Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

The effect of calpastatin gene on meat quality traits in Turkish sheep breeds

V.K. Esen

Department of Breeding and Genetics, Sheep Breeding Research Institute, Balikesir, Turkiye

Article information

Article history:
Received 07 April, 2024
Accepted 07 June, 2024
Published online 18 September, 2024

Keywords:
Calpastatin
Ultrasound measurements
Live weight
Lamb

Correspondence:
V.K. Esen
vasfiye.esen@gmail.com

Abstract

This research aimed to examine the effects of haplotype groups observed in three different loci on the Calpastatin gene on ultrasonographic MLD measurements and seasonal live weight in five different types of sheep (GBK, HM, K, KM, and R). In the CAST intron 1, intron 5, and intron 12 regions, 15 SNPs were found. The HWE p-value for SNP2, SNP3, SNP5, SNP6, SNP7, SNP8, and SNP14 is less than 0.05, and except SNP9 and SNP10, all SNPs have a MAF of more than 0.01. SNP1, SNP2, and SNP7 made up one haplotype block. The haploblock has 3 haplogroups. The most common haplotype group was H1 (-AGG-), which had a frequency of 0.52; H2 (-TGG-) and H3 (-TAA-) had rates of 0.35 and 0.13, respectively. Based on ultrasonographic MLD readings and live weights, there were no statistically significant differences between haplotype H1 and H3, but there were statistically significant differences between haplotype H2 lambs. The effect of the H2 haplotype on 90-day MLD depth revealed a statistically significant difference between the HM and KM and K and KM breeds. This distinction persisted until the 180th day of life before disappearing into adulthood. Similarly, the effect of H2 haplotype on the skin thickness at day 90 was significant between K and KM and between K and R, whereas the effect of H2 haplotype on fat thickness demonstrated a substantial difference between HM and KM at one year of age.

DOI: 10.33899/ijvs.2024.148563.3599,

Introduction

Sheep farming assumes a pivotal role in the socioeconomic landscape, particularly within developing countries, by simultaneously addressing nutritional, economic, and sociocultural dimensions. It is a reliable source of high-quality sustenance, fosters income growth, and promotes societal inclusivity (1,2). Within such contexts, expeditiously optimizing these multifaceted benefits emerges as an imperative. To expedite the realization of these objectives, marker-assisted selection (MAS) programs have emerged as a prominent and widely implemented methodology. Leveraging MAS programs, livestock breeders can effectively augment livestock metrics, including live weight gain and meat quality (3-5). Notably, contemporary selection programs emphasize the effects of candidate genes employed in MAS programs regarding meat tenderness and composition, reflecting a strategic focus aligned with the heightened consumer demand for premium meat products (6,7). This strategic orientation not only underpins the economic prosperity of sheep farming communities but also satisfies the discerning preferences of consumers. On the other hand, Calpastatin merits particular attention due to its vital contributions in determining the quantity and quality of meat (8). Calpastatin, a cellular protein inhibiting calpains (Ca^{2+}-dependent cysteine proteinases) involved in diverse cellular processes such as cytoskeleton modulation, cell migration, cell cycle progression, and apoptosis, plays a crucial role in the calpain-calpastatin system governing protein turnover,

growth, myoblast migration, myoblast fusion, and meat tenderness; given its influence on these activities and its potential impact on meat quality, the calpastatin (CAST) gene emerges as a promising candidate gene for elucidating variations in meat traits (9-11). It has been shown that calpains are crucial to the breakdown of myofibrillar proteins in living muscle tissues and play a major role in postmortem proteolysis, a biochemical process responsible for meat tenderization (12-14). Thus, Calpastatin acts as an endogenous inhibitor of calpains, influencing both the rate and extent of postmortem tenderization (11,15,16). More precisely, the augmentation in skeletal muscle growth can be attributed to reduced muscle protein degradation. This decrease is linked to lower calpain activity, which elevated calpastatin levels facilitate (17). Additionally, prior studies have indicated that elevated calpastatin activity within living cells impedes calpains' capacity to degrade myofibrillar proteins during postmortem storage (18,19). The CAST gene, localized at the 5q15 locus on chromosome 5 of the sheep genome and comprising 29 exons, exhibits polymorphism across numerous sheep breeds. Research on livestock species such as pigs, cattle, sheep, and goats has revealed the significant impact of various polymorphisms within the CAST gene. These polymorphisms influence weight gain, carcass quality, and meat quality, particularly tenderness, highlighting their substantial role in animal production and meat processing (20-24). These researches have demonstrated the significant influence of the CAST gene on growth, attributed to its capacity to promote muscle fiber proliferation. Specifically, it has documented its impact on the birth weight and growth rate of Romney sheep (10) until weaning and its influence on post-weaning weight and daily weight in Targhee sheep (25). These findings underscore the critical importance of assessing the varied impacts of the CAST locus and its polymorphisms on a range of traits throughout different developmental stages. Therefore, evaluating the effects of the CAST locus and its polymorphisms on various traits at different stages is crucial, and a comprehensive understanding of their potential effects from birth to adulthood before integrating them into MAS programs can significantly improve the efficiency of selection processes. Nevertheless, ultrasound technology, designed for evaluating the composition and quality of animal carcasses intended for market, facilitates swift and cost-effective assessment of carcass properties in live animals without causing harm (26-28). Utilizing ultrasound measurements in live animals holds practical significance, enabling the selection of particular carcass traits based on measurement criteria for breeding purposes and predicting the optimal timing for slaughtering or marketing (29-31). Previous studies have identified polymorphic variants in intron 1 (32), intron 5 (14), and intron12 (24,33) of the CAST gene in sheep; however, the relationship between haplotypic diversity and live weight, as well as ultrasonographic muscle measurements, has not been explored.

Therefore, the current study aims to fill this gap by investigating single nucleotide polymorphisms (SNPs) in these regions within selected meat-type sheep breeds in Turkey and determining the associations between haplotypes and live weight and ultrasonographic muscle measurements collected at various time points.

Materials and methods

Ethical approve

The Ethics Committee of the Sheep Breeding Research Institute in Türkiye (approval number: 13360037) granted consent for all animal trials on April 11, 2018. This research was conducted at the Bandirma Sheep Breeding Research Institute, Balikesir, Türkiye. Lambs used in this study were sourced from the institute's farm and constituted the primary animal material for our research endeavors.

Animals and DNA isolation

The study specifically focused on lambs born within the 2018 lambing season and restricted the inclusion criteria to those born within a 10-day window after the lambing season. In total, the study encompassed 202 lambs, encompassing diverse breeds such as German Black-Head Mutton × Kivircik (GBK), Hampshire Down × Merino (HM), Kivircik (K), Karacabey Merino (KM), and Ramlic (R). It is pertinent to note that our prior investigations have extensively documented these animals, offering comprehensive insights into their care and feeding regimens. As elucidated by Kader Esen (3) and Kader Esen (2), these details provided a foundational understanding of the subjects under scrutiny. The methodology involved the collection of blood samples from the lamb's Vena jugularis, ensuring meticulous preservation in 10 ml EDTA tubes to obtain high-quality genomic DNA. These samples were then stored at -20 °C until the subsequent DNA extraction process was executed with precision and accuracy.

Genetic analyses and identification of SNPs

DNA extraction from the samples followed the protocols outlined in the GeneAll® kit. Specific primers were designed to target three distinct regions with lengths of 565, 254, and 448 base pairs to investigate the CAST gene. The amplification process was conducted in a 20 µL reaction mixture containing DNA and each primer at a concentration of 100 ng using a commercially available kit. Primers for amplifying Intron 1 were described by Khederzadeh (17), while those for Intron 5 and Intron 12 were sourced from the work of Byun (10). The detailed polymerase chain reaction (PCR) conditions are provided in table 1.

Following the Sanger sequencing method, PCR products underwent sequencing using the ABI3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Geospiza's FinchTV software (Version 1.4) was employed to visualize and scrutinize the sequence of chromatograms. The obtained

sequences were meticulously deposited in the GenBank database with the following accession numbers: OP620911, OP620912, OP620913, OQ513936, and OQ513937. To discern the genetic variations, the DNA sequences obtained in this study were systematically compared with the reference sheep genome (Oar_v3.1) sourced from the Ensembl Genome Database, thereby facilitating the precise identification of SNP positions.

Table 1: PCR conditions for CAST loci

Reaction phase	Intron 1			Intron 5			Intron 12		
	H	T	C	H	T	C	H	T	C
First denaturation	95	5	1	94	2	1	94	2	1
Denaturation	94	1		94	0,5		94	0,5	
Annealing	51	1	33	55	0,5	35	55	0,5	35
Extension	72	2		72	0,5		72	0,5	
Final extension	72	8	1	72	5	1	72	5	1

H: Heat (°C); T: Time (min); C: Cycle.

Live weight and ultrasonographic muscle measurements

In the study's initial stages, lambs' birth weights were meticulously documented within the first 12 hours postpartum. Subsequent assessments involved carefully recording live weights (LW) and precise ultrasonographic measurements on the research period's 90^{th}, 180^{th}, and 360^{th} days. To ensure accuracy and reliability, lambs were weighed before their morning feeding, thus mitigating potential inaccuracies arising from the presence of stomach content. The ultrasonographic evaluations were conducted by a skilled technician employing a real-time ultrasound system (Mindray DP-20) integrated with a linear veterinary ultrasound transducer (Mindray 75L50EAV) operating at a frequency of 7.5 MHz, as detailed in the work of Esen (34). The ultrasonographic analysis focused on monitoring the Musculus longissimus dorsi depth (MLDD), fat thickness (FT), and skin thickness (ST) located between the 12th and 13th ribs. These assessments were conducted after recording live weights at predetermined intervals, as stipulated in the research protocol elucidated by Kader Esen and Elmaci (1).

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was assessed for each SNP by comparing observed (Het_{Ob}**)** and predicted (Het_{Pre}) heterozygosities. A threshold of 5 percent was defined for HWE. Haploview software (Version 4.2) was utilized to ascertain haplotypes and assess the linkage disequilibrium (LD) between SNPs. SNPs were considered eligible for inclusion in the linkage disequilibrium analysis if they exhibited a p-value greater than 0.05 in the HWE test and possessed a minor allele frequency (MAF) of at least 1% (27). The dataset underwent rigorous analysis to explore the relationship between the response variables (LW, MLDD, FT, and ST) and various explanatory factors, including breed, gender, birth type, dam age, and haplotype. An analysis of variance (ANOVA) was performed using a mixed model approach. This method, executed in the R programming language using the 'lmer' function from the 'lme4' package, was chosen to account for potential data correlations arising from the hierarchical structure. Post hoc analysis was conducted using Tukey's test on the mixed model to discern differences among the variables (35).

Results

Four lambs were omitted from the study due to indistinct genotyping results, ensuring the integrity of the dataset. Fifteen distinct SNPs were identified within three specific regions of the CAST gene, as depicted in figure 1. Notably, SNPs 1 to 8 were located in intron 1, 9 to 11 were in intron 5, and SNPs 12 to 15 were positioned in intron 12. Seven SNPs detected in our study had been previously documented in the reference genome (Sheep_texel Oar_v3.1), underlining their relevance and consistency with existing genetic data.

Genetic parameters, including observed heterozygosity, predicted heterozygosity, and the assessment of HWE, were calculated for all SNPs, as detailed in table 2. Notably, HWE was only observed for some SNPs under consideration. Specifically, SNPs 1, 9, 10, 11, 12, 13, and 15 exhibited p-values exceeding 0.05. Additionally, except for SNPs 9 and 10, the minimal allelic frequencies of these SNPs were more significant than 0.01.

SNP3, SNP4, SNP5, SNP6, SNP8, and SNP14 displayed subpar performance and failed in one or more tests, as delineated in figure 2. Examination of linkage disequilibrium (LD) coefficients, encompassing D' and r^2 values, demonstrated strong genetic associations between SNP1 and SNP2 (D'= 1.0, LOD=20.61, r^2=0.17), SNP2 and SNP7 (D'= 1.0, LOD=57.03, r^2=1.0), SNP9 and SNP10 (D'= 1.0, LOD=4.95, r^2=1.0), SNP12 and SNP13 (D'= 1.0, LOD=22.73, r^2=1.0), and SNP13 and SNP15 (D'= 1.0, LOD=22.73, r^2=1.0). Conversely, a weak linkage was observed between SNP10, SNP11, and SNP12 (LOD<2). It is important to note that, despite their high LOD scores represented by red diamonds, not all of these SNPs formed haplotype blocks due to their placement outside Gabriel's confidence interval, as depicted in figure 2. Specifically, a

haplotype block was established by SNP1, SNP2, and SNP7. Through haplotype analysis, three distinct haplotype groups were identified within the population, each with frequencies exceeding 1%. The H1 (-AGG-) haplotype group was prevalent, with a frequency of 0.52, while the H2 (-TGG-) and H3 (-TAA-) haplotype groups exhibited frequencies of 0.35 and 0.13, respectively.

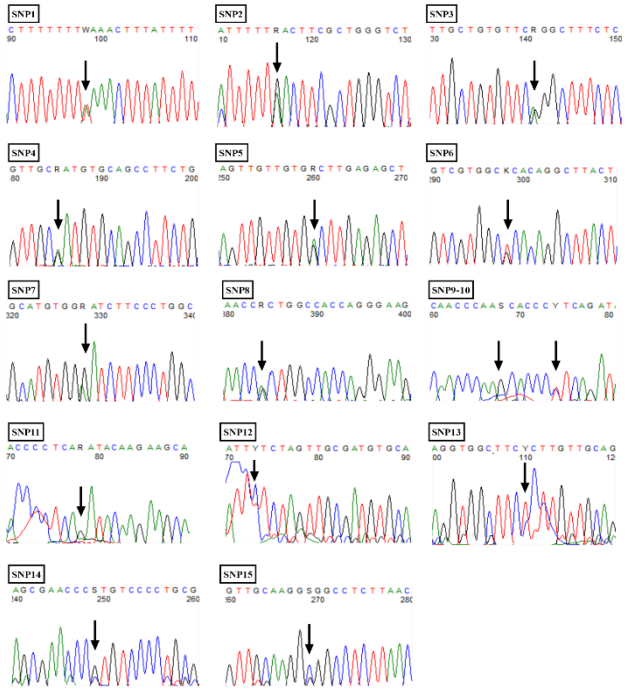

Figure 1: Nucleotide variants in introns 1, 5, and 12 of the CAST gene.

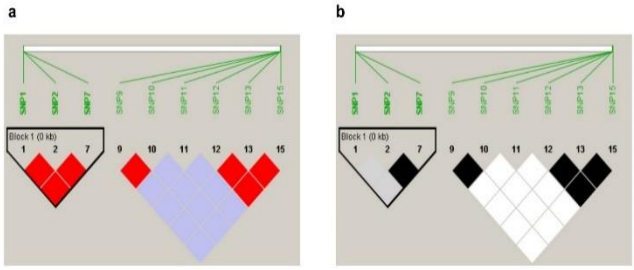

Figure 2: Linkage disequilibrium (LD) plot of calpastatin SNPs. The D' coefficient is depicted in graph (a), whereas graph (b) represents the r^2 coefficient. LD is represented through standard color codes: red signifies strong LD with LOD > 2 and D′ = 1), blue denotes intermediate LD with LOD < 2 and D′ = 1, while white signifies no LD with LOD < 2 and D′ <1.

Table 3 presents the findings on the effect of CAST haplotypes on lambs' live weight and ultrasonographic muscle features. Neither live weight nor ultrasound measurements were statistically significant among the three haplotype groups (P>0.05). Although there were no statistically significant differences between haplotypes in LW and MLDD, notable patterns were observed. Compared to other haplotype groups, lambs of haplotype H2 had higher birth weights and adult weights. Furthermore, lambs in the H1 haplotype group exhibited higher MLDD at both weaning (LW90) and 180 days, while no significant differences were observed in ST and FT among the haplotypes.

Table 2: Hardy-Weinberg equilibrium, minor allele frequency, and heterozygosity of CAST SNPs in meat-type sheep breeds

SNP #	Chromosome Location	rs ID	Alleles	Het_{Ob}	Het_{Pre}	HWE	MAF
SNP1	5:93448534	rs421197310	A: T	0.432	0.499	0.0569	0.480
SNP2	5:93448548	-	G: A	0.264	0.229	0.0261	0.132
SNP3	5:93448577	rs399966367	A: G	0.432	0.339	4.1339E-6	0.216
SNP4	5:93448621	rs407174907	G: A	0.432	0.339	4.1339E-6	0.216
SNP5	5:93448696	rs412475054	G: A	0.432	0.339	4.1339E-6	0.216
SNP6	5:93448734	rs398259427	G: T	0.432	0.339	4.1339E-6	0.216
SNP7	5:93448760	-	G: A	0.264	0.229	0.0261	0.132
SNP8	5:93448820	rs161885148	A: G	0.432	0.339	4.1339E-6	0.216
SNP9	5:102025584	-	G: C	0.009	0.009	1.0	0.005
SNP10	5:102025590	-	C: T	0.009	0.009	1.0	0.005
SNP11	5:102025594	-	G: A	0.027	0.027	1.0	0.014
SNP12	5:102036450	-	C: T	0.064	0.062	1.0	0.032
SNP13	5:102036487	-	T: C	0.064	0.062	1.0	0.032
SNP14	5:102036502	rs422618244	G: C	0.936	0.498	2.1698E-47	0.468
SNP15	5:102036646	-	C: G	0.064	0.062	1.0	0.032

Het_{Ob}: observed heterozygosity; Het_{Pre}: predicted heterozygosity; HWE: Hardy-Weinberg equilibrium p-value; MAF: minor allele frequency.

Table 3: Effects of CAST haplotypes on live weight and ultrasonographic muscle measurements

Trait	Day	N	H1 (-AGG-)		N	H2 (-TGG-)		N	H3 (-TAA-)		P value
			Mean	SE		Mean	SE		Mean	SE	
LW	0	66	4.27	0.25	83	4.51	0.14	49	4.29	0.19	NS
	90	66	32.87	1.36	83	30.52	0.76	49	31.74	1,04	NS
	180	58	41.16	2.12	66	40.93	1.08	24	40.90	1.88	NS
	360	57	58.14	2.80	63	62.87	1.33	21	62.49	2.55	NS
MLDD	90	66	2.37	0.14	83	2.34	0.08	49	2.33	0.11	NS
	180	58	2.63	0.14	66	2.39	0.06	24	2.24	0.12	NS
	360	57	2.79	0.17	63	2.96	0.08	21	2.76	0.15	NS
FT	90	66	0.45	0.05	83	0.38	0.03	49	0.50	0.04	NS
	180	58	0.43	0.06	66	0.39	0.03	24	0.36	0.05	NS
	360	57	0.38	0.07	63	0.46	0.03	21	0.49	0.06	NS
ST	90	66	0.22	0.01	83	0.23	0.01	49	0.21	0.01	NS
	180	58	0.18	0.02	66	0.18	0.01	24	0.16	0.01	NS
	360	57	0.23	0.03	63	0.22	0.01	21	0.25	0.03	NS

LW: live weight; MLDD: Musculus longissimus dorsi depth; FT: fat thickness; ST: skin thickness; N: sample size; SE: standard error of the mean.

Figure 3 illustrates the effect of CAST haplotypes on LW at various time intervals in meat-type sheep breeds. Male lambs were absent in the studied populations from both H1 and H2 groups. Furthermore, the absence of H1 and H3 haplotypes in HM and R breeds and the complete lack of H3 haplotypes in the R breed was notable. Similarly, GBK breeds displayed a complete absence of H2 haplotypes. While H1 and H3 haplotypes demonstrated no significant influence on the breeds, the H2 haplotype emerged as a pivotal factor. Significant birth weight differences were observed between KM and R (P<0.05). During the weaning period, substantial variations were identified among K and KM (P<0.05), K and R (P<0.001), and KM and R (P<0.05) H2 haplotype lambs. By the 180th day, noteworthy differences in LW were evident between HM and R (P<0.01), K and R (P<0.01), and KM and R (P<0.01) H2 haplotype lambs. Moreover, on the 360th day, notable differences in the live weights of H2 haplotype lambs were observed, with significant distinctions between HM and R (P<0.05) and KM and R (P<0.01).

Figure 4 depicts the effects of CAST haplotypes on ultrasonographic muscle measurements in meat-type sheep breeds at different time intervals. Particularly, on the 90th day, noteworthy distinctions in MLDD were evident among lambs with H2 haplotypes, with significant differences observed between HM and K (P<0.05) and HM and R (P<0.05). Moreover, substantial differences in ST were noted among H2 haplotype lambs, with significant disparities between K and KM lambs (P<0.01) and K and R lambs (P<0.05). Notably, K lambs exhibited greater ST than their counterparts, while the influence of CAST haplotypes on FT on the 90th day did not yield a statistically significant difference. Concerning the impact of H2 haplotype in lambs on MLDD on the 180th day, significant differences were noted between HM and K lambs (P<0.05) and between KM and K lambs (P<0.05). Likewise, the impact of the H2 haplotype on ST on the 180th day showed statistical significance, specifically between K and R lambs (P<0.05). In contrast, no statistically significant FT differences existed among haplotype groups at 180 days (P>0.05). In adulthood (on the 360th day), there were no statistically significant differences in MLDD or ST among the haplotype groups (P>0.05). However, in H2 haplotype lambs, a significant difference was observed between HM and K (P<0.05) as well as between K and R (P<0.05) in terms of FT.

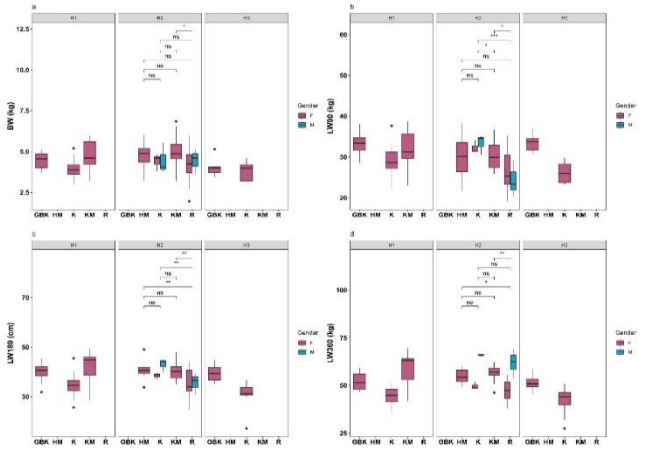

Figure 3: Effects of CAST haplotypes on live weights over time in meat-type sheep breeds. BW: birth weight; LW90: live weight on the 90th day LW180: live weight on the 180th day; LW360: live weight on the 360th day; F: female; M: male; GBK: German Black-Head Mutton × Kivircik; HM: Hampshire Down × Merino; K: Kivircik; KM: Karacabey Merino; R: Ramlic; ns: not significant; *: P<0.05; **: P<0.01; ***: P<0.001.

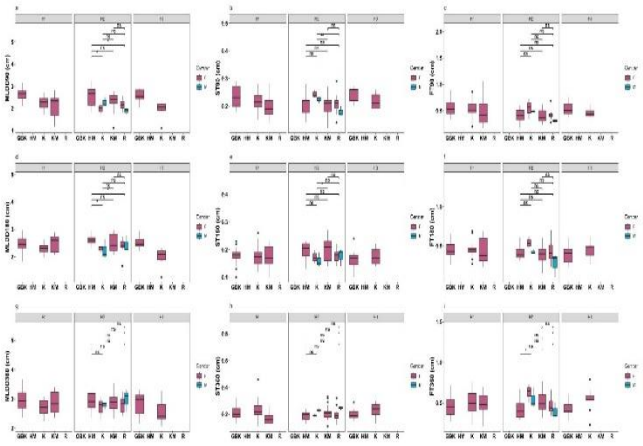

Figure 4: Effects of CAST haplotypes on ultrasonographic muscle measurements over time in meat-type sheep breeds MLDD: Musculus longissimus dorsi depth; ST: skin thickness; FT: fat thickness; F: female; M: male; GBK: German Black-Head Mutton × Kivircik; HM: Hampshire Down × Merino; K: Kivircik; KM: Karacabey Merino; R: Ramlic; ns: not significant; *: P<0.05; **: P<0.01; ***: P<0.001.

Discussion

The effectiveness of MAS lies in its ability to curtail the generation interval, thereby expediting genetic progress and facilitating targeted improvements (15,36,37). Notably, previous research has underscored the pivotal role of the CAST gene as a prime candidate in meat quality selection initiatives. This importance is attributed to inhibiting the calpain system, a vital mechanism governing muscle development, growth, and postmortem meat tenderness (10,38,39). Combining this gene with ultrasonographic muscle measurements has proven to enhance the precision of genetic parameters, indicating its potential in genetic selection strategies. Various studies have elucidated genetic variations within the CAST gene, spanning both coding and non-coding regions across diverse sheep breeds (11,32,40). Integrating this gene with ultrasonographic muscle measurements significantly augments the accuracy of genetic parameters, underscoring its substantial promise in genetic selection tactics (26,28).

Prior research has established that introns can influence mRNA stability and transcriptional efficiency, eliciting distinct biological effects on genes (41). Recent research has revealed that introns also significantly impact growth, carcass, and meat quality traits in sheep and cattle, with specific effects on genes (42,43). The current study focused on the intron 1, 5, and 12 regions of the CAST gene, believed to influence LW and ultrasonographic muscle measurements, to identify SNPs within these critical regions. A comprehensive analysis uncovered 15 SNPs in total: 8 within intron 1 (SNP 1 to 8), three within intron 5 (SNP 9 to 11), and four within intron 12 (SNP 12 to 15). Notably, seven of these SNPs identified in the current study had been previously documented in existing literature, highlighting their significance in genetic research (rs421197310, rs399966367, rs407174907, rs412475054, rs398259427, rs161885148, and rs422618244). Numerous SNPs within the ovine CAST gene have been extensively explored in prior studies, aligning with the present investigation's outcomes. Notably, Roberts (43) delineated nine SNPs within intron 12, forming distinctive haplotypes, a discovery later corroborated by Byun (33), who identified a novel haplotype encompassing previously reported ones. The genetic variations within intron 12, as highlighted by Greguła-Kania (44), exhibited robust correlations with growth rates, underscoring the genetic significance in the context of ovine development. Furthermore, Palmer (40) revealed three unique haplotypes within the intron region spanning exons 1C and 1D, marked by nine SNPs, and established their significant relationships with lamb growth and meat tenderness. In a parallel vein, Chung and Davis (25) made a groundbreaking discovery of a novel SNP (A/G) within intron 25, establishing significant associations with birth weight and average daily gain, illuminating critical genetic determinants of these traits. Moreover, Esteves (43) identified specific SNPs (c.679A>G; c.383A>G) within the CAST gene, leading to the substitution of glutamic acid with glycine and threonine with alanine, profoundly impacting pH values. However, in contrast, Zhou (45) found no significant links between tenderness in un-aged lamb and CAST haplotypes or genotypes within the region encompassing exon six and partial introns 5 and 6, emphasizing the nuanced nature of genetic associations in this specific genomic area.

The intricate process of muscle growth and development is significantly influenced by the regulation of new protein degradation and synthesis within the calpain-calpastatin system. The suppression of CAST leads to increased μ-calpain expression and controlling cell proliferation, survival, and apoptotic pathways, as demonstrated by Van Ba *et al.* (46), and it also results in reduced calpain activity. This reduction in calpain activity, in turn, decreases muscle fiber breakdown, thereby facilitating muscle mass accumulation. In this context, LW, employed to evaluate body growth and partial development, plays a crucial role, as highlighted by Greguła-Kania (44). Furthermore, grazing Texel ewes have observed an established association between an SNP in the CAST gene and birth weight and growth rate (6). Notably, the influence of the A allele on birth weight and pre-weaning daily gain was particularly discerned in animals of the simple lambing type among Romney lambs (10).

Furthermore, Chung and Davis (25) highlighted the influence of the CAST gene on average daily gain and post-weaning weight in Targhee sheep. This study observed no significant impact of CAST gene haplotypes on birth weight

and LW values recorded at various intervals. This outcome distinguishes the present study from previous research in this particular aspect. Nevertheless, the obtained results align with the findings reported by Nikmard (47), where no significant relationship was observed between SNPs and metrics such as birth weight, weaning weight, weight at 6 and 9 months, as well as pre-and post-weaning weight gain characteristics in Afshari sheep.

In previous studies, ultrasonographic muscle measurements have demonstrated optimal reliability when assessing muscling and fatness in live animals (28,42). The strong correlation between fat depth at the C-site of the carcass and its corresponding ultrasonic measurement was established in previous research (48). This study, however, found no statistically significant impact of the CAST gene haplotypes on ultrasound muscle measurements. Correspondingly, Knight (47) identified specific SNPs, CAPN2_28672486 (m-calpain) and CAPN3_38942291 (calpain 3), associated with fat depth at the C-site of the carcass; yet, subsequent analysis using a Restricted Maximum Likelihood model revealed their lack of statistical significance. The present study's findings indicate a breed-specific influence of CAST haplotypes on ultrasonographic muscle measurements in meat-type sheep breeds, with particular emphasis on the H2 haplotype. Notably, a previous study conducted on Lori-Bakhtiari (fat-tailed) and Zel (thin-tailed) sheep highlighted polymorphic variations within the CAST gene specific to the breed and tail type (23).

Moreover, consistent evidence demonstrates the additive effects of the CAST gene variants on both FT and carcass fat scores. In their study, Machado (27) identified six CAST variants (rs423099226, rs428213368, rs400315475, rs415186098, rs430517308, and rs418818682) with significant additive impacts on carcass fat scores in Santa Ines sheep, showing differences ranging from 0.170 (rs415186098) to 0.246 (rs418818682) between homozygotes. Additionally, the CAST variant rs403339381 exhibited a 0.038 cm difference between homozygotes in ultrasound images of FT.

Conclusion

To conclude, this study examined the intricate genetic landscape of the CAST gene within specific meat-type sheep breeds, revealing how genetic variations influence critical characteristics like live weight and ultrasonographic muscle measurements by shedding light on the complexity of genetic variation. This study identifies 15 distinct SNPs within the CAST gene's introns 1, 5, and 12, some of which have been previously reported. While extensive exploration was conducted, no significant associations were found between the CAST gene haplotype and live weight or ultrasonographic muscle measurements during various periods. In light of this nuanced result, it is evident that genetic influences on complex traits are multifaceted, and comprehensive investigations across different breeds and environments are necessary.

Acknowledgment

This study received funding from the Republic of Türkiye, the Ministry of Agriculture and Forestry, and the General Directorate of Agricultural Research (Project No: TAGEM/HAYSUD/B/18/A4/P2/308).

Conflict of interests

The author declares no conflicts of interest regarding this manuscript's publication and/or funding.

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تأثير الكالبستاتين على صفات جودة اللحوم في سلالات الأغنام التركية

فاسفييا قادر إيسن

قسم التربية والوراثة، معهد بحوث تربية الأغنام، باليكسير، تركيا

الخلاصة

هدف هذا البحث إلى فحص تأثيرات مجموعات النمط الفرداني التي لوحظت في ثلاثة مواقع مختلفة من جين الكالبستاتين على قياسات الموجات فوق الصوتية للـ MLD والوزن الحي الموسمي لخمسة أنواع مختلفة من الأغنام (GBK و HM و K و KM و R). في الكالبستاتين وجدت مواقع intron 1 و intron 5 و intron 12 و SNPs 15. كانت قيمة الاحتمالية لتوازن هاردي واينبرغ في SNP2 و SNP3 و SNP5 و SNP6 و SNP7 و SNP8 و SNP14 أقل من ٠,٠٥، وباستثناء SNP9 و SNP10 تحتوي جميعSNPs على تردد أليل طفيف أكثر من ٠,٠١. يتكون SNP1 و SNP2 و SNP7 من كتلة واحدة من النمط الفرداني. تحتوي الكتلة الفردانية على ٣ مجموعات فردانية. كانت مجموعة النمط الفرداني الأكثر شيوعا هي (-AGG-) H1 التي كان ترددها ٠,٥٢، (-TGG-) H2 و(-TAA-) H3 لها معدلات ٠,٣٥ و ٠,١٣ على التوالي. استنادا إلى قراءات الموجات فوق الصوتية للـ MLD والأوزان الحية، لم تكن هناك فروق معنوية بين النمط الفرداني H1 وH3، ولكن كانت هناك فروق معنوية بين الحملان ذات النمط الفرداني H2. كشف تأثير النمط الفرداني H2 على عمق الـ MLD في ٩٠ يوما عن اختلاف معنوي بين سلالات HM و KM و K و KM. استمر هذا التمييز حتى اليوم ١٨٠ من العمر قبل أن يختفي في مرحلة البلوغ. ومشابها" لذلك كان تأثير النمط الفرداني H2 على سمك الجلد في اليوم ٩٠ كبيرا بين K و KM وبين K وR ، في حين أظهر تأثير النمط الفرداني H2 على سمك الدهون فرقا كبيرا بين HM و KM في عمر سنة واحدة.