4).

**Molecular docking outputs:** In order to obtain a molecular insight into the identified SNP on the interaction of the GDF8 with its corresponding receptors, two molecular docking events were conducted between GDF8 and the AVR2A receptor. The first docking is between wild-type GDF8 and the AVR2A receptor, whereas the second is between the altered (p.Leu15Val) GDF8 and the AVR2A receptor. Through the utilization of the same setting conditions between the docked protein-protein interactions, the exact effects of the SNP were accordingly comprehended. While the HADDOCK score of the wild-type GDF8 with its receptor showed a slightly favourable value (771.3 +/- 43.9) compared with the altered form (787.1 +/- 37.9), other docking parameters were in disagreement with these scores. This is due to several conditions surrounding the conducted docking, including van der Waals interactions (-59.9 +/- 16.1 for wild-type versus -68.1 +/- 5.1 for altered complex), electrostatic energy (-380.2 +/- 62.9 for wild-type versus -423.8 +/- 74.2 for altered complex), buried surface area (3141.7 +/- 353.5 for wild-type versus 3518.4 +/- 378.9 for altered complex), and Z-score (0.0 for wild-type versus -1.0 for altered complex). These conditions showed a larger tendency for the altered GDF8 to bind with the receptor in more favourable interactions. Accordingly, these parameters that the HADDOCK score is largely attributed to the differences in the RMSD values of both docked complexes (18.8 +/- 0.5 for wild-type versus 19.2 +/- 0.8 for altered complex) rather than the other putative reasons. To further assess the observed differences between the docked complexes, more in-depth evaluations were conducted using the PDPSum tool. The ability of this tool to calculate the quality and quantity of the amino acid residues involved in the interaction has largely supported the proposition of the presence of a higher affinity of the altered GDF8 to bind with the receptor compared with the wild-type counterpart. While wild-type GDF8 uses five salt bridges in its binding with the receptor, only three salt bridges were used by the altered GDF8 in its interaction with the receptor. Nevertheless, the higher number of the detected hydrogen bonds (14) and non-bonded interactions (228) in the altered GDF8 may exhibit more favourable interactions compared with the wild-type GDF8 (11 for hydrogen bonds and 154 for non-bonded interactions). What has further supported this higher affinity of binding is attributed to the higher number of involved residues in the binding of the altered GDF8 with the receptor compared with the wild-type binding mode. While only 23 residues of the wild-type GDF8 were found to interact with 22 residues of the receptor in the wild-type complex, 30 residues of the altered GDF8 were involved in the binding with 29 residues of the receptor in the altered complex (Fig. 5).

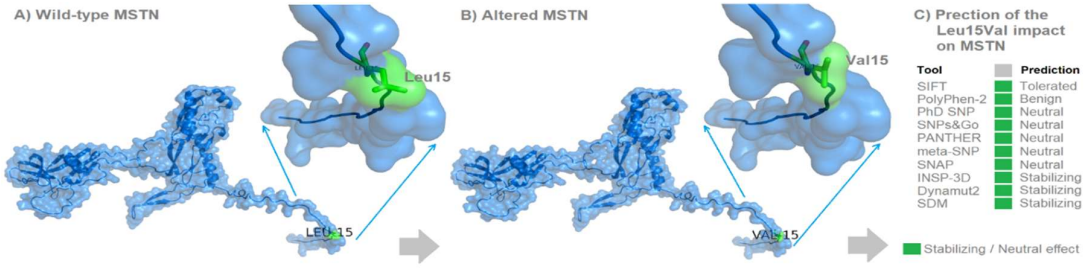


**Fig. 4:** Three-dimensional representation of the identified p.Leu15Val with its computational consequences on the GDF8 protein. A) Wild-type GDF8, B) altered GDF8, tools used in the *in-silico* prediction.

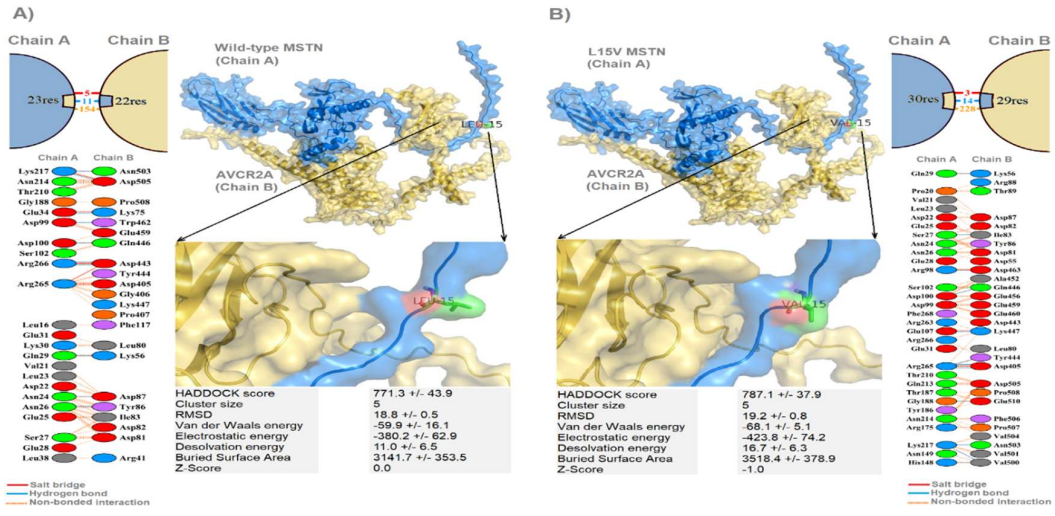


**Fig. 5:** Comparative molecular docking interactions between the wild type GDF8 and AVCR2A receptor (in branch A) and altered (p.Leu15Val) GDF8 and AVCR2A receptor (in branch B).

## DISCUSSION

The current work will present combined genetic and computational data to ascertain the applicability of Myf5 and MSTN exon 1 variations to growth-associated phenotypic difference in the Saanen and Alpine goats. The findings complement the argumentative main claim that myogenic regulatory genes play an important role in the regulation of skeletal development and body size by identifying a synonymous replacement in Myf5 (p.Phe32=) as well as a missense mutation in MSTN (p. Leu15Val) and showing that they significantly regulate the development of the body with different body measures among different breeds. The present study has revealed various outcomes that are consistent with the known biological role of Myf5 in initial myogenic determination and MSTN as a negative muscle development regulator. Furthermore, it indicates that exon 1 polymorphisms of both Myf5 and MSTN genes could be used as predictors of economically significant growth phenotypes in dairy goats.

Sequencing of exon 1 identified polymorphism at both candidate loci, and genotype distributions were consistent with HWE in each breed. In practical terms, HWE compatibility supports the technical reliability of genotyping and suggests that at the population level these loci are not experiencing strong directional selection, intense assortative mating, or severe substructure that would distort genotype proportions. The allele frequency patterns also indicate that both breeds carry substantial standing variation at MSTN and MYF5 exon 1, which is a prerequisite for any marker's utility in selection. In the present results, the identified c.163C>G substitution is a missense change (Leu→Val), which is chemically conservative (both hydrophobic residues). Conservative substitutions can still matter when they occur at structurally or functionally constrained positions, but they also frequently produce subtle phenotypic effects rather than dramatic changes. Therefore, the observed associations are biologically plausible even if the underlying effect size is modest.

In Alpine goats, MSTN c.163C>G showed a significant association with body weight (P=0.042), where the CC genotype had the highest mean body weight and differed from CG and GG. This pattern aligns with the known role of MSTN in muscle mass regulation: genotypes that slightly reduce MSTN activity (directly through protein function or indirectly via linkage disequilibrium with regulatory variants) would be expected to shift weight upward, particularly in traits strongly influenced by muscle deposition. Comparable directions of association between MSTN polymorphisms and growth traits have been reported in goats, although the specific causal variants and the magnitude of effects vary across breeds and genomic regions. For example, An *et al*. (2011) identified MSTN polymorphisms in goats and reported genotype-dependent differences in body weight in Boer and crossbred animals. Zhang *et al*. (2013) also described MSTN polymorphisms (including an exon I substitution) and detected significant associations with body weight and body size traits in goat populations. These studies collectively support MSTN as a credible growth-related candidate gene in caprine breeding, while simultaneously underscoring that breed background and variant type (UTR, intronic, exon, indel) can change which traits show detectable signals.

In Saanen goats, MSTN did not associate with body weight but showed a significant association with body length (P = 0.031). This breed-specific shift from weight in Alpine to linear body dimension in Saanen may reflect differences in genetic background, selection history, and trait covariance. Dairy breeds can diverge in how body weight partitions across muscle, frame size, and body condition. Under such circumstances, MSTN-linked variation could manifest more strongly in skeletal frame or longitudinal growth in one breed, while in another breed it could be expressed primarily through muscle-related mass. Notably, goat studies have reported MSTN associations with multiple conformation measures, not only weight. Bi *et al*. (2020), studying a 5-bp indel in MSTN, observed significant relationships with body height and chest-width–related indices in a large cashmere-goat dataset. Zhang *et al*. (2013) likewise found associations spanning body weight, body length, and body height depending on locus and population. The present Saanen result therefore fits within an emerging caprine pattern: MSTN variation can be detected across a spectrum of growth and body-size phenotypes, but the top trait may differ by breed and by the local haplotype context around MSTN.

The present MSTN signals are broadly consistent with prior caprine studies showing that MSTN polymorphisms can relate to growth or body-size phenotypes, but they also mirror a common theme: effects are often modest and not universal across traits. Khani *et al*. (2017), for instance, reported generally weak associations between MSTN polymorphisms and growth traits in Markhoz goats, illustrating that detection can be limited or inconsistent across populations. Such variability can arise from differences in allele frequency, LD patterns, environmental variance, phenotype definitions, age at measurement, and genetic background. The present results significant for body weight in Alpine and body length in Saanen, but not for the remaining traits fit this pattern of trait- and breed-specific detectability rather than a broad, uniform MSTN effect.

It is also notable that some goat MSTN studies focus on regulatory indels in the 5′UTR, which may exert clearer expression-level effects than conservative missense substitutions. Bi *et al*. (2020) demonstrated associations between a 5-bp MSTN indel and several growth-related indices in a large dataset. By contrast, a Leu→Val substitution may produce subtler functional consequences, making it more likely that the observed association reflects either a small direct effect or LD with regulatory variants. This distinction highlights an important practical implication: the biological class of the variant (UTR indel vs. conservative missense vs. intronic splicing variant) can influence both effect size and reproducibility across herds.

The identified c.232T>C variant is synonymous, but synonymous does not necessarily mean functionally silent. A large body of literature shows that synonymous substitutions can influence gene expression and phenotype through mechanisms such as altered mRNA secondary structure and stability, modified translational efficiency (codon usage), altered co-translational folding dynamics, or impacts on splicing regulatory elements (Chamary and Hurst, 2005; Chamary *et al*., 2006). Thus, the observed

breed-specific associations for chest girth (Alpine) and chest width (Saanen) are compatible with modern understanding of silent variants: the molecular effect may be regulatory rather than structural.

Empirically, evidence for MYF5 as a growth-related candidate marker has been stronger in some livestock species than in others, with cattle studies reporting associations between MYF5 polymorphisms and growth traits under certain population designs. For instance, Zhang *et al*. (2007) evaluated MSTN and MYF5 polymorphisms in Chinese cattle and reported allele-frequency patterns and trait associations consistent with candidate-gene relevance. In goats, the MYF5 candidate-gene literature is thinner than MSTN's, so the present findings add potentially useful breed-specific signals especially because they involve routinely recorded conformation traits that can be incorporated into selection indices if validated.

In Alpine goats, MYF5 genotype showed a significant effect on chest girth (P=0.011), with the heterozygous genotype displaying the highest mean. In Saanen goats, MYF5 was significantly associated with chest width (P=0.028), again with heterozygotes showing the largest mean and the TT genotype the smallest. Heterozygote-favorable patterns can arise from several sources: true biological overdominance at the locus, pseudo-overdominance due to LD with multiple functional variants in repulsion, or sampling/stratification effects that inflate heterozygote means. Because most other traits were non-significant, a cautious interpretation is warranted: these loci may influence particular dimensions of thoracic development (width and girth) rather than global size. This is not implausible biologically, since muscle and skeletal development in the thoracic region is shaped by coordinated regulation of myogenic programs and growth trajectories, and modest regulatory differences could be expressed in region-specific conformation measures.

Importantly, contemporary goat genomics reinforces the idea that body conformation traits are controlled by many loci of small-to-moderate effect. Genome-wide association studies in goats identify multiple genomic regions contributing to body weight and body conformation traits, illustrating a polygenic architecture that can make candidate-gene effects appear sporadically across populations and trait definitions. For example, Zhang *et al*. (2021) used a 70K SNP chip in ~1,900 cashmere goats and detected loci associated with birth, weaning, and yearling weights, supporting the highly distributed genetic basis of growth. Similarly, Yang *et al*. (2024) performed GWAS for multiple conformation traits (including chest width and chest girth) in goats, emphasizing that linear measurements are genetically complex and may be influenced by different sets of genes across breeds. In this context, the MYF5 exon 1 synonymous variant may act as one small contributor (or a proxy for nearby functional variation) whose measurable impact depends on breed background and the trait's genetic correlation structure.

Since leucine and valine are both non-polar and exhibit aliphatic side chains, the substitution of leucine to valine cannot be considered a dramatic alteration in the overall structure of the protein since both amino acid residues share highly similar physicochemical characteristics. Accordingly, p.Leu15Val SNP has been predicted by all the utilized tools as neutral or non-deleterious on the protein structure, biological activity, and stability. Due to the predicted neutral and stabilizing effects of the identified SNP on GDF8, the overall affinity of the altered protein with the receptor has been shown to exhibit a greater tendency to bind compared with the wild-type counterpart. The differences in the observed affinity of GDF8 binding with receptor may be attributed to several biophysical differences between both residues. Though both amino acid residues are aliphatic and nonpolar, leucine possesses an isobutyl side chain, whereas valine contains a smaller isopropyl group. This difference results in leucine having a slightly larger side-chain volume and surface area. Consequently, leucine contributes more strongly to hydrophobic packing within protein cores, while valine tends to occupy more spatially constrained environments in its interaction. With respect to conformational preferences, valine's β-branched side chain imposes greater steric constraints on the backbone dihedral angles, often favoring β-sheet formation and limiting flexibility. Leucine, lacking β-branching, allows greater conformational freedom and is frequently enriched in α-helical regions (Frederiksen *et al*., 2004). These differences between both amino acids can alter protein stability, affect packing efficiency and hydrophobic stabilization.

Though the position at which the p.Leu15Val was found to exhibit a very close vicinity in the interaction of the GDF8 with its corresponding receptor, no direct participation of this SNP was observed with any residue of that receptor. Nevertheless, it is not necessary for a SNP to be located within the regions harboring the protein – protein interaction residues to exert its effect on this kind of interaction. Instead, it can exert its effect of the interface regions through mechanisms beyond direct location in the interaction interface residues (Yates and Sternberg, 2013). A missense SNP may induce allosteric effects, altering protein conformation or dynamics remotely from the interface region, thus modulating binding affinity. For instance, a distant SNP can enhance electrostatic complementarity or propagate long-range effects through solvent regions, improving or disrupting interactions without altering interface residues directly (Joughin *et al*., 2005). In agreement with this data, several molecular docking studies, such as these conducted on Damascus goats (Bayraktar *et al*., 2024), Black Bengal, Ganjam and Raighar goats (Mohanty *et al*., 2021), predicted variable influences of several SNPs on the protein – protein interactions without being included in the interface regions.

Based on the observed data, this study showed that the p.Leu15Val has induced several biophysical changes in the binding of GDF8 with its receptors. This change has easily been inferred from the comparative molecular docking conducted, which showed that the altered GDF8 exhibit more overall binding affinity with the receptor compared with the wild-type counterpart. Due to this higher affinity of the binding with the receptor, more participation of the altered protein in exerting more stringent control on the muscular growth development is observed. The mechanism of this molecular mechanism has already been shown in goats with the altered genotype. In another words, the goats with altered GDF8 genotype have been significantly associated with lower values of chest width compared with the goats with wild-type GDF8. This finding can be

connected with the previously explained favoured binding affinity of the altered GDF8 with the receptor, giving it a more inhibitory role in regulating the muscular growth development.

Despite the important finding obtained in the present study, the limitations surrounding the the study cannot be eliminated from consideration. These limitations are mainly correlated with the difficulty of interpreting candidate-gene associations outside the increasingly polygenic view of growth traits. GWAS evidence in goats consistently indicates that growth and conformation traits are influenced by many loci spread across the genome (Moaeen-ud-Din *et al*., 2022). Studies conducted on diverse goat populations that analyzed body weight and conformation in Karachai and other breeds have reported the presence of multiple regions and candidate genes associated with these traits rather than single major-effect loci (Yang *et al*., 2024). However, these multiple polyfactorial effects did not invalidate candidate genes MSTN and MYF5, but it reframes the overall scenario of our expectations. This can be explained by the ability of these loci to contribute in a meaningful regulation when integrated into multi-locus selection strategies, or when used as part of targeted mating schemes for specific morphological outcomes.

Several methodological aspects can shape interpretation. First, effect sizes should be evaluated not only by P-values but also by their biological and economic relevance. For example, the Alpine MSTN body-weight contrast between CC and GG genotypes appears meaningful at the measured age, but its value for selection depends on persistence over time, correlations with milk performance in dairy systems, and potential trade-offs. Second, the breed-specific nature of signals suggests that marker deployment should be breed-calibrated; applying a marker across breeds without validation may dilute benefits or produce unintended responses. Third, functional follow-up would strengthen causal inference particularly for the MYF5 synonymous variant. Expression analyses, splicing assays, or *in-silico* predictions of mRNA folding changes could help clarify whether the synonymous change itself is functional or is simply tagging a nearby causal site. Finally, integrating these candidate loci with genome-wide information is a natural next step. Given the demonstrated polygenic architecture of growth traits in goats, using MSTN and MYF5 variants as features within genomic prediction models or as part of haplotype-based selection tools may yield more robust and transferable gains than relying on single-marker selection alone.

**Conclusions:** Overall, the findings support the view that Myf5 and MSTN exon 1 variation may contribute to phenotypic diversity in specific growth and morphometric traits in goats. The current research shows that exon 1 variants of Myf5 and MSTN have significant associations with growth- and conformation-related phenotypes in Saanen and Alpine goats as well as offers mechanistic evidence of how minor amino acid changes can have a significant impact on phenotypes. The identified p.Leu15Val SNP has been generated from the replacement of two non-polar and aliphatic residues which are always predicted to be neutral with regard to protein structure, stability and function. Nevertheless, comparative molecular docking studies show that this variant is associated with the improvement of the overall binding affinity of GDF8 to its receptor. This effect does not seem to be due to direct involvement of the modified reside in the protein-protein interface of the protein-protein interaction but may be an effect of indirect biophysical and conformational effects which may be propagated allosterically to alter receptor binding. The identified correlations between the changed GDF8 genotype and reduced growth-related measurements can be considered as the indication of the biological importance of this increased inhibitory contact, since the negative regulatory effect of myostatin in muscle growth is already established. Meanwhile, the strong relations identified with the synonymous Myf5 variant support the idea that silent polymorphisms can have a phenotypic impact, which can be explained by the connection with regulatory factors or the existence of minor impacts on gene expression. Although these results support the candidacy of MSTN and Myf5 as growth trait variability predictors, the results need to be viewed in the context of the polygenic nature of growth traits in goats. These genes are not single major-effect loci, but rather much more likely are combined parts of networks of multiple locus systems that together determine body size and conformation. The identified variants, in turn, will have a practical value not in the form of individual values, but as part of multi-marker selection models or custom breeding programs focused on the optimal development of particular morphological features in populations of dairy goats. However, because significant effects were limited to a subset of traits and differed between breeds, these results should be considered preliminary. Validation in larger, independent populations, alongside functional evaluation and consideration of additional genetic and environmental factors, will be important to clarify the robustness and practical relevance of these variants for growth-related selection in goat breeding programs.

## REFERENCES

Adzhubei I, Jordan DM and Sunyaev SR, 2013. Predicting functional effect of human missense mutations using PolyPhen-2. Current Protocols in Human Genetics 76:7–20.

Aljubouri TRS and Al-Shuhaib MBS, 2023. The identification of a novel SNP in the resistin (RETN) gene associated with growth traits in Karakul and Awassi sheep. Tropical Animal Health And Production 55(1–12):165.

An XP, Hou JX, Zhao HB *et al*., 2011. Polymorphism identification in the goat MSTN gene and association analysis with growth traits. Czech Journal of Animal Science 56(12):529-535.

Ayele S, Özcan BD and Bayraktar M, 2024. Identification of the association of the GH, IGF-I and Pit-I gene polymorphism with growth traits in Saanen, Alpine and Boer goat breed. Small Ruminant Research 236:107297.

Baneh H, Elatkin N and Gentzbittel L, 2025. Genomic variance partitioning of carcass and meat quality traits in Angus beef cattle. Frontiers in Veterinary Science 12:590226.

Bayraktar M and Shoshin O, 2021a. Determination effects of SLC27A3 and β-Lactoglobulin gene polymorphisms on the milk composition in Hamdani sheep. Applied Ecology and Environmental Research *19*(4):3293-3302.

Bayraktar M and Shoshin O, 2021b. Estimate of the association of IGF-I and IGFALS genes with growth traits in Hamdani sheep. Brazilian Archives of Biology and Technology 64:e21210262.

Bayraktar M and Shoshin O, 2022. Association between CAST and MSTN gene polymorphisms with growth traits in Awassi sheep. Kuwait Journal of Science 49(2):1-15.

Bayraktar M, 2022. Detection a novel genetic variant of the Myf5 gene in Turkish Anatolian Water Buffalo. Nutrition Program *24*(2): e2022044.

Bayraktar M, Koluman N and Al-Shuhaib MBS, 2024. Investigating the impact of a novel GHRHR gene variant on growth traits in Damascus goats. Small Ruminant Research 240:107366.

Bi Y, Feng B, Wang Z *et al*., 2020. Myostatin (MSTN) gene indel variation and its associations with body traits in Shaanbei white cashmere goat. Animals 10(1):168.

Bienert S, Waterhouse A and De Beer TAP, 2017. The SWISS-MODEL Repository—new features and functionality. Nucleic Acids Research 45:D313–D319.

Capriotti E and Fariselli P, 2023. PhD-SNPg: updating a webserver and lightweight tool for scoring nucleotide variants. Nucleic Acids Research 51:W451–W458.

Capriotti E, Altman, RB and Bromberg Y, 2013. Collective judgment predicts disease-associated single nucleotide variants. BMC Genomics 14:1–9.

Cetin N, Yilmaz O, Bolac C *et al*., 2025. Correlations between udder morphometry and body measurements in Norduz Sheep and Saanen Goats. Pakistan Veterinary Journal 45(4):2044-2051.

Chamary JV and Hurst LD, 2005. Evidence for selection on synonymous mutations affecting stability of mRNA secondary structure in mammals. Genome Biology 6(9):R75.

Chamary JV Parmley JL and Hurst LD, 2006. Hearing silence: Non-neutral evolution at synonymous sites in mammals. Nature Reviews Genetics 7(2):98–108.

Cheng J, Novati G, Pan J *et al*., 2023. Accurate proteome-wide missense variant effect prediction with AlphaMissense. Science 381(6664): eadg7492.

Fadhıl M and Zülkadir U, 2017. Molecular characterization of MSTN gene in Holstein Friesians and Brown Swiss cattle breeds. Selcuk Journal of Agriculture and Food Sciences 31(3):151-153.

Fadhil M and Zülkadir U, 2021. Association between polymorphisms of Myf5, MSTN and CAST genes and fattening performance in Brown Swiss and Holstein cattle breeds. Animal Biotechnology 32:121-129.

Frederiksen H, Berenstein D and Munch-Petersen B, 2004. Effect of valine 106 on structure–function relation of cytosolic human thymidine kinase: Kinetic properties and oligomerization pattern of nine substitution mutants of V106. European Journal of Biochemistry 271:2248–2256.

Gonzalez Trotter D, Donahue S, Wynne C *et al*., 2025. GDF8 and activin A are the key negative regulators of muscle mass in postmenopausal females: a randomized phase I trial. Nature Communications 16:4376.

Gore S, García ES, Hendrickx PM *et al*., 2017. Validation of structures in the Protein Data Bank. Structure 25(12):1916–1927.

Hernández-Hernández JM, García-González EG, Brun CE *et al*., 2017. The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. Seminars in Cell and Developmental Biology 72:10–18.

Honorato RV, Trellet ME, Jiménez-García B *et al*., 2024. The HADDOCK2. 4 web server for integrative modeling of biomolecular complexes. Nature Protocols 19:3219–3241.

Howe KL Achuthan P, Allen J *et al*., 2021. Ensembl 2021. Nucleic Acids Research 49(D1):D884-D891.

Huang J, Wang K, Zhang Y *et al*., 2025. Effects of COLQ gene missense mutations on growth and meat traits in Leizhou Black Goats. Animals 15(17):2618.

Hussain T, Babar ME, Sadia H *et al*., 2013. Microsatellite markers based genetic diversity analysis in Damani and Nachi goat breeds of Pakistan. Pakistan Veterinary Journal 33(4):520-522.

Hyder AU, Akhtar P and Gondal KZ, 2002c. Influence of some non genetic factors on birth weight of Teddy goat kids. Pakistan Veterinary Journal 22(3):116-119.

Hyder AU, Akhtar P and Haider OU, 2002b. Environmental and genetic influences on pre-weaning daily weight gain in Teddy goat kids. Pakistan Veterinary Journal 22(4):188-191.

Hyder AU, Khan MS, Akhtar P *et al*., 2002a. Genetic, phenotypic and residual correlations among various performance traits in Teddy goats. Pakistan Veterinary Journal 22(3):128-130.

Joughin BA, Green DF and Tidor B, 2005. Action-at-a-distance interactions enhance protein binding affinity. Protein Science 14:1363–1369.

Khani K, Abdolmohammadi A, Foroutanifar S *et al*., 2017. Assessment of polymorphisms in myostatin gene and their allele substitution effects showed weak association with growth traits in Iranian Markhoz goats. Journal of Agricultural Sciences 155(3):519-526.

Kimchi-Sarfaty C, Oh JM, Kim IW *et al*., 2007. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science *315*(5811):525–528.

Laskowski RA, Jabłońska J, Pravda L *et al*., 2018. PDBsum: Structural summaries of PDB entries. Protein Science 27:129–134.

Lee DJ, KimY, Dinh PTN *et al*., 2023. Identification of Missense variants affecting carcass traits for hanwoo precision breeding. Genes 14(10):1839.

Lee SJ, 2023. Myostatin: A skeletal muscle chalone. Annual Review of Physiology 85: 269-291.

McPherron AC, Lawler AM and Lee SJ, 1997. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature *387*:83–90.

Moaeen-ud-Din M, Danish Muner R and Khan MS, 2022. Genome wide association study identifies novel candidate genes for growth and body conformation traits in goats. Scientific Reports 12:9891.

Mohanty L, Mishra C, Pradhan SK *et al*., 2021. Identification of novel polymorphism and *in-silico* analysis of caprine DNAJB3 gene. Small Ruminant Research 203:106492.

Mrode R, Tarekegn GM, Mwacharo JM *et al*., 2018. Invited review: Genomic selection for small ruminants in developed countries: how applicable for the rest of the world? Animal 12(7):1333-1340

Ng PC and Henikoff S, 2003. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Research 31:3812–3814.

Pandurangan AP, Ochoa-Montaño B, Ascher DB *et al*., 2017. SDM: a server for predicting effects of mutations on protein stability. Nucleic Acids Research 45:W229–W235.

Peka M and Balatsky V, 2024. Bioinformatic approach to identifying causative missense polymorphisms in animal genomes. BMC Genomics 25:1226.

Rodrigues CHM, Pires DEV and Ascher DB, 2021. DynaMut2: Assessing changes in stability and flexibility upon single and multiple point missense mutations. Protein Science 30:60–69.

Rudnicki MA, Schnegelsberg PNJ and Stead RH *et al*., 1993. MyoD or Myf-5 is required for the formation of skeletal muscle. Cell 75(7):1351–1359.

Savojardo C, Fariselli P, Martelli PL *et al*., 2016. INPS-MD: a web server to predict stability of protein variants from sequence and structure. Bioinformatics 32:2542–2544.

Schrödinger L and DeLano W, 2020. PyMOL. PyMOL Mol. Graph. Syst. Version 2.

Tamura K, Stecher G and Kumar S, 2021. MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38:3022–3027.

Wang J, Zhou H, Forrest RH *et al*., 2017. Variation in the ovine MYF5 gene and its effect on carcass lean meat yield in New Zealand Romney sheep. Meat Science 131:146-151.

Wiederstein M and Sippl MJ, 2007. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Research 35:W407–W410.

Yang R, Zhou D, Tan X *et al*., 2024. Genome-Wide Association study of body conformation traits in Tashi goats (Capra hircus). Animals 14(8):1145.

Yates CM and Sternberg MJE, 2013. The effects of non-synonymous single nucleotide polymorphisms (nsSNPs) on protein–protein interactions. Journal of Molecular Biology 425:3949–3963.

Ye J, Coulouris G, Zaretskaya I *et al*., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 13:134.

Zhang L, Liu M, Xu J *et al*., 2021. Genome-wide association study of body weight traits in Inner Mongolia Cashmere goats. Frontiers in Veterinary Science 8:752746.

Zhang RF, Lan XY, Zhang HJ *et al*., 2007. Association between polymorphisms of MSTN and MYF5 genes and growth traits in three Chinese cattle breeds. Asian Australasian Journal of Animal Sciences 20:1798–1804.

Zhang ZJ, Ling, YH, Wang LJ *et al*., 2013. Polymorphisms of the myostatin gene (MSTN) and its relationship with growth traits in goat breeds. Genetics and Molecular Research 12(2):965–971.