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Genetic analysis of Intron 2 of Muscling Gene Myostatin (MSTN) and its association with morphometric in Dorper sheep

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ABSTRACT

Myostatin (MSTN) acts as an inhibitor of skeletal muscle growth, and genetic variations in this gene are known to affect growth performance in livestock. This research explored the genetic variation in intron 2 of the MSTN gene and its correlation to morphometric traits in Dorper sheep. The study involved 42 animals, including nine imported Australian Dorper (G0) and 33 offspring (G1). Genomic DNA was extracted from blood samples, and a 1078 bp segment of MSTN intron 2 was amplified and sequenced. The morphometric traits assessed were body weight (BW), body length (BL), withers height (WH), chest depth (CD), chest width (CW), chest girth (CG), rump height (RH), and rump width (R). Sequence analysis revealed a single nucleotide polymorphism (SNP), rs406265773, with genotypic frequencies of 0.81 (AA), 0.14 (AC), and 0.05 (CC), and allelic frequencies of 0.88 (A) and 0.12 (C). The observed and expected heterozygosity were 0.14 and 0.21, respectively, indicating a deviation from Hardy–Weinberg equilibrium (χ^2 = 4.27). Both haplotype and nucleotide diversity were found to be low. Significant correlations were found between MSTN genotypes and several morphometric traits. These findings suggest that rs406265773 could be a useful genetic marker for growth related traits in Dorper sheep, potentially aiding marker assisted selection in breeding programs.

Introduction

MSTN, also referred to as Growth Differentiation Factor-8 (GDF8), plays a crucial role in regulating skeletal muscle growth by acting as an inhibitor. Mutations that result in a loss of function or regulatory variations in MSTN have been associated with increased muscle mass and changes in carcass characteristics in various livestock species, positioning MSTN as a key gene for enhancing growth performance. Although numerous studies concentrate on mutations in coding regions, non coding areas like introns may contain polymorphisms that impact gene expression, mRNA splicing, or other regulatory processes, thereby indirectly influencing muscle development and body structure. Specifically, intron 2 has been identified as a potential hotspot for functional polymorphisms that could affect growth and muscle traits (Aiello *et al*., 2018).

Sheep are a key focus for genetic enhancements in meat production systems. In breeds like the Colored Polish Merino, variations in the first intron of the MSTN gene have been linked to carcass and growth characteristics, such as early body weight and the weight of specific carcass parts (Grochowska *et al*., 2020). Likewise, in Egyptian and Saudi Arabian populations, SNPs within the MSTN intron showed a significant correlation with birth weight and average daily weight gain (Osman *et al*., 2021). These results highlight the potential of MSTN intronic variations as genetic markers for growth and production traits in sheep.

Dorper sheep, which were initially bred from Dorset Horn and Blackhead Persian sheep (Milne, 2000), are extensively utilized for meat production because of their fast growth, favorable carcass characteristics, and ability to adapt to diverse environmental conditions. In Indonesia, both imported Dorper sheep (founder generation, G0) and their offspring (G1) are being increasingly integrated into meat type sheep production systems. Nonetheless, there is limited genetic information on MSTN variability, particularly in intron 2, within Indonesian Dorper populations. Understanding this variation and its link to morphometric traits can offer valuable insights for breeding programs focused on enhancing growth and carcass quality.

Morphometric characteristics serve as practical measures of growth potential and overall body structure. Examining the relationship between these physical traits and MSTN genotypes could uncover genotype–phenotype correlations that are beneficial for marker assisted selection. Prior research on different sheep breeds has shown such connections, although the extent and nature of these associations can differ based on breed, environmental factors, and population structure (Grochowska *et al*., 2020).

The objectives of this study are to identify and describe genetic variations within intron 2 of the MSTN gene in an Indonesian Dorper sheep population, specifically the G0 and G1 groups. Additionally, the study aims to determine the genotypic and allelic frequencies of the SNP (rs406265773) found and to explore the relationship between MSTN genotypes and a wide range of morphometric traits. This research sought to offer foundational genetic data for Indonesian Dorper sheep and assessed the potential of intron 2 MSTN polymorphism as a molecular marker for growth and muscling traits in marker assisted breeding programs.

Materials and methods

Ethical clearance

The current study adhered to the animal care and experimental procedures as outlined by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences at Diponegoro University, with approval number 61-11/A-32/KEP-FPP.

Morphometric measurement

Standard livestock measurement techniques were employed to gather morphometric data from each of the 42 Dorper sheep. The measurements taken included live body weight (BW), body length (BL), withers

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height (WH), chest depth (CD), chest width (CW), chest girth (CG), rump height (RH), and rump width (RW) shown in Figure 1. These measurements were conducted in the morning when the animals were calm and properly positioned. The morphometric data collected were utilized to assess variations among different genotypes, age categories, and pedigree groups (G0 vs. G1).

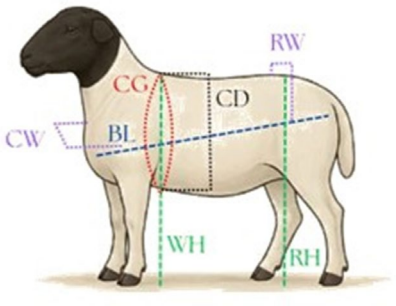

Figure 1. Morphometric traits in Dorper sheep

Sample collection and DNA isolation

Samples were collected from the Farmer's Agricultural and Training Center (P4S) Mitra Ternak Utama in Malang, East Java, a total of 42 Dorper sheep were sampled. The population was divided into two groups: nine imported Australian Dorper sheep (G0) and 33 of their offspring (G1). Blood samples were drawn from the jugular vein using vacutainer tubes coated with EDTA and were kept at –20°C until they were analyzed. Genomic DNA was extracted with the Geneaid GS300 gSYNC™ DNA Extraction Kit from Geneaid Biotech Ltd, Taiwan. The integrity of the DNA was checked using 1% agarose gel electrophoresis, and its purity and concentration were measured before being stored at –80°C. Only DNA samples of high quality were selected for further PCR amplification.

Intron 2 mstn gene amplification in Dorper sheep

A 1078 bp segment from intron 2 of the MSTN gene (GenBank Accession No. MH025940) was successfully amplified using primers crafted with Primer3 Plus. The forward primer sequence was 5′-CAAAGTTGGTGACACGGCAG-3′, and the reverse primer sequence was 5′-CCTTTGGGGTTTGCTTGGTG-3′. The PCR amplification process was conducted in a 50 µL reaction mixture, which included 4 µL of genomic DNA (20–30 ng/µL), 1 µL of each primer (10 pmol/µL), 25 µL of MyTaq Red Mix (Bioline), and 19 µL of ddH_2O. Optimal cycles and annealing temperature were established using gradient PCR, and the amplification profile for MSTN intron 2 is shown in Table 1.

The PCR products were then separated on a 2% agarose gel at 100 V for 30 minutes and visualized under UV light using an Enduro GDS Touch II imaging system. The presence of a distinct 1078 bp band indicated successful amplification. Out of 50 PCR products sequenced in both directions, only 42 high quality sequences were selected for SNP identification.

Table 1. Thermocycling protocol for MSTN Intron 2 amplification.

Protocol	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	1 min	1 cycle
Denaturation	95	15 s	40 cycles
Annealing	55.9	15 s	40 cycles
Extension	72	35 s	40 cycles
Final extension	72	5 min	1 cycle

Genotype analysis and association with morphometric traits

SNP identification

The nucleotide sequences were aligned using MEGA X software, version 10.1 (Kumar *et al*., 2018), and subsequently compared with the GenBank assembly sequence, accession No: MH025940.1, through the BLAST program (www.ncbi.nlm.nih.gov/blast/). The detected SNPs were then integrated to form haplotypes (Swain *et al*., 2020). A single SNP at locus rs406265773 was identified and used to classify genotypes into AA, AC, and CC.

Genotype and Allelic Frequencies

Genotypic and allelic frequencies were calculated according to Warwick *et al*. (1990):

$$F_{An} = (\sum \text{Lokus}_{An})/(\sum \text{Lokus } A_1 + \sum \text{Lokus } A_2 + \ldots\ldots + \sum \text{Lokus } A_n)$$

Genetic diversity parameters, including observed heterozygosity (Ho), expected heterozygosity (He), haplotype number (h), haplotype diversity (Hd), and nucleotide diversity (Pi), were estimated following Nei (1987). Hardy–Weinberg equilibrium was assessed using the chi-square test, where:

$p2 + 2pq + q2 = 1$

Statistical Analysis

The data were analyzed utilizing the General Linear Model (GLM) of SAS Software (SAS University Edition, 2018). A fixed model was employed for morphometric traits. :

$y_{ij} = \mu + G_i + A_j + e_{ijk}$

Let Y_{ij} represent the morphometric traits measured for each sample, where µ denotes the overall mean, G_i signifies the fixed effect of the ith genotype (i = 1, 2, 3), A_j indices the fixed effect of the ath age (j = 1, 2, 3, 4, 5), and e_{ijk} represents the random error of each observation. Statistical significance was confirmed when $P < 0.05$. In this study, multiple comparisons of the means were conducted using the Tukey-Kramer method with a significance level of 5%.

Results

Identification of PCR Fragments and SNP

The MSTN gene was successfully amplified, and the resulting PCR amplicons were separated using 2% agarose gels (Figure 2). The amplified fragment lengths matched the target DNA fragments, confirming amplicon specificity. The amplicons were therefore suitable for direct DNA sequencing (1st BASE Asia). Sequence alignment against the GenBank reference (MH025940.1) identified a polymorphic site in intron 2 of the MSTN gene in Indonesian Dorper sheep at position 2:118148578A (rs406265773). Three genotypes were observed: AA, AC, and CC (Figure 3).

Genotypic and allelic frequencies and genetic diversity of the MSTN gene

Genotype and allele frequencies and genetic diversity parameters for the studied populations are presented in Table 2. In Indonesian Dorper sheep (N= 42), the MSTN intron 2 SNP rs406265773 showed genotype frequencies of AA= 0.81, AC= 0.14, and CC= 0.05. Allele frequencies were A= 0.88 and C= 0.12. Genetic diversity estimates indicated low variation at this locus (Hd= 0.09; Pi= 0.0001). Observed heterozygosity (Ho= 0.14) was lower than expected heterozygosity (He= 0.21).

When stratified by generation, the imported animals (G0; N = 9) were

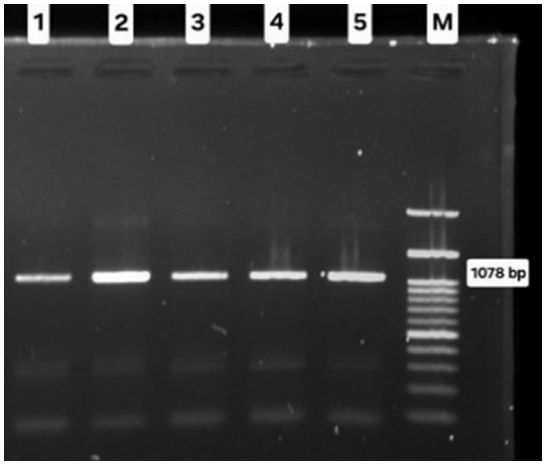

Figure 2. A 1078 bp PCR product of MSTN gene. 1-5 = Dorper Sheep DNA Samples; M = Marker.

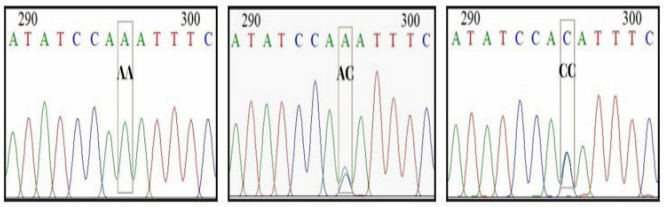

Figure 3. Chromatograms of rs406265773 in MSTN gene.

monomorphic with AA = 1.00 and A = 1.00 (Ho = 0.00; He = 0.00). The progeny (G1; N = 33) were polymorphic with genotype frequencies AA= 0.76, AC= 0.18, and CC = 0.06, and allele frequencies A= 0.85 and C= 0.15. In G1, Ho (0.18) remained lower than He (0.26). Hardy–Weinberg equilibrium was tested for the entire population (χ^2= 4.27) and for G1 (χ^2= 2.83).

Association among fixed effects and morphometric traits in Dorper sheep

A fixed effects analysis using a general linear model with Tukey–Kramer adjustment (α= 0.05) showed that generation (Table 3) and age (Table 4) significantly affected morphometric traits, whereas the effect of rs406265773 genotype was significant only for specific traits (Table 5).

Imported Dorper sheep (G0) had larger body size than their offspring (G1). Significant differences between generations were observed for BW, BL, WH, CD, and RH, with means including BW (G0: 62.60±3.35 kg; G1: 48.93±2.83 kg), BL (G0: 70.11±1.67 cm; G1: 62.94±1.64 cm), WH (G0: 58.67±0.97 cm; G1: 52.33±1.56 cm), CD (G0: 29.56±0.80 cm; G1: 25.42 ± 0.73 cm), and CG (G0: 60.00±1.04 cm; G1: 52.76±1.74 cm), respectively. Generation did not significantly influence CW, CG, or RW. Across age groups, significant differences were detected for BW, BL, WH, CD, CW, CG, and RH (P < 0.05).

Genotype rs406265773 was significantly associated with BW (P= 0.02), BL (P= 0.01), and CG (P= 0.04). Animals with genotype AA had the highest means for BW (55.66±2.70 kg), BL (67.38±1.47 cm), and CG (84.38±2.26 cm); AC animals showed intermediate values (BW 43.99±5.26 kg; BL 58.33±3.55 cm; CG 76.17±5.06 cm); and CC animals had the lowest values (BW 30.30 ± 6.10 kg; BL 54.50±6.50 cm; CG 66.00±8.00 cm). No significant genotype effects were observed for WH (P= 0.23), CD (P= 0.17), CW (P= 0.50), RH (P= 0.14), or RW (P= 0.33).

Sex did not significantly affect most traits (e.g., BW P = 0.07; CG P= 0.087), except CD, where females showed higher values than males (P= 0.005). Age showed strong effects on BW and CG (P < 0.0001 for both)

Table 2. Genotypic, allelic frequencies and genetic diversity of rs406265773 in MSTN genes.

Variable	N	Genotype			Alleles		Ho	He	χ^2	h	Hd	Pi
		AA	AC	CC	A	C						
Population	42	0.81	0.14	0.05	0.88	0.12	0.14	0.21	4.27	2	0.09	0.00
Imported	9	1	0	0	1	0	0	0	-	1	0	0
Progenies	33	0.76	0.18	0.06	0.85	0.15	0.18	0.26	2.83	2	0.12	0.00

N: Number of samples; h: Number of Haplotypes; Hd: Haplotype diversity; Pi: Nucleotide diversity

Table 3. Comparison of Morphometric Traits Between Generations (G0 and G1) in Dorper Sheep.

Generations	Morphometric Traits							
	BW (Kg)	BL (cm)	WH (cm)	CD (cm)	CW (cm)	CG (cm)	RH (cm)	RW (cm)
G0	62.60±3.35^{a}	70.11±1.67^{a}	58.67±0.97^{a}	29.56±0.80^{a}	16.00±1.33	88.56±2.00	60.00±1.04^{a}	16.56±1.25
G1	48.93±2.83^{b}	62.94±1.64^{b}	52.33±1.56^{b}	25.42±0.73^{b}	17.06±1.69	78.85±2.46	52.76±1.74^{b}	16.67±0.72
P value	0.02	0.03	0.04	0.01	0.75	0.05	0.04	0.94

Superscripts in different rows indicate significant differences at P < 0.05.
BW: body weight; BL: body length; WH: withers height; CD: chest depth; CW: chest width; CG: chest girth; RH: rumph height; RW: rump width

Table 4. Comparison of Body Weight and Morphometric Traits Among Different Age Groups of Dorper Sheep.

Age (months)	BW (Kg)	Morphometric traits						
		BL (cm)	WH (cm)	CD (cm)	CW (cm)	CG (cm)	RH (cm)	RW (cm)
≤12	36.81±3.24^{b}	56.31±1.44	49.84±3.07^{b}	23.31±1.00^{b}	13.773.87^{b}	70.38±2.78^{b}	51.23±3.4^{b}	15.15±1.01
13 - 24	54.40±1.85^{a}	66.67±1.67^{a}	57.33±0.33^{ab}	27.67±0.67^{ab}	14.33±0.88^{ab}	85.00±2.08^{a}	58.00±0.58^{ab}	14.67±0.67
25 - 36	56.59±6.25^{a}	67.14±4.25^{a}	53.43±1.65^{b}	26.57^{ab}	15.14^{ab}	85.00±5.39^{a}	53.86±2.03^{b}	16.43±1.66
37 - 48	61.16±5.07^{a}	71.43±1.72^{a}	57.00±1.22^{ab}	28.71±1.22^{a}	17.00±0.56^{ab}	90.71±3.88^{a}	57.14±1.38^{ab}	18.43±1.09
49 - 60	67.58±2.56^{a}	72.40±1.95^{a}	61.60±0.94^{a}	27.80±0.31^{ab}	19.6±1.98^{a}	90.70±1.90^{a}	63.30±0.96^{a}	18.1±1.73
P value	<.0001	<.0001	0.00	0.02	0.04	<.0001	0.00	0.29

Superscripts in different rows indicate significant differences at P < 0.05.
BW: body weight; BL: body length; WH: withers height; CD: chest depth; CW: chest width; CG: chest girth; RH: rumph height; RW: rump width

Table 5. Association Between Intron 2 (rs406265773) Genotypes of the MSTN Gene with Body Weight and Morphometric Traits in Dorper Sheep.

Genotypes	BW (Kg)	Morphometric Traits						
		BL (cm)	WH (cm)	CD (cm)	CW (cm)	CG (cm)	RH (cm)	RW (cm)
AA	55.66±2.70^{a}	67.38±1.47^{a}	55.59±1.49	26.68±0.74	16.32±1.63	84.38±2.26^{a}	56.29±1.64^{a}	16.86±0.69
AC	43.99±5.26^{ab}	58.33±3.55^{bc}	55.00±3.11	25.17±1.33	15.33±1.63	76.17±5.06^{ab}	57.00±3.71^{a}	16.17±1.49
CC	30.30±6.10^{c}	54.50±6.50^{b}	46.50±3.50	21.50±1.50	12.50±2.50	66.00±8.00^{c}	46.50±1.50^{b}	12.50±2.50
P value	0.02	0.01	0.23	0.17	0.5	0.04	0.14	0.33

Superscripts in different rows indicate significant differences at $P < 0.05$.
BW: body weight; BL: body length; WH: withers height; CD: chest depth; CW: chest width; CG: chest girth; RH: rumph height; RW: rump width

and significant effects on BL ($P < 0.0001$), WH ($P= 0.001$), CD ($P= 0.02$), CW ($P= 0.04$), and RH ($P= 0.001$), while RW was not associated with age ($P= 0.29$). Overall population means were 51.86±2.48 kg (BW), 64.48±1.40 cm (BL), 53.69±1.30 cm (WH), 26.31±0.65 cm (CD), 16.83±1.35 cm (CW), 80.93±2.07 cm (CG), 54.31 ± 1.46 cm (RH), and 16.64±0.62 cm (RW).

Discussion

The predominance of the AA genotype and the absence of variation in G0 are consistent with founder effects and reduced allelic diversity frequently observed in imported livestock populations, particularly when founders originate from highly selected meat breeds (Osman *et al*., 2021; Thepa and Tyasi, 2024). Similar patterns of low minor allele frequency and reduced heterozygosity for MSTN variants have been reported in other sheep breeds under long term selection for growth and carcass traits (Dhakad *et al*., 2017; Osman *et al*., 2021). Although rs406265773 is located in an intronic region, intronic and other non coding polymorphisms in MSTN have been repeatedly associated with growth and body weight traits, likely through linkage disequilibrium with causal regulatory or coding variants (Osman *et al*., 2021; Thepa and Tyasi, 2024; Zhao *et al*., 2024). The limited diversity observed here suggests that Indonesian Dorper sheep may represent only a small subset of global MSTN haplotype variation, reflecting both importation history and past selection for meat type performance (Miar *et al*., 2014).

The high prevalence of the A allele indicates that selection based solely on this single SNP may yield limited short term response because most animals already carry the putatively favorable genotype. However, the presence of the C allele in G1, together with its association with phenotypes reported in this study, indicates that this locus or linked variation may still be informative for distinguishing individuals. Under tropical production constraints (e.g., heat stress, parasite pressure, and seasonal feed shortage), maintaining diversity at candidate loci such as MSTN can be beneficial for simultaneous improvement of growth and adaptation. Thus, rs406265773 could be incorporated with other loci and conventional records within a selection index rather than used as a stand alone marker (Miar *et al*., 2014; Bellinge *et al*., 2005).

The consistently larger size of G0 animals relative to G1 likely reflects genotype environment interactions and differences in management or nutrition between founder stock and locally raised progeny (Febriana *et al*., 2026). Growth traits in sheep are sensitive to environmental conditions, and changes in feed availability or quality can reduce the expression of genetic growth potential (Sallé *et al*., 2021). The pattern of larger reductions in length and height traits compared with more stable width traits may indicate environmentally mediated constraints on skeletal and overall body development, reinforcing the importance of nutrition and management to support growth under local conditions (De Marzo, 2023).

Age related differences across multiple traits confirm that body morphology in Dorper sheep changes predictably with development. Morphometric traits such as chest girth, body length, withers height, and rump height are commonly correlated with body weight, supporting their use as field indicators when scales are unavailable (Markos *et al*., 2023; Özentürk *et al*., 2025). The strong age effect observed here emphasizes the need to account for age (and potentially growth stage) when evaluating morphometrics for selection and management decisions.

The association of rs406265773 with BW, BL, and CG is biologically consistent with the established role of myostatin (GDF8) as a negative regulator of skeletal muscle growth; reduced myostatin activity leads to increased muscling and carcass yield (Bellinge *et al*., 2005; Miar *et al*., 2014). The stronger effects on BW, BL, and CG compared with height or width related traits suggest that this locus (or linked causal variation) may influence muscling and body volume more than skeletal frame dimensions. This agrees with reports that MSTN polymorphisms in sheep are often more strongly associated with weight and growth traits than with linear skeletal measures (Osman *et al*., 2021; Thepa and Tyasi, 2024; Zhao *et al*., 2024), including studies on intronic MSTN variants in indigenous breeds (Dhakad *et al*., 2017; Osman *et al*., 2021).

The consistent ranking of genotypes (AA > AC > CC) for BW, BL, and CG indicates that rs406265773 or correlated MSTN variation may be useful in marker assisted or genomic selection aimed at improving growth and meat yield, particularly where environmental stressors can mask phenotypic differences (Miar *et al*., 2014; Thepa and Tyasi, 2024). Nevertheless, because MSTN is closely tied to muscling, strong directional selection for alleles that markedly reduce myostatin function could introduce unfavorable correlated responses (e.g., reduced fat reserves or dystocia), as reported in double muscled cattle and heavily muscled sheep (Bellinge *et al*., 2005; Miar *et al*., 2014). Therefore, if this marker is implemented, it should be embedded in balanced breeding objectives that consider fitness and adaptation alongside production traits.

Conclusion

Overall, the intronic MSTN SNP rs406265773 shows low genetic diversity in Indonesian Dorper sheep and is associated with key growth related traits, with the AA genotype was associated with higher body weight, longer body length, and larger chest girth. Meanwhile, generation and age exert substantial effects on phenotypic expression, highlighting the importance of management and appropriate modeling of fixed effects. Future work should validate these associations in larger and more diverse populations, incorporate additional MSTN and genome wide markers, and include functional and carcass assessments to support balanced breeding programs under tropical production systems.

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Conflict of interest

The authors have no conflict of interest to declare.

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