

Genetic polymorphisms of the Olfactomedin-like 3 gene and their association with cholesterol content, mineral composition, flavor and odor in Indonesian local sheep

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ARTICLE INFO

Received: 21 September 2025

Accepted: 13 October 2025

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Keywords:

Cholesterol, Flavor and Odor, Minerals, OLFML3, Sheep

ABSTRACT

Cholesterol content, minerals composition, flavor, and odor of sheep meat are always key factors for consumers to consider when choosing meat. The quality of sheep meat influences these aspects; otherwise, they are important to consumers when selecting good-quality beef. Thus, genetic improvement programs oriented toward increasing minerals and flavor in sheep meat and decreasing cholesterol are programs to increase consumer interest in these products. The Olfactomedin-like 3 (OLFML3) gene may serve as a genetic marker that influences cholesterol, mineral, flavor, and olfactory qualities in local sheep raised in Indonesia. This research was conducted to analyze this relationship and investigate variations in the OLFML3 gene, as well as the levels or contents of cholesterol, minerals composition, flavor, and odor in local sheep in Indonesia. This research analyzed longissimus dorsi muscle samples derived from 100 male sheep (10-12 months old). Genotyping of the OLFML3|MspI locus was performed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism, with association analyses conducted via the General Linear Model (GLM) methodology. Three distinct genotypes (CC, TT, and CT) were identified at the OLFML3 gene. Statistical evaluation revealed significant genotype effects on cholesterol concentration ($P < 0.05$), while statistically insignificant associations were detected for minerals, flavor, and odor ($P > 0.05$). The TT genotype in sheep was associated with significantly lower cholesterol concentrations than the CC and CT genotypes. The Olfactomedin-like 3 gene's g.90317673 C>T SNP is a candidate genetic marker for low-cholesterol trait selection in Indonesian local sheep.

Introduction

Indonesia is experiencing steady population growth, which in turn drives a higher demand for food, particularly animal protein sources. To respond effectively, it is essential to boost domestic meat production capacity. According to the Central Bureau of Statistics (BPS, 2023), the country's population reached 272 million in 2023, marking an increase of around one million since 2020. This demographic development appears to parallel patterns in small ruminant production, where official records from the Directorate General of Livestock and Animal Health (Ditjen, 2022) indicate a 62% rise in the number of sheep slaughtered, rising from 976,068 head in 2017 to 1,581,373 by 2022. Alongside this, national sheep numbers have consistently grown, with the total population exceeding 14 million head in the same year. Echoing the perspective of Argaw *et al.* (2025), promoting flock expansion and reproductive efficiency in sheep plays a vital role in securing a long-term meat supply and supporting national food security frameworks.

Efforts to expand sheep meat production must be aligned with improvements in meat quality to ensure both market relevance and consumer acceptance. As noted by Gunawan *et al.* (2021), the quality of meat plays a crucial role in shaping purchasing behavior, serving as a core economic driver in the livestock sector. Nutritional content, flavor, and odor are key attributes of the product that impact its competitiveness and the sustainability of sheep-based food systems (Listyarini *et al.*, 2018). However, Indonesian consumers often have perceptions of sheep meat that are hesitant and negative, primarily related to concerns about high cholesterol, saturated fat, and a strong or unpleasant flavor (Miller, 2020). With these challenges in mind, molecular genetic approaches are gaining popularity, focusing on quality traits that are achieved by identifying essential candidate genes. In the case of SNP-based polymorphism, this is typically used to examine the genetic variation of genes encoding desirable traits (Gunawan *et al.*, 2018a). One of the most exciting is

the olfactomedin-like 3 (OLFML3) gene, which has been associated with cholesterol regulation, trace mineral content, and sensory traits (flavor and odor). These associations have been demonstrated by transcriptomic studies using RNA-seq data and are supported by suggested references to the genetic basis of meat quality (Listyarini *et al.*, 2023).

OLFML3 (olfactomedin-like 3) is located on chromosome 1 (Gene ID: 101121985) in sheep. It comprises three exons, four introns, and a total length of 2,581 bp. OLFML3 is a downstream variant gene associated with numerous quantitative trait loci (QTL) relating to high-quality meat (Braz *et al.*, 2019; Jin and Li, 2019). OLFML3 is a member of the olfactomedin gene family and was previously known as hOLF44. OLFML3 gives rise to a secreted glycoprotein comprising 406 amino acids, which is expressed in various tissues, including the liver, skeletal muscle, and adipose tissue (Listyarini *et al.*, 2022). The existing literature suggests that the OLFML3 gene may serve as a regulator of traits that contribute to the quality of sheep meat. Like many olfactomedins, OLFML3 functions as an extracellular glycoprotein, participating in various biological processes, including embryogenesis, cell adhesion, protein-protein interactions, and intercellular signalling, all of which contribute to the normal development and maintenance of tissues (Tomarev and Nakaya, 2009). OLFML3 is also a crucial molecule in myogenesis, playing a vital role in the formation and maintenance of skeletal muscle during embryonic development and postnatal growth (Zhao *et al.*, 2012). Notably, OLFML3 has been demonstrated to have functional significance in contributing to the tenderness of meat in cattle, and variants in OLFML3 have been shown to correlate with desirable meat texture (Braz *et al.*, 2019). Findings from a study (Listyarini *et al.*, 2022) further demonstrated the role of this gene in lipid metabolism, indicating its involvement in regulating specific enzymatic pathways associated with the uptake and deposition of fatty acids within tissues. In this capacity, OLFML3 plays a direct role in the fatty acid composition of meat. It has a strong case to be employed as a candidate gene in genomic selection approaches targeting the nutritional and sensory

quality of sheep and mutton products.

The olfactomedin gene family encodes a subset of glycoproteins that are secreted and perform regulatory functions involved in lipid metabolism, adipocyte differentiation, and insulin sensitivity (Barrientos-Riosalido *et al.*, 2023). For sheep meat, a key quality determinant of the product is its fatty acid profile, as it is essential for both nutritional value and cholesterol content (Gunawan *et al.*, 2018a). This is primarily due to the biochemical process by which fatty acids are converted into acetyl-CoA, a key precursor in the endogenous synthesis of cholesterol (Sahadevan *et al.*, 2014). Additionally, the proportions and types of fatty acids present in muscle will also influence the sensory quality of the meat product in terms of flavor and odor acceptance by consumers (Gunawan *et al.*, 2018b). The relationship between fatty acid metabolism and meat quality characteristics is reasonably well understood. Still, there is relatively little known about the OLFML3 gene and its role in regulating cholesterol concentrations, mineral amounts, and sensory characteristics in sheep. The current lack of knowledge presents a significant gap in understanding the molecular aspects of meat quality in sheep. Thus, this study focused on investigating polymorphisms of the OLFML3 gene and examining associations with cholesterol concentrations, mineral profile, and critical sensory characteristics, including flavor and odors, in selected local Indonesian sheep populations. The purpose was to gain a deeper understanding of the functional role of the gene and its potential application in molecular breeding programs aimed at enhancing quality of meat.

Materials and methods

Animals and Samples

Animal procedures were conducted in accordance with recognized ethical guidelines and approved by the IPB University Institutional Animal Care and Use Committee (IACUC). Approval number 117-2018 IPB. Genomic DNA was isolated from longissimus dorsi muscle samples obtained from 100 sheep representing three indigenous Indonesian breeds: 75 Javanese Thin Tailed Sheep (JTTS), 15 Jonggol Sheep (JS), and 10 Javanese Fat Tailed Sheep (JFTS). The animals sampled were 10–12 months of age and had a live weight of 20–35 kg. The JTTS and JFTS samples were collected from several farms in the West Java municipality. In contrast, the JS samples were collected from the Jonggol Animal Science Teaching and Research Facility (6°28'24.3"S, 107°00'49.7"E). All animals were raised collectively in group pens under standard conditions where they had ad libitum access to forage and concentrate throughout the experiment, ensuring homogenous feeding and environmental conditions.

Extraction of DNA and PCR-RFLP Amplification

DNA was extracted from ram longissimus dorsi tissues using a genomic DNA micro kit (Geneaid Biotech, Taiwan) that included three separate sequential steps. In the preliminary sample preparation stage, 1,000 µL of a 0.2% NaCl solution was amalgamated with blood specimens in 1.5 mL tubes, followed by centrifugation for a duration of 5 minutes at 8,000 rpm to facilitate the separation of cellular constituents, after which the supernatant was meticulously removed. The subsequent phase of protein degradation entailed resuspending the sample in a lysis solution comprising 40 µL of 10% sodium dodecyl sulfate, 10 µL of proteinase K (5 mg mL⁻¹), 350 µL of STE buffer, and augmented with 400 µL of phenol solution and 40 µL of 5 M NaCl. This amalgamation was incubated with

continuous agitation for 1 hour at ambient temperature to promote comprehensive protein degradation and was then centrifuged for 5 minutes at 12,000 rpm to segregate the DNA-containing aqueous phase from the organic phenol layer. For the precipitation of DNA, 400 µL of the fresh tube was used to hold the aqueous phase, combined with 40 µL of 5 M NaCl and 800 µL of absolute ethanol, and subsequently stored at -20°C overnight to facilitate the complete precipitation of nucleic acids.

The DNA-containing tube was centrifuged for 5 minutes at 12,000 rpm at 20°C, and the supernatant was discarded. After resuspending the material in 800 µL of 70% ethanol, it was centrifuged again for 5 minutes at 20°C and 12,000 rpm. Moreover, the supernatant was thrown away. The lid of the sample was then opened sustainably to allow the sample to dry until the ethanol evaporated and the DNA precipitate remained. The pellet of DNA was then resuspended in 100 µL of 80% TE buffer (10 mM Tris-HCl, one mM EDTA, pH 8.0) and placed at -20°C for safe molecular storage until further analysis.

The OLFML3 gene variant (g90317673 C>T) was amplified using custom-designed primers to intron 4, using the methods outlined by Listyari *et al.* (2022). The PCR reaction was prepared at a total volume of 16 µL, consisting of 2 µL of genomic DNA, 0.2 µL of forward primer, 0.2 µL of reverse primer, 6.1 µL of DW and 7.5 µL of Red Mix. Amplification was performed on GeneAmp PCR system (The Applied Biosystems 2720 Thermal cycler) using the following conditions: one cycle of 94°C for 1 minute per sample, 35 cycles of 94 °C for 10 seconds, 59°C for 15 seconds, and 72°C for 15 seconds, with a final cycle at 72 °C for 1 minute. PCR products were analyzed by electrophoresis on a 1.5% agarose gel and visualized using a UV illuminator. For RFLP-genotyping, 498 bp amplicons were digested by MspI restriction enzyme at 37°C for 4 hours and analysed by electrophoresis on a 2% agarose gel. The digests produced characteristic patterns for each genotype: CC (195 bp and 303 bp), TT (498 bp), and CT (195 bp, 303 bp, and 498 bp), as shown in Table 1.

Cholesterol analyses

The cholesterol concentration in sheep carcass fat was decided employing the HPLC (High-Performance Liquid Chromatography) technique, as described by Li *et al.* (2019). A 10 mL volumetric flask was filled with about 10 mg of extracted fat and a small amount of 2-propanol. After a few minutes of ultrasonic extraction, the product was homogenized. The 2-propanol solution was passed through a 0.45 µm membrane filter after cholesterol had been dissolved in it. Next, the obtained sample was tested using HPLC. This method separates compounds based on their polarity. Once separated, the detection of compounds is indicated by visual peaks in the chromatogram. The number of peaks indicates the number of different compounds, and the area under each peak indicates the concentration of that compound. One benefit of HPLC is that it can detect non-volatile compounds, in addition to a range of other applications, including molecular weight determination, quantitative analysis, clinical diagnostics, forensic science, food safety, environmental analysis, and other analytical chemistry applications.

Minerals analyses

Atomic Absorption Spectrophotometry (AAS) is a method used to determine the mineral content in meat samples. AAS measures the concentration of metal elements via the absorption of radiation by free gaseous atoms following (Fairulnizal *et al.*, 2019). To analyze (K) potassium,

Tabel 1. Primer sequence and accession number of OLFML3 gene.

Gene ¹	Accession Number	Size of PCR	Temperature Anneling (°C)	Enzyme	Primer sequence
<i>OLFML3</i>	NC_019458.2	498 bp	59	<i>MspI</i>	F: 5'-ATG ATG GCT ACC AGA TTG TC-3 R: 5'-CTC CTT CTG TAC TGC AGA CT-3

¹Designed using MEGA 7 software.

(Fe) iron, (Zn) zinc, and (Se) selenium, the contents of the meat samples, 2.5 g of each meat sample were digested in 25 mL of concentrated HNO₃ by boiling them for 30-35 minutes, cooled, and 10 mL of 70-72% HClO₄ was added, slowly boiled to complete decolorization, then cooled again, and 50 mL of H₂O was added, and cooked to eliminate NO₂ gas. After it cooled, the digestate was filtered into a 100 mL precision volume flask and used to determine the concentration of minerals of interest by AAS.

Flavor and odor analyses

The quantification of important flavor and odor active compounds such as: 4-methylnonanoic acid (MNA), 4-methyloctanoic acid (MOA), 3-methylindole (MI), 4-methylphenol (MP), and ethyl octanoate (EOA) was accomplished in 500 g samples of lean meat, by following the Likens-Nickerson simultaneous distillation and extraction method, and analysis on a gas chromatograph-mass spectrometer (GC-MS) system (Muharram *et al.*, 2024). The simultaneous distillation and extraction method was effective for isolating and identifying volatile flavor compounds because it combines solvent extraction and steam distillation, followed by the separation of the isolated volatile substances in a chromatographic column, and subsequent detection in a mass spectrometer.

Statistical analysis

Genotype, Allele frequencies and Association Study

The study employed the method of Nei and Kumar (2000) to calculate genotype and allele frequencies, as well as Hardy-Weinberg equilibrium values. The fixed-effect model (ANOVA) was also performed using PROC GLM in Minitab 19. The fixed-effects model was employed to investigate the relationships between the identified genotypes and key meat quality traits, specifically cholesterol levels, mineral content, flavor, and aroma. Where the analysis revealed significant differences, further comparisons were conducted using Duncan's test to examine any differences between genotype group means (Ahmad Ansori Mattjik, 2018). The model used in the ANOVA follows that of Gunawan *et al.* (2018a), as described below:

$$Y_{ijk} = \mu + g_i + e_{ijk}$$

Where:

Y_{ijk} = Cholesterol Content, minerals composition, flavor dan odor

μ = the population's mean value

g_i = the genotype (i = CC, TT, and CT)

e_{ij} = the residual error

Protein Interaction Analysis Using STRING

Protein interaction network analysis was carried out using the STRING platform (v12.0) to explore the functional relationship between the OLFML3 gene and several other regulatory genes related to lipid metabolism and meat quality (Figure 2). The gene list was input with the organism specifications of *Ovis aries*. The mapping results showed that OLFML3 interacts with genes such as TGFBR1, HSPA12A, P2RY12, SREBF2, TMEM150A and HMGCR, which play a role in cholesterol, minerals, odor and flavor (Szkarczyk *et al.*, 2024).

Results

Polymorphism of the OLFML3 Gene

The OLFML3 gene polymorphism analysis successfully amplified the target SNP (g.90317673 C>T) using specifically designed primers, revealing distinct restriction fragment patterns: TT genotypes showed a single 498 bp fragment, CC genotypes displayed two fragments (195 bp and 303 bp), while CT heterozygotes exhibited all three fragments (195 bp, 303 bp, and 498 bp) as illustrated in Figure 1. This genotyping method was successfully utilized on three Indonesian sheep populations: Javanese fat-tailed sheep (JFTS), Javanese thin tailed sheep (JTTS), and Jonggol sheep (JS). The genotypes were detected in HW, with the findings presented in Table 2. Overall, the heterozygous CT genotype had the highest frequency, followed by the CC genotype, and the TT genotype. For allele frequencies, the C allele was more dominant (0.545) than the T allele (0.455) in the combined population. When viewed per breed, JFTS showed a slightly different pattern with higher frequencies of the CC (0.40) and CT (0.50) genotypes, and a lower frequency of TT (0.10), which was showed with a higher frequency of the C allele (0.65) and a lower frequency of the T allele (0.35) compared to the other breeds. Meanwhile, JTTS and JS showed very similar genotype and allele distributions, with CC and CT frequencies around 0.33-0.40, respectively, and TT frequencies around 0.27.

Genotype frequencies in each breed and the total population were in equilibrium with the HW. This indicated that there is no significant deviation from HW in the OLFML3 gene in the three Indonesian sheep breeds, or in the combined population. This equilibrium indicates that factors such as mutation, selection, migration, and genetic drift do not significantly affect the allele and genotype frequencies of the OLFML3 gene in this population, indicating genetic stability at this locus.

Table 2. Frequency of genotype and allele of the OLFML3 gene.

Sheep Breed ¹	N ²	Genotype frequency			Allele frequency		χ^2
		CC	CT	TT	C	T	
JFTS	10	0.40 (4)	0.5 (5)	0.1 (1)	0.65	0.35	0.09
JTTS	75	0.33 (25)	0.40(30)	0.27 (20)	0.53	0.47	2.89
JS	15	0.33 (5)	0.40(6)	0.27 (4)	0.53	0.47	0.57
Totals	100	0.34 (34)	0.41 (41)	0.25 (25)	0.55	0.46	3

¹JFTS: Javanese Fat-Tailed Sheep, JTTS: Javanese Thin-Tailed Sheep, JS: Jonggol Sheep, ²N is the number of samples; (...) is the number of samples with the genotypes TT, CC, CT, ³ $\chi^2_{tab} = 3.84$.

Table 3. Association of OLFML3 gene with cholesterol.

Parameter (%)	Genotype (Mean± SE Mean) ¹			P-Value
	CC (n=34)	CT (n=41)	TT (n=25)	
Cholesterol	16.51±2.18 ^a	10.00±1.31 ^b	7.79±0.82 ^b	0.002* ²

¹Cholesterol means, SE = standard error, n = number of samples, ²*: A statistically significant difference (P < 0.05) is indicated by means within the same row that are indicated with separate superscript letters.

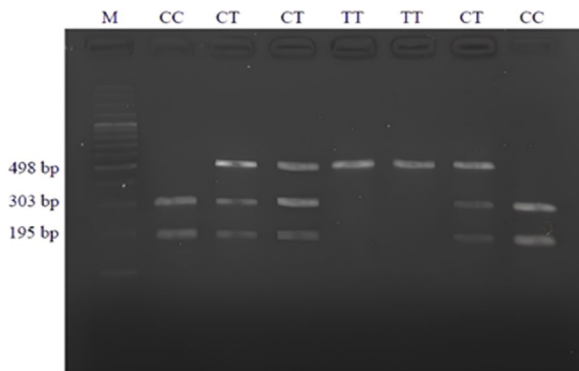


Figure 1. PCR-RFLP result for the OLFML3 gene; bp = base pair; genotypes TT (498), CC (195, 303 bp), and CT (195, 303, and 498 bp); M = 100 bp ladder size standard.

Association of OLFML3 Gene Polymorphism with Cholesterol

The association between the OLFML3 g.90317673C>T and There is a significant correlation ($P < 0.05$) between cholesterol levels and the TT genotype, with individuals having significantly lower cholesterol levels compared to those with the CC and CT genotypes (Table 3). The CC genotype had the highest mean cholesterol level of 16.51 ± 2.18 . In contrast, the CT and TT genotypes showed significantly lower cholesterol levels (10.00 ± 1.31 and 7.79 ± 0.82).

Association between Minerals and OLFML3 Gene Polymorphism

The polymorphism of the OLFML3 gene had no significant ($P > 0.05$) between mineral content (Fe, Zn, K, Se) in sheep meat (Table 4). Moreover, minerals consisting of Fe (0.216), Zn (0.374), K (0.597), and Se (0.123).

Association of OLFML3 Gene Polymorphism with Flavor and Odor

Association analysis of OLFML3 gene polymorphism with flavor and odor content revealed insignificant association ($P > 0.05$). Including 4-methyloctanoic (MOA), 4-Ethyloctanoic (EOA), 4-Methylnonanoic (MNA), 3-Methylphenol (MP), and 3-Methylindole (MI). Based on the P-value obtained for each parameter (MOA: 0.234; EOA: 0.450; MNA: 0.264; MP: 0.584; MI: 0.629) (Table 5).

Protein Interaction of OLFML3 Gene

The OLFML3 gene is functionally linked to several regulatory genes, such as CSF1R, TGFBR1, P2RY12, and HSPH1, all of which are involved in

lipid metabolism and cellular stress responses, as demonstrated by the STRING interaction network (Figure 2). These linkages suggest a coordinated regulatory system that influences cholesterol homeostasis, likely through cellular signaling cascades (mediated by TGFBR1) and macrophage activation (via CSF1R). Notably, OLFML3 may alter lipid processing during metabolic stress due to its association with the heat shock proteins HSPA12A and HSPH1. Meat quality is impacted by the gene's ability to control fatty acid transport and cholesterol accumulation in muscle tissue through its extracellular signaling function.

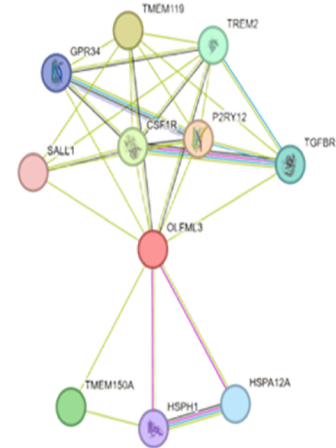


Figure 2. Protein interaction network between the OLFML3 gene and several other regulatory using the STRING platform (v12.0).

Discussion

Polymorphism analysis of the OLFML3 gene revealed multiple alleles at the g.90317673C>T position in the studied sheep populations, reflecting a high level of genetic variation (Table 2). Frequency analysis revealed the rare, but predominant, occurrence of C (55%) over T (45%) alleles. Importantly, this aligns with Gunawan *et al.* (2017), who note that the term polymorphism can be applied when frequencies remain ≤ 0.99 . There are obvious genotypic frequencies (TT = 0.25, CT = 0.41, and CC = 0.34), indicating that polymorphisms are occurring in the population. The population sampling distribution showed Hardy–Weinberg equilibrium (HWE), which suggests that the population was obviously not affected by, or was distanced from, events that could disrupt the evolution of polymorphism in that population (mutation, natural selection, or migration); therefore, genotypic variation remains constant year-to-year across decades. Noor (2010) states that stick populations will stay at genetic equilibrium if they

Table 4. Association of OLFML3 gene with minerals.

Parameter (mg/100 g)	Genotype (Mean $\pm$ SE Mean) ¹			P-Value
	CC (n=34)	CT (n=41)	TT (n=25)	
Fe	20.37 $\pm$ 1.67	18.34 $\pm$ 1.11	16.93 $\pm$ 1.13	0.22
Zn	25.72 $\pm$ 1.85	27.47 $\pm$ 1.48	24.13 $\pm$ 1.73	0.37
K	26.88 $\pm$ 1.53	27.43 $\pm$ 1.25	28.15 $\pm$ 1.90	0.60
Se	6.22 $\pm$ 0.56	5.75 $\pm$ 0.50	6.95 $\pm$ 0.45	0.12

¹: Means of minerals; SE: standard error; n: number of samples.

Table 5. Association of OLFML3 gene with flavor and odor.

Parameter (μ g/g)	Genotype (Mean $\pm$ SE Mean) ¹			P-Value
	CC (n=34)	CT (n=41)	TT (n=25)	
4-methyloctanoic (MOA)	81.4 $\pm$ 42.6	156.4 $\pm$ 70.7	527 $\pm$ 483	0.23
4-Ethyloctanoic (EOA)	44.4 $\pm$ 11.2	92.1 $\pm$ 28.1	211 $\pm$ 119	0.45
4-Methylnonanoic (MNA)	529 $\pm$ 233	1222 $\pm$ 503	972 $\pm$ 588	0.26
3-Methylphenol (MP)	0.81 $\pm$ 0.33	1.52 $\pm$ 0.49	2.57 $\pm$ 0.197	0.58
3-Methylindole (MI)	1.152 $\pm$ 0.623	1.744 $\pm$ 0.829	0.56 $\pm$ 0.295	0.63

¹: Means of flavor and odor; SE: standard error; n: number of samples.

are not influenced by mutation, selection, migration, or genetic drift; this supports our findings.

In terms of cholesterol content, the results corresponded with previous reports by Munyaneza *et al.* (2019), which indicated genotype differences in cholesterol content relative to sheep homozygous for the TT genotype of the BHMT. In sheep meat, Aksoy *et al.* (2019) reported a total cholesterol value of approximately 75 mg per 100 g, encompassing all lipid fractions, including triglycerides, LDL, and HDL cholesterol. The OLFML3 protein family is likely to influence cholesterol-regulating metabolic activity in the body, which is mediated through the olfactomedin two protein (Yang *et al.*, 2021). Associations may exist between OLFML3 genotypes and cholesterol metabolism, including the quantification of dynamic cholesterol. The lambs in this study were slaughtered at ages ranging from 10 to 12 months, having been fed a nutritionally balanced diet before slaughter, which is likely a contributing factor to the lower cholesterol levels observed. Therefore, one could speculate that the TT genotype of OLFML3 may be associated with lower tissue cholesterol storage, which represents a significant new contribution to understanding gene-lipid relations in sheep. This finding represents a new contribution to understanding the functional implications of OLFML3 and therefore requires further investigation to describe the molecular pathways involved.

Olfactomedin-like 3 is a secreted extracellular matrix glycoprotein that biochemically connects to many key genes involved in lipid metabolism, tissue remodelling, and regulating the immune system (Figure 2). OLFML3 interacts with CSF1R which is a primary receptor for macrophages and ultimately influences lipid phagocytosis and tissue homeostasis through macrophage activation and the known down-regulating accumulation of tissue cholesterol (Shibata and Glass, 2009). OLFML3 also interacts with TGFBR1 which is an important regulatory component in TGF- β signalling pathway further supporting the hypothesis that the protein is altering the expression of its targeting genes including SREBF2 and HMGCR which directly lead to cholesterol production (Gabitova-Cornell *et al.*, 2020). Lastly, OLFML3 has a direct interaction with P2RY12 which is a direct modulator, and cellular cholesterol metabolism by preventing foam cell formation originating from vascular smooth muscle cells (VSMCs) by inhibiting cholesterol efflux using the autophagy-MTOR pathway in atherosclerosis (Pi *et al.*, 2021). Via OLFML3, there may exist an anatomical link to the inflammatory status of muscle or adipose tissue and their connection to cholesterol metabolism with OLFML3 acting in a unique manner within immune-metabolic pathways.

The direct involvement of HSPA12A is a clear indication of the strong association between hyperlipidemia, diet-induced obesity, and the overall capacity of lipids in the system. This heat shock protein interacts with OLFML3, a component identified in adipocyte differentiation (Zhang *et al.*, 2019). OLFML3 interacts with TMEM150A, which contributes to inflammatory responses and maintains membrane lipid homeostasis, including the production of sterols and phosphatidylinositol phosphate. The general architecture of this specific interaction network suggests that OLFML3 is directly involved in controlling cholesterol and lipids at both the cellular and tissue levels. It is more than a structural protein, contributing to myogenesis. Altogether, OLFML3 contributes to the cellular and structural context in which cholesterol dysregulation occurs by regulating vascular remodelling, macrophage function, and processes related to cellular stress exposure (Yu *et al.*, 2025). OLFML3 does not directly enter into the production or transport of cholesterol; however, it has an indirect role in the development of cardiovascular diseases linked to cholesterol, contributing to the major pathological processes of inflammation and angiogenesis. Therefore, OLFML3 could be considered a relevant candidate gene for livestock breeding, providing a means to reduce intramuscular cholesterol and enhance meat quality.

However, in the mineral composition, the TT genotype was associated with elevated concentrations of Selenium (Se) and potassium (K), compared to the CC and CT genotypes, although these associations were

statistically nonsignificant (Table 4). Potassium emerged as the most abundant mineral, consistent with its classification as a macromineral with recommended human intake levels exceeding 100 mg/day (Prashanth *et al.*, 2015). The potassium level recorded, 28.15 mg/100 g, aligns with findings that potassium plays vital physiological roles in osmotic regulation, enzyme activation, and acid-base balance (Ando *et al.*, 2010). On the other hand, crucial trace minerals including selenium (Se), zinc (Zn), and iron (Fe) which have notably lower dietary requirements (15 mg for Fe/Zn; 55–70 μ g for Se) as noted by Sigdel and Janaswamy (2020), can still be partially supplied by sheep meat. The observed mineral profile may suggest genotype-related influences on mineral metabolism, although other genetic elements or environmental factors likely exert more dominant roles in maintaining mineral homeostasis. Research conducted by Anas *et al.* (2024), long non-coding RNA (lncRNA) and gene networks have been shown to regulate mineral homeostasis in the fetal liver, but OLFML3 was not highlighted among the key genes involved in mineral regulation. Current research supports OLFML3 gene association with meat quality and fatty acid composition, but there is no direct evidence linking OLFML3 to mineral content in sheep meat. Further studies are needed to clarify any potential role in mineral regulation.

Concerning flavor and odor, the current findings contrast with those of (Herpina *et al.*, 2023), who reported that the CT genotype of the KCTD2 gene exhibited weaker correlations with odor and flavor attributes than other genotypes. In contrast, Gunawan *et al.* (2018b) suggested that the best methods for reducing the off flavor in sheep meat are still genetic selection and focused breeding. Several volatile organic compounds including MOA, MP, MNA, MI, and EOA serve as measurable phenotypic markers for these sensory properties and are influenced by genetic variation. The distinct, often undesirable, smell of sheep meat is primarily attributed to branched-chain fatty acids (BCFAs), whose origin lies in ruminal microbial metabolism (Muharram *et al.*, 2024). This metabolism produces butyrate, propionate, and acetate, with three primary compounds 4-Methylnonanoic acid, undecanoic acid, and 4-Methylphenol directly associated with undesirable odor. Another key compound, 3-methylindole (skatole), is produced by intestinal *Lactobacillus* bacteria and accumulates in fat tissue when hepatic degradation is insufficient (Robic *et al.*, 2008; Wesoly and Weiler, 2012). Currently, there has been no research examining OLFML3 variation and its correlation with volatile compounds that determine flavor in Indonesian sheep, despite its prospective benefits as a basis for genetic selection.

Conclusion

This study confirms the existence of polymorphism at the OLFML3 g.90317673 C>T locus in Indonesian local sheep, with the TT genotype significantly associated with reduced cholesterol levels. The results imply that OLFML3 is involved in lipid metabolism, possibly through its molecular interactions with regulatory genes such as CSF1R, TGFBR1, and P2RY12, which are involved in tissue cholesterol regulation. Given these associations, the TT genotype emerges as a promising candidate for marker-assisted selection (MAS) strategies aimed at enhancing the quality of sheep meat. Integrating this SNP into polygenic breeding programs may support the development of low-cholesterol sheep meat products, thereby contributing to precision livestock breeding initiatives that align with modern nutritional demands.

Acknowledgments

This study was funded by the Program Tesis Magister with grant number 18878/IT3.D10/PT.01.02/M/T/2023.

Conflict of interest

The authors declare that they have no financial, personal, or profes-

sional interests that may influence the work submitted for publication.

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