Study on Two single nucleotide polymorphisms (SNPs) in Calpastatin Gene and Their Association with Weight Traits among Iranian Indigenous Sheep Breeds

Research Article

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ABSTRACT

This study examines two single nucleotide polymorphisms (SNPs) in the CAST gene, which plays a crucial role in muscle growth and meat tenderness, in 721 sheep from 16 Iranian indigenous breeds. The goal was to analyze the allelic and genotypic frequencies of these mutations using the PCR-RFLP method. A 620 bp fragment of the CAST gene was amplified by PCR and digested with *Msp*I and *Nco*I enzymes to target the mutation regions. Both mutations were detected in all breeds. At the *Msp*I locus, the highest frequency of the N allele was observed in the Lori breed (0.243), while the lowest was in the Zel breed (0.048). At the *Nco*I locus, the highest frequency of the N allele was in the Zandi breed (0.192), and the lowest in the Zel breed (0.024). Pearson correlation coefficient test revealed a positive correlation between the MN genotype at the *Msp*I locus and weight traits such as birth weight, mature weight, and weight gain. A weak positive correlation was also observed between the NN genotype and mature weight and weight gain in both rams and ewes. These findings suggest that the *Msp*I mutation has a stronger influence on weight traits than the *Nco*I mutation, highlighting its potential as a valuable genetic marker for improving meat production in Iranian sheep breeding programs.

KEY WORDS calpastatin, polymorphisms, sheep, weight.

INTRODUCTION

The global population is expected to reach 9 billion by 2050, driving a 73% increase in meat demand, which is unlikely to be met by raising animal numbers alone. In response, improving the efficiency of meat production, including mutton, is crucial (Talebi *et al.* 2022). Iran, the birthplace of sheep domestication, is home to 27 indigenous sheep breeds, making it the world leader in breed diversity (Talebi *et al.* 2018). More than 67% of Iran's livestock are sheep, which account for about 36% of the country's red meat production (Agricultural Statistics of Iran, 2024). De-

spite their importance, Iranian indigenous breeds suffer from slow growth rates, poor carcass composition, and low feed efficiency, limiting their potential (Talebi *et al.* 2023; Talebi *et al.* 2024). Thus, advancing breeding strategies to enhance growth, carcass traits, and meat yield is essential to improve both the economic and biological efficiency of sheep production, ensuring these breeds can better contribute to global protein needs (Mohammadabadi *et al.* 2021). Calpastatin (CAST) is one of the genes involved in the quality and quantity of meat production in livestock, which is considered in breeding programs. Calpastatin along with m-calpain and μ-calpain, are three well-known proteins of

the Calpain family. Calpastatin is a specific inhibitor of m-calpain and μ-calpain, both calcium-dependent proteolytic enzymes. The calpain-calpastatin system plays a crucial role in rate of skeletal muscle growth, as well as the rate and extent of postmortem tenderization (Goll *et al.* 1998). The activity balance between calpains and calpastatin regulates the extent of proteolytic activity, which results in the breakdown of myofibrillar proteins. In live animals, high muscle yields are promoted by high activity of muscle calpastatin, whereas in post-mortem situation, low calpastatin activity is necessary for high quality of meat tenderness (Bickerstaffe *et al.* 2008). The CAST gene has been mapped on chromosome 5 of the sheep genome [accession number NC_056058.1 in NCBI]. Calpastatin gene polymorphisms have been studied extensively across various mammalian species, including humans (Zhang *et al.* 2013), goats (Zhou and Hickford, 2008), pigs (Ropka-Molik *et al.* 2014), cattle (Tait *et al.* 2014), and sheep (Palmer *et al.* 1998). In cattle, CAST influences meat tenderness, reduced loin muscle area, and increased fat yield (Schenkel *et al.* 2006), and has also been implicated in fertility and longevity in dairy cattle (Garcia *et al.* 2006). Moreover, CAST gene polymorphisms are associated with the development of keratoconus in humans (Zhang *et al.* 2018). In sheep, certain calpastatin genotypes have been significantly linked to traits such as birth weight and average daily gain (Chung and Davis, 2012), and variants of the CAST gene have been associated with enhanced live weight gain, carcass weight (Palmer *et al.* 1999), and loin weight in lambs (Bickerstaffe *et al.* 2008). While CAST gene polymorphisms have been linked to birth weight and some meat traits in sheep, their effects on growth rate and carcass quality vary across breeds. For example, no significant associations were found between CAST polymorphisms and growth or carcass traits in Afshari sheep (Nikmard *et al.* 2012). In contrast, the "ac" genotype of CAST has been associated with increased intramuscular fat content in specific breeds suggesting its potential to enhance meat yield (Gregula-Kania *et al.* 2019). Building on these findings, this study was designed to investigate the genetic variation of the CAST gene in 16 meat-type Iranian indigenous sheep breeds due to its critical role in muscle growth and development. The objectives of this study were to: 1) characterize the polymorphisms of the CAST gene across these 16 breeds, and 2) evaluate the association of these polymorphisms with weight traits.

MATERIALS AND METHODS

Samples, DNA extraction, and PCR reaction
A total of 721 individuals from 16 Iranian indigenous breeds were sampled: Afshari (55), Dalagh (30), Farahani (55), Gray Shiraz (55), Kalkooyi (47), Lori (37), Makooyi (41), Mehraban (55), Moghani (47), Nayini (49), Sanjabi (23), Shal (46), Taleshi (45), Turk Ghashghayi (55), Zandi (39), and Zel (42). Blood samples were collected from the jugular vein in 10 ml EDTA venoject tubes and stored at 4 °C. The samples were taken from multiple herds, with unrelated animals selected based on pedigree and owner-provided data to minimize inbreeding and enhance genetic variation. Genomic DNA was extracted from whole blood using the GPP kit (Gene Pajoohan Pooya), according to the manufacturer's manual with certain modifications. DNA concentration was measured with a Thermo Scientific NanoDrop spectrophotometer (2000), and its integrity was confirmed through electrophoresis on a 1% agarose gel. PCR analysis was conducted using an Astec thermal cycler (PC-818). Details on the primers, PCR products, reaction components, and temperature program for SNPs are provided in Tables 1, 2, and 3.

Genotyping via restriction fragment length polymorphisms (RFLP)
Genotyping was carried out using restriction fragment length polymorphism (RFLP) analysis, employing two restriction enzymes, *Msp*I and *Nco*I, for DNA digestion. These enzymes were selected based on their ability to cleave DNA at specific recognition sites flanking the targeted SNP positions. The digestion reactions for each SNP were carefully optimized to ensure accurate and reliable genotyping. Table 4 provides detailed information about each enzyme, including their specific recognition sites, the resulting fragment sizes produced for each SNP position, and the corresponding digestion reactions. The use of *Msp*I and *Nco*I allowed for precise identification of allelic variants at the selected loci, providing insights into the genetic variation across the sampled individuals.

Statistical analysis
Genotypes were identified based on the presence or absence of bands resulting from enzyme digestion, which were then scored on 3% agarose gels. The band patterns corresponding to different alleles were used to determine the genotype of each individual. The genotyping data were subsequently analyzed using POPGENE software (version 1.31) to assess genetic variation and population structure. To investigate potential relationships between genetic variation and phenotypic traits, Pearson's correlation coefficients were calculated between allele frequencies and weight traits using R software (version 4.3.2). The Pearson correlation coefficient is a widely used statistical measure to quantify the strength and direction of a linear relationship between two variables.

Table 1 Details of primers and PCR products

Primer	Sequence	Details of PCR product
CAST-F	5´-TGGGGCCCAATGACGCCATCGATG-3´	620 bp (accession numbers: KX722533.1 and
CAST-R	5´-GGTGGAGCAGCACTTCTGATCACC-3´	KX722534.1)

Table 2 PCR reaction components and volumes

Material	DNA	Buffer	MgCl2	dNTPs	Primer F	Primer R	Taq	ddWater	Reaction volume;
Concentration	20 ng	10 X	100 mM	10 mM	10 pmol	10 pmol	3 U	-	15 µL
Volume used	2.5 µL	1.5 µL	0.3 µL	0.3 µL	1 µL	1 µL	0.4 µL	8 µL	

Table 3 PCR program in thermal cycler

PCR Step	Whole denaturation	Denaturation	Annealing	Extension	Cycles	Final extension
Temp/time	94 °C/5 min	94 °C/40 sec	59 °C/40 sec	72 °C/1 min	X 35	72 °C/5 min

Table 4 Details of restriction enzymes and digestion conditions

Enzyme	*Msp*I	*Nco*I
Cutting site	C.CGG	C.CATGG
Cutting temp	37 °C	37 °C
Duration time	3:30 h	4 h
PCR product	2.5 µL	2 µL
Digestion buffer	1.5 µL	1.5 µL
Restriction enzyme	0.25 µL	0.2 µL
DD water	10.75 µL	11.3 µL
MM (bp)	336 / 284	620
MN (bp)	620 / 336 / 284	620 / 370 / 250
NN (bp)	620	370 / 250

Figure 1 Representative genomic DNA samples extracted for this study, indicating high-quality and purity suitable for downstream analyses
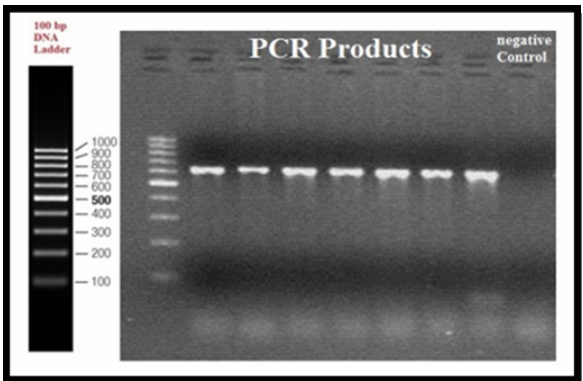
Figure 2 Visualization of PCR products (620 bp) on a 1.5% agarose gel, showing successful amplification with no evidence of non-specific bands

Figure 3 Restriction enzyme digestion patterns for the *Msp*I locus, demonstrating polymorphic bands on a 3% agarose gel

Its value ranges from -1, indicating a perfect negative correlation, to +1, indicating a perfect positive correlation, with 0 representing no correlation (Zou *et al.* 2003).

RESULTS AND DISCUSSION

The genomic DNA extracted from the samples exhibited high purity, as assessed by visual inspection (Figure 1). The DNA samples showed clear, distinct bands and minimal degradation, indicating the suitability of the samples for subsequent molecular analyses. PCR amplification was performed successfully, yielding the expected 620 bp product for all samples. No non-specific bands were observed, ensuring the specificity of the amplification process. The PCR products were visualized on a 1.5% agarose gel, as shown in Figure 2, where clear and distinct bands corresponding to the target fragment size were detected for each sample.

Restriction enzyme digestion of the PCR products revealed polymorphisms at two loci, with distinct fragment patterns observed following enzyme digestion. The digestion products for the *Msp*I locus are shown in Figure 3, where the presence of various fragment sizes indicated the existence of genetic variation at this locus.
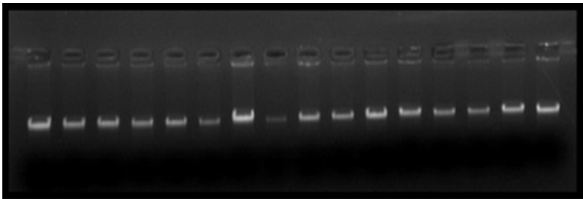
Similarly, digestion results for the *Nco*I locus are depicted in Figure 4, also showing polymorphisms in the form of different fragment sizes. Both sets of digestion results were visualized on a 3% agarose gel, which allowed for clear differentiation of the polymorphic bands.
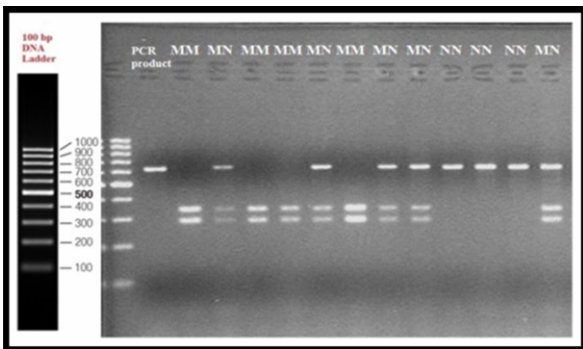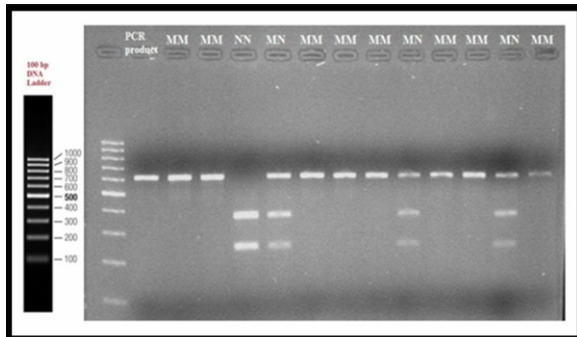

Figure 4 Restriction enzyme digestion patterns for the *Nco*I locus, demonstrating polymorphic bands on a 3% agarose gel

Table 5 summarizes the genotype and allele frequencies for the *Msp*I locus across sheep breeds. The highest frequency of homozygous dominant genotype (MM) was observed in Zel sheep (0.905), heterozygous (MN) in Lori sheep (0.486), and homozygous recessive (NN) in Shal sheep (0.065). Also, the highest frequency of dominant allele (M) was observed in Zel sheep (0.952) and recessive allele (N) in Lori sheep (0.243). The dominant M allele was more prevalent (0.847) than the N allele (0.153) in the total population (721 individuals). The homozygous MM genotype is the most frequent (0.717), followed by the heterozygous MN (0.261) and the homozygous NN (0.022), which is rare or absent in many breeds. The frequency of the MM genotype is higher in smaller breeds (e.g., Zel, Taleshi), suggesting a possible association between the N allele and weight traits.

Accordingly, the low frequency of the NN genotype across smaller breeds suggests that the recessive N allele confer an adaptive advantage. These findings indicate that the N allele may be associated with larger body sizes in certain sheep breeds, providing insights into the genetic structure of these populations and potential implications for breeding strategies aimed at improving body size and related traits.

Table 6 presents the genotype and allele frequencies for the *Nco*I mutation across various sheep breeds. The highest frequency of homozygous dominant genotype (MM) was observed in Zel sheep (0.952), heterozygous (MN) in Lori sheep (0.378), and homozygous recessive (NN) in Moghani sheep (0.042). Also, the highest frequency of dominant allele (M) was observed in Zel sheep (0.976) and recessive allele (N) in Zandi sheep (0.192).

The dominant M allele was more prevalent (0.897) than the N allele (0.103) in the total population (721 individuals). The homozygous MM genotype is the most frequent (0.803), followed by the heterozygous MN (0.189) and the homozygous NN (0.008), which is rare or absent in many breeds, indicating a strong selection pressure against the recessive allele.

This pattern is consistent with the findings for the *Msp*I mutation, where overall, the N allele was also more prevalent in larger breeds. The results suggest that the *Nco*I mutation may also contribute to some extent to the genetic differentiation of body size among sheep breeds.

The results of the Hardy-Weinberg Equilibrium (HWE) test for both the *Msp*I and *Nco*I loci across all breeds are summarized in Table 7.

For the *Nco*I locus, all breeds were in HWE (P>0.05), indicating no significant deviation from equilibrium. In contrast, at the *Msp*I locus, the Shal breed exhibited a significant deviation from HWE (P<0.05), suggesting potential forces influencing allele frequencies in this breed. Overall, the findings indicate that most of the populations studied are in Hardy-Weinberg equilibrium, with the exception of the Shal breed at the *Msp*I locus.

The frequencies of genotypes and alleles for the *Msp*I and *Nco*I loci, categorized by body size (large, Medium, Small), are presented in Table 8. Significant differences in the frequency of the N allele were observed between small and medium/large breeds. At the *Msp*I locus, the N allele frequency was 0.162 in large breeds, 0.170 in medium breeds, and 0.099 in small breeds. Similarly, at the *Nco*I locus, the N allele frequency was 0.111 in large breeds, 0.119 in medium breeds, and 0.051 in small breeds. In general, the frequency of the N allele in small breeds is lower than in medium and large breeds, with a frequency of 9.9% for *Msp*I and 5.1% for *Nco*I. In particular, the frequency of the MN genotype in small breeds is lower than in medium and large breeds.

Pearson's correlation coefficient was used to explore the relationship between mutation frequencies and weight traits across the 16 sheep breeds listed in Table 9. The weight traits considered include birth weight (BW), maturity weight (MW), and weight gain (WG) for both ewes and rams.

As shown, larger breeds, such as Afshari and Shal, typically exhibited higher weight gains, maturity weights, and birth weights. In contrast, smaller breeds like Zel and Nayini displayed lower weight metrics across all traits. A trend was observed linking higher frequencies of the N allele in larger breeds with higher weight traits, suggesting a potential genetic influence on body size, though further statistical analysis is required to confirm the strength of these associations.

Table 5 Frequencies of genotypes and alleles for *Msp*I mutation. Breeds are ranked from highest to lowest based on N allele frequency

Breed (n)	Body size	Genotypes and alleles				
		MM (f)	MN (f)	NN (f)	M (f)	N (f)
Lori (37)	Large	19 (0.514)	18 (0.486)	0 (0.000)	56 (0.757)	18 (0.243)
Zandi (39)	Medium	25 (0.641)	13 (0.333)	1 (0.026)	63 (0.808)	15 (0.192)
Moghani (47)	Large	31 (0.660)	14 (0.298)	2 (0.042)	76 (0.809)	18 (0.191)
Farahani (55)	Medium	38 (0.691)	15 (0.273)	2 (0.036)	91 (0.827)	19 (0.173)
Afshari (55)	Large	37 (0.673)	17 (0.309)	1 (0.018)	91 (0.827)	19 (0.173)
Makooyi (41)	Large	27 (0.659)	14 (0.341)	0 (0.000)	68 (0.829)	14 (0.171)
Kalkooyi (47)	Medium	33 (0.702)	12 (0.255)	2 (0.043)	78 (0.830)	16 (0.170)
Gray Shiraz (55)	Medium	38 (0.691)	16 (0.291)	1 (0.018)	92 (0.836)	18 (0.164)
Shal (46)	Large	35 (0.761)	8 (0.174)	3 (0.065)	78 (0.848)	14 (0.152)
Dalagh (30)	Medium	21 (0.700)	9 (0.300)	0 (0.000)	51 (0.850)	9 (0.150)
Nayini (49)	Small	37 (0.755)	10 (0.204)	2 (0.041)	84 (0.857)	14 (0.143)
Mehraban (55)	Large	40 (0.727)	15 (0.273)	0 (0.000)	95 (0.864)	15 (0.136)
Turk Ghashghayi (55)	Large	44 (0.800)	9 (0.164)	2 (0.036)	97 (0.882)	13 (0.118)
Sanjabi (23)	Large	18 (0.783)	5 (0.217)	0 (0.000)	41 (0.891)	5 (0.109)
Taleshi (45)	Small	36 (0.800)	9 (0.200)	0 (0.000)	81 (0.900)	9 (0.100)
Zel (42)	Small	38 (0.905)	4 (0.095)	0 (0.000)	80 (0.952)	4 (0.048)
Total (721)		**517 (0.717)**	**188 (0.261)**	**16 (0.022)**	**1222 (0.847)**	**220 (0.153)**

n: the number of investigated individuals.
MM: homozygous dominant; MN: heterozygous; NN: homozygous recessive; M: dominant allele; N: recessive allele and f: frequency.

Table 6 Frequencies of genotypes and alleles for *Nco*I Mutation. Breeds are ranked from highest to lowest based on N allele frequency

Breed (n)	Body size	Genotypes and alleles				
		MM (f)	MN (f)	NN (f)	M (f)	N (f)
Zandi (39)	Medium	25 (0.641)	13 (0.333)	1 (0.026)	63 (0.808)	15 (0.192)
Lori (37)	Large	23 (0.622)	14 (0.378)	0 (0.000)	60 (0.811)	14 (0.189)
Moghani (47)	Large	35 (0.745)	10 (0.213)	2 (0.042)	80 (0.851)	14 (0.149)
Kalkooyi (47)	Medium	33 (0.702)	14 (0.298)	0 (0.000)	80 (0.851)	14 (0.149)
Turk Ghashghayi (55)	Large	44 (0.800)	9 (0.164)	2 (0.036)	97 (0.882)	13 (0.118)
Mehraban (55)	Large	42 (0.764)	13 (0.236)	0 (0.000)	97 (0.882)	13 (0.118)
Gray Shiraz (55)	Medium	44 (0.800)	10 (0.182)	1 (0.018)	98 (0.891)	12 (0.109)
Afshari (55)	Large	44 (0.800)	11 (0.200)	0 (0.000)	99 (0.900)	11 (0.100)
Dalagh (30)	Medium	24 (0.800)	6 (0.200)	0 (0.000)	54 (0.900)	6 (0.100)
Makooyi (41)	Large	33 (0.805)	8 (0.195)	0 (0.000)	74 (0.902)	8 (0.098)
Sanjabi (23)	Large	19 (0.826)	4 (0.174)	0 (0.000)	42 (0.913)	4 (0.087)
Taleshi (45)	Small	39 (0.867)	6 (0.133)	0 (0.000)	84 (0.933)	6 (0.067)
Farahani (55)	Medium	48 (0.873)	7 (0.127)	0 (0.000)	103 (0.936)	7 (0.064)
Nayini (49)	Small	43 (0.878)	6 (0.122)	0 (0.000)	92 (0.939)	6 (0.061)
Shal (46)	Large	43 (0.935)	3 (0.065)	0 (0.000)	89 (0.967)	3 (0.033)
Zel (42)	Small	40 (0.952)	2 (0.048)	0 (0.000)	82 (0.976)	2 (0.024)
Total (721)		**579 (0.803)**	**136 (0.189)**	**6 (0.008)**	**1294 (0.897)**	**148 (0.103)**

n: the number of investigated individuals.
MM: homozygous dominant; MN: heterozygous; NN: homozygous recessive; M: dominant allele; N: recessive allele and f: frequency.

Table 10 presents the Pearson's correlation coefficients between the genotypes at the *Msp*I and *Nco*I loci and various phenotypic traits, including birth weight (BW), mature weight (MW), and weight gain (WG) for both rams and ewes. At the *Msp*I locus, the homozygous dominant (MM) genotype showed negative correlations with all traits in both rams and ewes, with the strongest negative correlations observed for mature weight (MW) and weight gain (WG) in ewes (-0.64 and -0.50, respectively). In contrast, the heterozygous (MN) genotype exhibited positive correlations, with moderate correlations for birth weight in rams (+0.40) and ewes (+0.35), and the strongest positive correlation with mature weight in both rams (+0.40) and ewes (+0.43).

The homozygous recessive (NN) genotype showed a weak positive correlation with mature weight and weight gain in both rams and ewes, suggesting a lesser effect of this genotype on these traits compared to the MN genotype. For the *Nco*I locus, the correlations were generally weaker. The MM genotype showed negative correlations with all traits, though these correlations were relatively small, ranging from -0.29 for mature weight in ewes to -0.16 for weight gain in ewes. The MN genotype exhibited weak positive correlations with all traits, with the highest being for mature weight in ewes (+0.29). The NN genotype showed minimal correlations with the phenotypic traits, with the strongest correlation being a weak positive relationship with mature weight in rams (+0.09).

Table 7 Results of Hardy-Weinberg equilibrium test for *Msp*I and *Nco*I in all breeds

Breed	*Msp*I SNP	*Nco*I SNP
Afshari	0.586[ns]	0.434[ns]
Dalagh	0.366[ns]	0.581[ns]
Farahani	0.680[ns]	0.642[ns]
Gray Shiraz	0.686[ns]	0.573[ns]
Kalkooyi	0.456[ns]	0.250[ns]
Lori	0.059[ns]	0.174[ns]
Makooyi	0.206[ns]	0.520[ns]
Mehraban	0.260[ns]	0.342[ns]
Moghani	0.735[ns]	0.230[ns]
Nayini	0.205[ns]	0.678[ns]
Sanjabi	0.604[ns]	0.695[ns]
Shal	0.020*	0.853[ns]
Taleshi	0.484[ns]	0.663[ns]
Turk Ghashghayi	0.088[ns]	0.088[ns]
Zandi	0.703[ns]	0.703[ns]
Zel	0.780[ns]	0.912[ns]
Total	**0.836[ns]**	**0.530[ns]**

SNP: single nucleotide polymorphism.
NS: non significant.
* ($P<0.05$).

In overall, these results highlight that the *Msp*I mutation has a greater impact on weight traits compared to the *Nco*I mutation, particularly for the MN genotype, which showed the most consistent positive correlations with birth weight, mature weight, and weight gain. Further details are shown in Table 10 and Figure 5.

The *Msp*I genotyping results from this study demonstrated the presence of the desired mutation across a diverse group of 16 Iranian sheep breeds. The frequency of the N allele varied significantly among breeds, ranging from a high of 0.243 in Lori sheep to a low of 0.048 in Zel sheep. This wide variation in allele frequencies suggests the potential influence of breed-specific factors such as genetic background, management practices, and geographic location on the distribution of the mutation. The detection of this allele in Iranian sheep is consistent with findings from previous studies, which have also observed polymorphisms at the *Msp*I locus across various sheep populations globally (Table 11). Additionally, studies on sheep breeds from different regions, such as those by Tohidi *et al.* (2013) and Azari *et al.* (2012), reported varying frequencies of the *Msp*I mutation, which underscores the genetic diversity present in sheep populations. The observation that the N allele is more prevalent in breeds like Lori (0.243) and Moghani (0.191) compared to breeds like Zel (0.048) or Taleshi (0.100) suggests that selective breeding practices and environmental factors may play a role in shaping the frequency of the allele in these populations. This is particularly interesting in the case of Lori and Moghani sheep, where the higher frequency of the N allele may be linked to specific breeding objectives or adaptation to local environmental conditions. Furthermore, comparisons with studies on other breeds

suggest that sheep populations from different regions may harbor distinct genetic characteristics, influencing the distribution of polymorphisms such as the *Msp*I mutation. This highlights the importance of regional and breed-specific studies in understanding the genetic landscape of sheep populations. Studies by Yilmaz *et al.* (2014) on Sakiz sheep and Santos *et al.* (2016) on Bergamacia sheep identified allele frequencies that were skewed toward the N allele.

Genotyping of *Nco*I revealed that the desired mutation is present in this locus among the examined breeds of Iranian sheep, with the difference in the frequency of the N allele ranging from 0.192 in Zandi to 0.024 in Zel. There are few studies on mutation at this locus in sheep populations around the world. Table 12 highlights the *Nco*I polymorphisms identified in various sheep populations. It was noted that three breeds - Polish Merino, Berrichon du Cher, and Ile de France - were found to lack the N allele (Szkudlarek-Kowalczyk *et al.* 2011).

Regarding previous studies, several authors have examined the role of the calpastatin gene in sheep, specifically investigating its polymorphisms and their impact on traits like meat tenderness, growth rate, and carcass weight. For example, Palmer *et al.* (1997) found significant differences in fillet tenderness and meat pH between various genotypes of the ovine calpastatin gene. Individuals with the "ab" genotype exhibited higher pH values in the longissimus dorsi muscle, while ewes with the "ac" genotype produced fillets with higher shear force measurements compared to other genotypes. Additionally, in a subsequent study (Palmer *et al.* 1999), the "ac" genotype was linked to higher live weight gain and age-corrected carcass weight. Study by Gorlov *et al.* (2016) also reported associations between specific genotypes and higher body weight and growth rates. Moreover, Chung and Davis (2012) confirm the significant correlation of calpastatin genotypes with birth weight and average daily gain in purebred Polypay, purebred Targhee, and crossbred sheep. Valencia *et al.* (2022) found a significant correlation between the M/N polymorphism in the CAST gene and birth weight in Colombian hair sheep. Other studies also demonstrated links between genotype and average daily weight gain in Balkhi and Kajli sheep (Saeed-ul-Hassan *et al.* 2012) or final body weight and longissimus muscle width in Awassi sheep (Jawasreh *et al.* 2019).

Ibrahim *et al.* (2015) identified four alleles (M, N, O, and P) in Barki lambs, with the O allele associated with higher lean meat and lower fat percentages, while the M allele showed the opposite effect. Similarly, Byun *et al.* (2008) identified three alleles (A, B, and C) in Romney lambs, with CAST alleles A and C significantly influencing birth weight, though no major effect was observed on growth rate.

Table 8 Frequencies of genotypes and alleles based on body size

SNP position	Body size	Genotypes and alleles				
		MM (f)	MN (f)	NN (f)	M (f)	N (f)
*Msp*I	Large	0.699	0.279	0.022	0.838	0.162
	Medium	0.686	0.288	0.026	0.830	0.170
	Small	0.816	0.169	0.015	0.901	0.099
*Nco*I	Large	0.788	0.201	0.011	0.889	0.111
	Medium	0.770	0.221	0.009	0.881	0.119
	Small	0.897	0.103	0.000	0.949	0.051

SNP: single nucleotide polymorphism.
MM: homozygous dominant; MN: heterozygous; NN: homozygous recessive; M: dominant allele; N: recessive allele and f: frequency.

Table 9 Comprehensive data on weight traits for 16 sheep breeds, including birth weight, maturity weight, and weight gain metrics

Breeds	Weight of rams (kg)			Weight of ewes (kg)		
	BW[1]	MW[1]	WG[1]	BW	MW	WG
Afshari	4.4	75	0.28	4	70	0.25
Dalagh	4.6	60	0.17	4.4	50	0.15
Farahani	3.3	55	0.18	2.3	50	0.13
Gray Shiraz	4.2	67.3	0.15	3.9	52.2	0.13
Kalkooyi	3.5	52	0.25	3.3	50	0.17
Lori	5.0	70	0.23	4.5	67	0.21
Makooyi	4.0	52.5	0.21	3.5	47.5	0.17
Mehraban	4.3	60	0.26	4.0	55	0.17
Moghani	4.5	65	0.25	4.2	60	0.23
Nayini	2.9	43.5	0.14	2.7	39	0.11
Sanjabi	4.5	60	0.26	4.3	55	0.22
Shal	5.3	75	0.28	4.8	67	0.25
Taleshi	3.3	47.5	0.16	3.1	43.6	0.14
Turk Ghashghayi	4.0	60	0.20	3.5	55	0.16
Zandi	2.9	47.5	0.18	2.5	42.5	0.15
Zel	2.5	38	0.15	2.1	33.5	0.14

BW: birth weight; MW: mature weight and WG: weight gain.

Table 10 Pearson's correlation coefficients showing the relationship between genotypes and phenotypic traits

Pearson		Rams			Ewes		
Locus	Genotype	BW	MW	WG	BW	MW	WG
*Msp*I	MM	-0.36	-0.58	-0.44	-0.31	-0.64	-0.50
	MN	+0.40	+0.40	+0.21	+0.35	+0.43	+0.23
	NN	+0.06	+0.23	+0.20	-0.02	+0.23	+0.17
*Nco*I	MM	-0.20	-0.21	-0.24	-0.22	-0.29	-0.16
	MN	+0.21	+0.20	+0.26	+0.23	+0.29	+0.16
	NN	-0.01	+0.09	-0.07	-0.01	+0.05	+0.01

BW: birth weight; MW: mature weight and WG: weight gain.
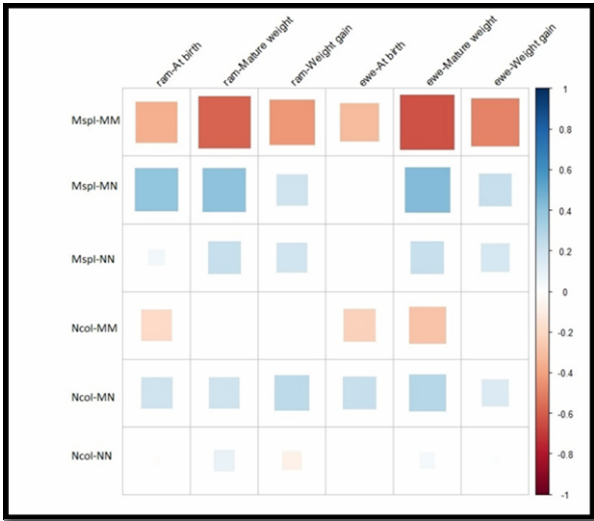
Figure 1 Pearson correlation results illustrating the relationship between *Msp*I and *Nco*I loci and weight traits in rams and ewes

Table 11 The *Msp*I polymorphisms observed in several studies across different sheep populations. Breeds are ranked from highest to lowest based on N allele frequency

Breed (n)	Genotypes and alleles					Reference
	N	M	MM	MN	NN	
Mehraban (25)	0.670	0.330	0.170	0.370	0.460	(Tohidi *et al.* 2013)
Sakiz (87)	0.655	0.345	0.092	0.506	0.402	(Yilmaz *et al.* 2014)
Bergamacia (50)	0.550	0.450	0.280	0.340	0.380	(Santos *et al.* 2016)
Arkhamerino (42)	0.520	0.480	0.280	0.470	0.250	(Tohidi *et al.* 2013)
Karya (90)	0.456	0.544	0.543	0.388	0.069	(Ata and Cemal, 2013)
Dalagh (110)	0.446	0.554	0.360	0.380	0.260	(Azari *et al.* 2012)
Ivesi (26)	0.410	0.590	0.500	0.190	0.310	(Balcioglu *et al.* 2014)
Awassi (95)	0.400	0.600	0.470	0.270	0.260	(Bayraktar, 2020)
Afshari (30)	0.370	0.630	0.540	0.170	0.290	(Tohidi *et al.* 2013)
Lori (100)	0.362	0.638	0.322	0.632	0.046	(Asadi *et al.* 2014)
Guney Karaman (23)	0.330	0.670	0.520	0.300	0.180	(Balcioglu *et al.* 2014)
Akkaraman (50)	0.320	0.680	0.440	0.540	0.020	(Kirikci, 2022)
Ghezel (65)	0.310	0.690	0.470	0.250	0.280	(Tohidi *et al.* 2013)
Akkaraman (21)	0.310	0.690	0.520	0.340	0.140	(Balcioglu *et al.* 2014)
Pantaneira (50)	0.300	0.700	0.580	0.230	0.190	(Santos *et al.* 2016)
Kivircik (25)	0.300	0.700	0.400	0.600	0.000	(Avanus, 2015)
Sanjabi (98)	0.280	0.720	0.540	0.370	0.090	(Tohidi *et al.* 2013)
Ascanian (30)	0.270	0.730	0.500	0.470	0.030	(Dimitrova *et al.* 2021)
NEBM (32)	0.270	0.730	0.470	0.530	0.000	(Bozhilova-Sakova *et al.* 2020)
Karakul (15)	0.267	0.733	0.467	0.533	0.000	(Avanus, 2015)
Cine Capari (97)	0.263	0.737	0.296	0.496	0.208	(Ata and Cemal, 2013)
Afshari (51)	0.260	0.740	0.570	0.330	0.100	(Nikmard *et al.* 2012)
Bandur (79)	0.260	0.740	0.540	0.390	0.070	(Sunilkumar, 2010)
Awassi (20)	0.250	0.750	0.600	0.300	0.100	(Dokgoz and Ozcan, 2024)
Red Karaman (14)	0.250	0.750	0.572	0.357	0.071	(Avanus, 2015)
Lori (37)	0.243	0.757	0.514	0.486	0.000	This Study
Nellore Palla (50)	0.240	0.760	0.560	0.400	0.040	(Ramadevi *et al.* 2020)
Polish Merino (82)	0.238	0.762	0.561	0.402	0.037	(Szkudlarek-Kowalczyk *et al.* 2011)
Sakiz (71)	0.230	0.770	0.590	0.360	0.050	(Dincel *et al.* 2015)
Rahmani (22)	0.227	0.773	0.545	0.455	0.000	(Othman *et al.* 2016)
Awassi (129)	0.220	0.780	0.700	0.160	0.140	(Bayraktar and Shoshin, 2022)
Zandi (100)	0.220	0.780	0.600	0.360	0.040	(Khederzadeh *et al.* 2016)
Norduz (102)	0.216	0.784	0.647	0.275	0.078	(Yilmaz *et al.* 2014)
Ascanian Merino (31)	0.210	0.790	0.610	0.350	0.040	(Bozhilova-Sakova and Dimitrova, 2021)
Karakul (100)	0.210	0.790	0.610	0.360	0.030	(Eftekhari Shahroudi *et al.* 2006)
Karacabey Merino (248)	0.200	0.800	0.669	0.262	0.069	(Yilmaz *et al.* 2014)
Dalagh crossbreed (120)	0.200	0.800	0.655	0.290	0.055	(Khederzadeh, 2011)
Zandi (124)	0.194	0.806	0.650	0.310	0.040	(Peirvisi *et al.* 2020)
Zandi (39)	0.192	0.808	0.641	0.333	0.026	This Study
Moghani (47)	0.191	0.809	0.660	0.298	0.042	This Study
Kajli (100)	0.190	0.810	0.680	0.260	0.060	(Suleman *et al.* 2012)
Nellore Brown (50)	0.190	0.810	0.640	0.340	0.020	(Ramadevi *et al.* 2020)
Assaf (117)	0.190	0.810	0.616	0.384	0.000	(Darissa and Irekat, 2021)
Black Headed Mutton (59)	0.186	0.814	0.712	0.203	0.085	(Szkudlarek-Kowalczyk *et al.* 2011)
Akkaraman (19)	0.180	0.820	0.630	0.370	0.000	(Dokgoz and Ozcan, 2024)
Ossimi (28)	0.179	0.821	0.643	0.357	0.000	(Othman *et al.* 2016)
AK (30)	0.174	0.826	0.674	0.304	0.022	(Iovenko *et al.* 2020)
Farahani (55)	0.173	0.827	0.691	0.273	0.036	This Study
Afshari (55)	0.173	0.827	0.673	0.309	0.018	This Study
Makooyi (41)	0.171	0.829	0.659	0.341	0.000	This Study
Prydniprovska (47)	0.170	0.830	0.770	0.130	0.100	(Pomitun *et al.* 2019)
Kalkooyi (47)	0.170	0.830	0.702	0.255	0.043	This Study
Nellore Jopidi (80)	0.170	0.830	0.680	0.310	0.010	(Ramadevi *et al.* 2020)
AFF (41)	0.167	0.833	0.667	0.333	0.000	(Iovenko *et al.* 2020)
Gray Shiraz (55)	0.164	0.836	0.691	0.291	0.018	This Study
West Siberian mutton (100)	0.163	0.837	0.720	0.230	0.050	(Afanasyeva *et al.* 2019)
Altai mountain (40)	0.160	0.840	0.720	0.230	0.050	(Selionova *et al.* 2020)

n: the number of investigated individuals.

MM: homozygous dominant; MN: heterozygous; NN: homozygous recessive; M: dominant allele; N: recessive allele and f: frequency.

Continued Table 11

Breed (n)	Genotypes and alleles					Reference
	N	M	MM	MN	NN	
Altai mountain (40)	0.160	0.840	0.720	0.230	0.050	(Selionova *et al.* 2020)
Kivircik (336)	0.153	0.847	0.729	0.235	0.036	(Yilmaz *et al.* 2014)
Shal (46)	0.152	0.848	0.761	0.174	0.065	This Study
NEBM (86)	0.150	0.850	0.720	0.260	0.020	(Bozhilova-Sakova and Dimitrova, 2021)
Arabic (111)	0.150	0.850	0.703	0.288	0.009	(Mohammadi *et al.* 2008)
Dalagh (30)	0.150	0.850	0.700	0.300	0.000	This Study
Nayini (49)	0.143	0.857	0.755	0.204	0.041	This Study
Karayaka (33)	0.140	0.860	0.850	0.030	0.120	(Balcioglu *et al.* 2014)
Awassi (40)	0.140	0.860	0.750	0.225	0.025	(Ibrahim and Kali, 2017)
Kajli (300)	0.140	0.860	0.740	0.240	0.020	(Saeed-ul-Hassan *et al.* 2012)
JASTRU (264)	0.140	0.860	0.750	0.230	0.020	(Sutikno *et al.* 2011)
Mehraban (55)	0.136	0.864	0.727	0.273	0.000	This Study
Mokaraman (34)	0.130	0.870	0.790	0.150	0.060	(Balcioglu *et al.* 2014)
Lohi (100)	0.130	0.870	0.770	0.200	0.030	(Suleman *et al.* 2012)
Caucasian (30)	0.130	0.870	0.730	0.270	0.000	(Dimitrova *et al.* 2021)
Barki (32)	0.125	0.875	0.750	0.250	0.000	(Othman *et al.* 2016)
Soviet Merino (72)	0.120	0.880	0.820	0.120	0.060	(Gorlov *et al.* 2016)
Ile de France (50)	0.120	0.880	0.820	0.120	0.060	(Santos *et al.* 2016)
Makui (32)	0.120	0.880	0.820	0.120	0.060	(Tohidi *et al.* 2013)
Suffolk (50)	0.120	0.880	0.800	0.160	0.040	(Santos *et al.* 2016)
SUE Malchyn (100)	0.120	0.880	0.770	0.220	0.010	(Kulikova *et al.* 2018)
Balkhi (300)	0.120	0.880	0.760	0.240	0.000	(Saeed-ul-Hassan *et al.* 2012)
Kurdi (100)	0.120	0.880	0.760	0.240	0.000	(Nassiry *et al.* 2007)
Breznik (30)	0.120	0.880	0.770	0.230	0.000	(Dimitrova *et al.* 2021)
Turk Ghashghayi (55)	0.118	0.882	0.800	0.164	0.036	This Study
Kivircik (153)	0.118	0.882	0.771	0.222	0.007	(Avanus, 2018)
Karakas (22)	0.110	0.890	0.820	0.140	0.040	(Balcioglu *et al.* 2014)
MUE Despen (51)	0.110	0.890	0.804	0.176	0.020	(Kulikova *et al.* 2018)
Salsk (108)	0.110	0.890	0.780	0.220	0.000	(Gorlov *et al.* 2016)
Sanjabi (23)	0.109	0.891	0.783	0.217	0.000	This Study
Hemsin (19)	0.105	0.895	0.842	0.105	0.053	(Avanus, 2015)
Kivircik x Merino (100)	0.100	0.900	0.820	0.160	0.020	(Kaplan and Atalay, 2017)
Akkaraman (374)	0.100	0.900	0.810	0.180	0.010	(Bayram *et al.* 2019)
Thalli (100)	0.100	0.900	0.800	0.200	0.000	(Suleman *et al.* 2012)
Taleshi (45)	0.100	0.900	0.800	0.200	0.000	This Study
Karayaka (22)	0.091	0.909	0.818	0.182	0.000	(Avanus, 2015)
SPBM (34)	0.090	0.910	0.820	0.180	0.000	(Bozhilova-Sakova *et al.* 2022)
Tsigai (58)	0.090	0.910	0.830	0.170	0.000	(Gabor *et al.* 2009)
OPC (300)	0.083	0.917	0.839	0.155	0.006	(Vergara *et al.* 2019)
SPBM (121)	0.080	0.920	0.840	0.150	0.010	(Georgieva *et al.* 2015)
Karayaka (105)	0.080	0.920	0.840	0.160	0.000	(Kirikci *et al.* 2021)
Kangal (31)	0.080	0.920	0.840	0.160	0.000	(Balcioglu *et al.* 2014)
Berrichon du Cher (41)	0.073	0.927	0.854	0.146	0.000	(Szkudlarek-Kowalczyk *et al.* 2011)
Pleven Blackhead (30)	0.070	0.930	0.870	0.130	0.000	(Dimitrova *et al.* 2021)
PDxNC (91)	0.060	0.940	0.879	0.121	0.000	(Nikolayevna *et al.* 2022)
Volgograd (131)	0.060	0.940	0.880	0.120	0.000	(Kolosov *et al.* 2021)
Karnobat Merino (35)	0.060	0.940	0.890	0.110	0.000	(Dimitrova *et al.* 2017)
Ile de France (30)	0.050	0.950	0.900	0.100	0.000	(Szkudlarek-Kowalczyk *et al.* 2011)
AFFT (21)	0.050	0.950	0.900	0.100	0.000	(Iovenko *et al.* 2020)
Zel (42)	0.048	0.952	0.905	0.095	0.000	This Study
AMW (34)	0.045	0.955	0.909	0.091	0.000	(Iovenko *et al.* 2020)
Imroz (27)	0.037	0.963	0.926	0.074	0.000	(Avanus, 2015)
Stara Zagora (48)	0.032	0.968	0.937	0.063	0.000	(Hristova *et al.* 2015)
Improved Valachian (19)	0.030	0.970	0.950	0.050	0.000	(Gabor *et al.* 2009)
Gokceada (49)	0.010	0.990	0.980	0.020	0.000	(Yilmaz *et al.* 2014)
Local Kamobat (48)	0.000	1.000	1.000	0.000	0.000	(Hristova *et al.* 2015)
Karakachan (25)	0.000	1.000	1.000	0.000	0.000	(Bozhilova-Sakova and Dimitrova, 2016)
Karakachan (13)	0.000	1.000	1.000	0.000	0.000	(Bozhilova-Sakova *et al.* 2022)
Cooper Red Shumen (30)	0.000	1.000	1.000	0.000	0.000	(Dimitrova *et al.* 2021)
CRS (13)	0.000	1.000	1.000	0.000	0.000	(Bozhilova-Sakova *et al.* 2022)
Lacaune (11)	0.000	1.000	1.000	0.000	0.000	(Gabor *et al.* 2009)

n: the number of investigated individuals.

Table 12 The *Nco*I polymorphisms observed in several studies across different sheep populations. Breeds are ranked from highest to lowest based on N allele frequency

Breed (n)	Genotypes and alleles					Reference
	N	M	MM	MN	NN	
Bandur (38)	0.303	0.697	0.500	0.395	0.105	(Sunilkumar, 2010)
Zandi (39)	0.192	0.808	0.641	0.333	0.026	This Study
Lori (37)	0.189	0.811	0.622	0.378	0.000	This Study
BlackHeaded Mutton (59)	0.153	0.847	0.712	0.271	0.017	(Szkudlarek-Kowalczyk *et al.* 2011)
Moghani (47)	0.149	0.851	0.745	0.213	0.042	This Study
Kalkooyi (47)	0.149	0.851	0.702	0.298	0.000	This Study
Kordi (100)	0.120	0.880	0.760	0.240	0.000	(Nassiry *et al.* 2007)
Turk Ghashghayi (55)	0.118	0.882	0.800	0.164	0.036	This Study
Mehraban (55)	0.118	0.882	0.764	0.236	0.000	This Study
Gray Shiraz (55)	0.109	0.891	0.800	0.182	0.018	This Study
Afshari (55)	0.100	0.900	0.800	0.200	0.000	This Study
Dalagh (30)	0.100	0.900	0.800	0.200	0.000	This Study
Makooyi (41)	0.098	0.902	0.805	0.195	0.000	This Study
Sanjabi (23)	0.087	0.913	0.826	0.174	0.000	This Study
Akkaraman (31)	0.080	0.920	0.839	0.161	0.000	(Karsli and Demir, 2024)
Taleshi (45)	0.067	0.933	0.867	0.133	0.000	This Study
Farahani (55)	0.064	0.936	0.873	0.127	0.000	This Study
Nayini (49)	0.061	0.939	0.878	0.122	0.000	This Study
Karakul (100)	0.060	0.940	0.880	0.120	0.000	(Aslaminejad *et al.* 2006)
Anatolian Merino (37)	0.040	0.960	0.919	0.081	0.000	(Karsli and Demir, 2024)
JASTRU (264)	0.040	0.960	0.920	0.080	0.000	(Sutikno *et al.* 2011)
Shal (46)	0.033	0.967	0.935	0.065	0.000	This Study
Zel (42)	0.024	0.976	0.952	0.048	0.000	This Study
Kivircik (153)	0.013	0.987	0.974	0.026	0.000	(Avanus, 2018)
Polish Merino (82)	0.000	1.000	1.000	0.000	0.000	(Szkudlarek-Kowalczyk *et al.* 2011)
Berrichon du Cher (41)	0.000	1.000	1.000	0.000	0.000	(Szkudlarek-Kowalczyk *et al.* 2011)
Ile de France (30)	0.000	1.000	1.000	0.000	0.000	(Szkudlarek-Kowalczyk *et al.* 2011)

n: the number of investigated individuals.
MM: homozygous dominant; MN: heterozygous; NN: homozygous recessive; M: dominant allele; N: recessive allele and f: frequency.

Table 13 Association of two mutations and the six identified local haplotypes in different sheep breeds

Haplotypes	1	2	3	4	5	6	Linkage (%)	Number of groups (haplotype)
Position	*Msp*I/*Nco*I	*Msp*I/*Nco*I	*Msp*I/*Nco*I	*Msp*I/*Nco*I	*Msp*I/*Nco*I	*Msp*I/*Nco*I		
Breed(n)[1]/Genotype[2]	MM/MM	MN/MN	MN/MM	NN/MN	NN/NN	NN/MM		
Dalagh (30)	21 (70.0%)	6 (20.0%)	3 (10.0%)	0	0	0	66.7	3 (1, 2, 3, -, -, -)
Lori (37)	19 (51.4%)	14 (37.8%)	4 (10.8%)	0	0	0	77.8	3 (1, 2, 3, -, -, -)
Makooyi (41)	27 (65.9%)	8 (19.5%)	6 (14.6%)	0	0	0	57.1	3 (1, 2, 3, -, -, -)
Mehraban (55)	40 (72.7%)	13 (23.6%)	2 (3.7%)	0	0	0	86.7	3 (1, 2, 3, -, -, -)
Sanjabi (23)	18 (78.3%)	4 (17.4%)	1 (4.3%)	0	0	0	80.0	3 (1, 2, 3, -, -, -)
Taleshi (45)	36 (80.0%)	6 (13.3%)	3 (6.7%)	0	0	0	66.7	3 (1, 2, 3, -, -, -)
Zel (42)	38 (90.4%)	2 (4.8%)	2 (4.8%)	0	0	0	50.0	3 (1, 2, 3, -, -, -)
Kalkooyi (47)	33 (70.2%)	12 (25.5%)	0	2 (4.3%)	0	0	100.0	3 (1, 2, -, 4, -, -)
Turk Ghashghayi (55)	44 (80.0%)	9 (16.4%)	0	0	2 (3.6%)	0	100.0	3 (1, 2, -, -, 5, -)
Zandi (39)	25 (64.1%)	13 (33.3%)	0	0	1 (2.6%)	0	100.0	3 (1, 2, -, -, 5, -)
Gray Shiraz (55)	38 (69.1%)	10 (18.2%)	6 (10.9%)	0	1 (1.8%)	0	64.7	4 (1, 2, 3, -, 5, -)
Moghani (47)	31 (66.0%)	10 (21.3%)	4 (8.5%)	0	2 (4.2%)	0	75.0	4 (1, 2, 3, -, 5, -)
Afshari (55)	37 (67.3%)	11 (20.0%)	6 (10.9%)	0	0	1 (1.8%)	61.1	4 (1, 2, 3, -, -, 6)
Farahani (55)	38 (69.1%)	6 (10.9%)	9 (16.4%)	1 (1.8%)	0	1 (1.8%)	41.2	5 (1, 2, 3, 4, -, 6)
Nayini (49)	37 (75.6%)	5 (10.2%)	5 (10.2%)	1 (2.0%)	0	1 (2.0%)	50.0	5 (1, 2, 3, 4, -, 6)
Shal (46)	35 (76.1%)	1 (2.2%)	7 (15.2%)	2 (4.3%)	0	1 (2.2%)	27.3	5 (1, 2, 3, 4, -, 6)
Total (721)	**517 (71.7%)**	**130 (18.1%)**	**58 (8.0%)**	**6 (0.8%)**	**6 (0.8%)**	**4 (0.6%)**	**69.0**	**6 (1, 2, 3, 4, 5, 6)**

n: the number of investigated individuals.
MM: homozygous dominant; MN: heterozygous and NN: homozygous recessive.

Also polymorphisms in calpastatin have been associated with some traits such as growth, body size and weaning weight in Santa Ines sheep (Machado *et al.* 2020), and body weight and chest circumference in Chinese Tibetan sheep (Gao *et al.* 2023).

Other studies also confirm the association of calpastatin genotypes with physical traits such as daily body weight gain, fat percentage, and muscle percentage in thin tail sheep (Dagong *et al.* 2016), synthetic lines: BCP and SCP (Gregula-kania *et al.* 2019) and Batur sheep (Haren *et al.* 2022).

All these findings underscore the important role of the calpastatin gene in regulating traits such as growth rate, carcass quality, and meat tenderness in sheep, highlighting the potential for utilizing these genetic markers in breeding programs aimed at improving meat production traits.

In this study, we investigated the linkage between two SNPs at the *Msp*I and *Nco*I loci in different sheep breeds. The results showed a varying degree of linkage between the two SNPs, with linkage percentages ranging from 27.3% in the Shal breed to 100% in the Kalkooyi, Turk Ghashghayi, and Zandi breeds (Table 13).

This variation in linkage percentages may be attributed to the differences in allele frequencies and genetic variation among the breeds. Across all breeds, six distinct haplotypes were observed with varying frequencies. The number of haplotypes per breed ranged from three to five, with the majority of breeds exhibiting three haplotypes. For instance, the Zel, Taleshi and Turk Ghashghayi breeds each showed three haplotypes, with the most frequent genotype being MM/MM, which suggests a high degree of homozygosity for the M allele at both loci in these breeds (90.4%, 80.0% and 80.0%, respectively). This observation aligns with the higher linkage percentages in these breeds, indicating strong genetic cohesion between the SNPs. The Kalkooyi, Turk Ghashghayi, and Zandi breeds exhibited perfect linkage (100%) between the *Msp*I and *Nco*I loci, which suggests a consistent genetic pattern across individuals within these populations. This could imply that these breeds are genetically more stable or have undergone selective breeding that favors certain allelic combinations at the *Msp*I and *Nco*I loci. On the other hand, the Shal breed exhibited the lowest linkage percentage (27.3%) and showed a more diverse haplotype distribution. This low linkage could suggest that the *Msp*I and *Nco*I loci in the Shal breed are less likely to be inherited together, possibly due to greater genetic diversity or the presence of recombination events between the two loci. Furthermore, the overall analysis of the haplotypes across all 721 individuals revealed the most frequent haplotype combination to be "MM/MM," with the majority of individuals carrying these two genotypes. This suggests that these genotypes are likely to be more preva-

lent across the sheep population as a whole, supporting the idea that these two genotypes may not play a significant role in the genetic structure of the studied breeds. The differences in linkage percentages observed across breeds suggest that the *Msp*I and *Nco*I loci may be subject to selective forces, which could have implications for breed management, conservation, and breeding programs.

CONCLUSION

This study highlights the significant role of the CAST gene polymorphisms at the *Msp*I and *Nco*I loci in influencing key traits such as body size and weight gain in Iranian sheep breeds. The observed variation in allele frequencies across different breeds, along with correlations between specific genotypes and weight traits, suggests that these mutations could serve as valuable genetic markers for improving meat production in breeding programs. The results also underscore the importance of regional and breed-specific genetic diversity, emphasizing the need for targeted breeding strategies to enhance productivity in sheep populations. Further studies are required to confirm these findings and explore the broader genetic implications of the CAST gene in sheep.

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