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Association and expression study of PCYOX1 Gene with flavor odor in Indonesian sheep

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ABSTRACT

Sheep meat is experiencing increasing global demand, making product quality enhancements through flavors or odors essential. The PCYOX1 gene and its role in the development of sheep meat sensory characteristics of flavors and odor have been identified. Therefore, the objectives of this study were to analyze species polymorphisms of the PCYOX1 gene and their relationships with flavor and odor traits in sheep. A total of 85 rams ranging from 10 to 12 months from Javanese Thin-Tailed Sheep (JTTS) and Jonggol Sheep (JS) were utilized. The species of the PCYOX1 gene were analyzed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. The relationship of phenotypes of flavor & odor by gene polymorphisms with the general linear model (GLM). The results showed a single nucleotide polymorphism (SNP) variation g.37912033 G>A occurring with genotypes AA, AG, and GG. The study of associations demonstrated a significant ($P < 0.05$) relationship between SNP of the PCYOX1 gene with odor-flavor compounds involving 4-methyl-octanoic acid (MOA) & 4-methyl-nonanoic acid (MNA). Results indicated the AG genotype was associated with lower flavor odor intensity compared to genotypes AA and GG. The results indicated that PCYOX1 might be a potential candidate for breeding sheep with better flavor and odor, which could probably increase their quality as well as economic value in the meat trade.

Introduction

The global meat sector has experienced a sizable growth in red meat output and consumption over the past decade, with sheep meat being a notable driver (Parlasca and Qaim, 2022). In terms of sheep, the Global Sheep Industry Report (ESS, 2025) estimated that sheep production reached 15 million metric tons in 2024, and predicted a substantial increase to 19 million metric tons by 2034. This also reflects a projected increase in consumption demand for meat, sheepmeat consumption globally forecast to grow by 1.3% p.a until 2029 (OECD, 2023), while growth is expected at similar rates across commodity types and applications (Henchion *et al*., 2014; OECD, 2023). For instance, reductions in cultured meat manufacturing costs—among sustainable protein options—are expected to come from rising incomes and a growing population (but disproportionately so among developing regions, where demand for relatively high cost premium Kcals is much lower than that of developed countries). Cultural desire likewise serves a critical function, as mutton is now an ordinary item in numerous terrestrial diets, so that it substantially contributes to the stable floor maintained via investments inside every region's solid carcass of national cattle product marketing.

Sheep meat is also an important component of the national meat supply, particularly in Indonesia, where it accounts for around one-third of all consumed meat. Sheep meat production is an important source of protein to the rural economy and an income-generating activity for millions of smallholder farmers in the region. It contributes to the growth of the community and also serves as income for rural families. However, because the price was affordable for all levels of consumers in Indonesia (Nyam *et al*., 2020), not surprisingly, sheep meat was used as an alternative protein provided along with fish. In the Islamic world, occasions like Eid al-Adha are a great image of sharing and relationship through this heritage. The difficult case of sheep meat points to the fact that increasing production efficiency and product quality, as required by domestic markets or consumption patterns, also has a cultural component.

Meat quality characteristics comprise, besides flavour qualities taste and aroma, nutritional attributes as well food safety aspects will also play a role in developing to provide the consumer satisfaction by creating advantage over competitors within meat markets (Prache *et al*., 2022). The experience of consuming sheep meat is an integrative sensory process, and some of these traits are flavor and aroma attributes that contribute to consumer acceptance. In the future, submolecular bases are also likely to be identified by these techniques using candidate genes and genetic markers associated with animal meat quality. In a different study, the expression of genes that may explain Indonesian local sheep meat flavor and odor was analyzed to improve these characteristics through selective breeding programs (Harahap *et al*., 2024; Septiyawan *et al*., 2024). The largest module, identification of Prenylcysteine oxidase 1 (PCYOX1) as a founder gene involved in meat quality using the bioinformatics method, carries important information for further studies on its genetic mechanisms, which will be relevant to understanding muscle metabolism and development in ruminants at the molecular level.

The PCYOX1 gene encodes an enzyme that catabolize prenylated cysteine derivatives, S-prenyl-L-cysteine to free sulfur and nonpolar isoprenoids (NCBI Gene ID: 51449) It is critical metabolites in numerous biochemical pathways, especially with regard to lipid metabolism. The candidate gene PCYOX1 is associated to genes that take part of metabolism, biosynthetic and lipid droplet biogenesis other process differentially expressed under teaser meat as carcass quality (Gianazza *et al*., 2025). Lipid content and composition are important determinants of meat quality in terms of flavor, juiciness, and tenderness attributes. PCYOX1 networks with previously identified genes suggest potential use in genetic markers associated with meat quality traits for breeding programs focusing on these phenotypes. Much has been learned about the multifaceted roles of the PCYOX1 gene through varied studies on other species. Take humans, PCYOX1 in relation to lipid metabolism and possible implications

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for cardiovascular health with biomarker applications in medical diagnostics (Saarensilta *et al*., 2025). Due to its overlap with homologous genes on chromosome 13 and because the locus has been tested in association studies across economically important traits in cattle, this gene was considered ruminant-specific only. Some examples include research on the impact of SNPs on birth traits (Aponte *et al*., 2024; Ogunbawo *et al*., 2025) in Jersey cattle or on Nellore cattle regarding growth and carcass characteristics. Genotype and environment Interactions. For some commercially relevant phenotypes, examining genotype and environment interactions is also feasible. Furthermore, murine models have been applied to reveal the activities of lipid-related genes.

This effect may be due to the pre-established role of the PCYOX1 gene in lipid metabolism (Gianazza *et al*., 2025), and it has been associated with meat quality traits even from a different species. This study tried to fill up such an assumption as well. The present study aimed to detect polymorphisms of the PCYOX1 gene in the native sheep population and relate these variations to flavor-odor compound metrics levels. Fortunately, no studies have reported the genetic variation of PCYOX1 and its relationship with volatile compounds that determine sheep flavor in Indonesia up to this moment, though it is still possible as an important component for future methods of selection. However, in the case of Indonesian sheep, this knowledge gap is even more pronounced, as no study has yet investigated their molecular characterization across these genes. Moreover, the present study also aimed to measure gene expression levels in tissues (i.e., longissimus dorsi and liver) that are relevant phenotypic traits due to their biological roles. This gap is especially pronounced in the context of Indonesian sheep, where no studies have explored the molecular characterization of these genes in this species. Furthermore, this study aimed to evaluate gene expression levels in relevant tissues, the liver and the loin, to understand their functional impact. This study aimed to elucidate the molecular mechanisms underlying sheep meat quality by identifying PCYOX1 polymorphisms and assessing their association with flavor-related compounds.

Materials and methods

Animals

All experimental procedures using animals were approved by the Animal Ethics Commission of IPB University (117-2018 IPB). The total number of 85 Javanese thin-tailed sheep (JTTS) and Jonggol Sheep (JS), including both sexes, was utilized in this study. The sheep were reared in homogeneous farming conditions and slaughtered at 10–12 months of age, following Indonesian regulations on animal welfare (SNI - Indonesian National Standard, number 99003-2018) (BSN, 2018). The study part evaluates samples for their flavor and odor characteristics. The longissimus dorsi was sampled for chemical analyses through the flavor and odor composition, as well as before atelectasis, and for DNA extraction.

Flavor Odor Analysis

This association study used sheep meat odor and flavor as the phenotype, which was described previously by Listyarini *et al*., (2018). A total of five flavor-active volatile compounds, 4-methyl-octanoic acid (MOA), 4-methyl-nonanoic acid (MNA), 3-methyl-indole (MI), ethyl hexanoate, and oxime were identified by assortment using the GC/MS system in the loin meat sample weighing approximately 500 g. Extraction of volatile compounds was accomplished using the distillation/solvent extraction method described by Likens and Nicholson.

DNA Extraction and Genotyping

DNA was extracted from the longissimus dorsi muscle samples using a commercial DNA extraction kit (Geneaid Biotech, Taiwan). Primers were developed for the PCYOX1 gene with the help of designing software Primer3. The forward primer is 5'-ACGGTGCAAACTAAAGTAAG-3', and the reverse primer is 5'-TACGTGATGCTGAAGGT TSG-3', which were designed from the accession number NC_019460.2 for precise amplification of a 384 bp piece of DNA. The PCR mixture for each sample contained 2 μL of extracted DNA, 6.1 μL of nuclease-free water, 7.5 μL of MyTaq Red Mix (Meridian Bioscience, USA), and 2 μL of each primer. The amplification protocol consisted of an initial denaturation step of 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, primer annealing at 62°C for 15 s, and DNA elongation at 72 °C for 10 s, and a final extension step at a temperature of 72 °C for 3 min. Genotyping was done using PCR-RFLP, following the methods described by Gunawan *et al*., (2018). The digestion reaction contained amplified DNA (5 μL), enzyme buffer (0.7 μL), BspHI restriction enzyme (0.3 μL), and nuclease-free water (NFW) (1 μL). The digestion reaction was incubated for 54 hours at 37 °C. Fragments were visualized by 2% agarose gel electrophoresis with a UV transilluminator (AlphaImager; Alpha Innotech, Santa Clara, Calif., USA).

RNA Isolation and Reverse Transcription Polymerase Chain Reaction

Liver was also sampled for RNA isolation for gene expression studies under each experimental condition. Vivo, all liver samples were collected from the experimental animal at 100 immediately frozen in liquid nitrogen and stored at -80 °C to prevent degradation (Zhao *et al*., 2012). Approximately 30 mg of homogenized liver tissue was used for RNA isolation via the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The tissue had been frozen and stored for 8 years prior to being homogenized, the isolated lysed

Table 1. PCR primer sequence and size for the PCYOX1 gene.

Gene	Accession number	Primer Sequence (5' to 3')	Application	TA (°C)	Size of PCR (bp)	Restriction Enzyme	SNP	Digest fragments length
PCYOX1	NC_019460.2	Forward: ACG GTG CAA ACT AAA GTA AG Reverse: TAC GTG ATG CTG AAG GTT SG	Genotyping	62	384	*BspHI*	g.37912033 G > A	GG = 384 AG = 117, 267 and 384 AA = 117 and 267
PCYOX1	XM_004005804.6	Forward: CTG GGT CTC TCT GCT GTT CA Reverse: CTA CCC ACA TGT GCA TCC GG	qRT-PCR	60	154			
GADPH	NC_019460.2	Forward: GAG AAA CCT GCC AAG TAT GA Reverse: TAC CAG GAA ATG AGC TTG AC	qRT-PCR	60	203			
β - Actin	NC_019471.2	Forward: GAA AAC GAG ATG AGA TTG GC Reverse: CCA TCA TAG AGT GGA GTT CG	qRT-PCR	60	194			

Note: designed using primer3; GADPH=glyceraldehyde-3-phosphate dehydrogenase in a guanidine-based buffer under denaturing conditions, followed by column-purification of high-quality RNA. The quality and concentration of the extracted RNA were then spectrophotometrically evaluated at an A260/A280 using a NanoDrop (Thermo Scientific, Waltham, MA), which makes it possible to verify that these values are above 1.8 and below 2.0 from pure RNAs suitable for subsequent applications Agarose gel electrophoresis confirmed that the RNA consisted of 2 major bands with the proper sized molecules for 28S and 18S ribosomal RNA. The isolated RNA was then reverse-transcribed to cDNA for gene expression profiling using the First Stand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA).

Analysis of Expression with Quantitative Real-Time PCR

The qRT-PCR-based method was utilized to determine the mRNA expression of PCYOX1 because it is widely accepted that PCYOX1 is an important member in regulating flavor and odor traits in sheep. BGN expression across the genotypic variations was likewise assessed. This study was performed on 85 male rams classified into three genotypic groups (n = number of samples/total): AA, n=48; AG, n=31, and GG: 6 in the form of liver tissue samples. Six samples (three per genotype) were selected for analysis. Three genes (PCYOX1, GAPDH, and β-actin) from sheep were designed as the specific primers used in qPCR experiments(ref: Table 1). The qRT-PCR was carried out using the SYBR Green Premix Kit for nine target miRNAs, and each 20 µL reaction system contained 5 µL of SYBR Green Load Mix (02), 0.5µl of forward primer and reverse primer, and each cDNA was amplified in a final volume of 10 µL. The thermal cycling protocol consisted of an initial denaturation step at 95°C for 30 s, followed by 35 cycles of the same stages with initiation at a temperature to give a Tm and using TaqGold polymerase (Raadsma *et al*., 2009). The melting curve analysis ended with a final cooling down to 50°C during 30s, and the expression of target genes was normalized against GAPDH or β-actin based on the potentiostatic method of delta cycle threshold (ΔCt). Statistical analysis was performed with the help of a general t-test using Minitab and a significance value set at $P < 0.05$. This approach translated very well to gene expression comparisons between genotypic groups and helped shed light on the underlying molecular mechanisms related to observed traits.

Statistical Analysis

Genotype and allele frequencies

The statistical procedures included investigating genotype (G) frequencies, and alleles were calculated by the following formula (Nei and Kumar, 2000):

$$X_{(i)}=(2n_{ii}+\sum_{j\neq i} n_{ij})/2N \quad \text{and} \quad x_{ii}=n_{ii}/N$$

The input was 'i' for allele frequency and the label will be 'ii', genotype frequencies. Also, N means the total number of samples assayed; where ii indicates in how many individuals genotype 'ii' is there and individual upon whom ij exists at 'ij'. The Hardy-Weinberg equilibrium was tested (Hartl *et al*., 1997). Meat quality traits i.e., concentration of protein (to calculate fat free mass), crude lipid, PUFA contents and fatty acid composition in muscle was determined per genotype by a fixed-effects model using analysis of variance with t-test for multiple comparisons function PROC GLM implemented within the SAS system software version 9.4. Statistical Analysis All statistical analyzes were done using Minitab 18 software. In the past it was decided, p-value less than 0.05 in all studies will be considered statistically significant evidence of a relationship between factors Subsequently, the significant differences between genotype effects at pair-wise level were further analyzed with Tukey's post hoc test for:

$$Y_{ijk} = \mu + \text{genotypei} + E_{ijk}$$

Where (Y_{ijk}) is the composition of flavor odor, µ denotes the population average,genotypei represents the fixed effect of the i th genotype, and represents residual error.

Expression analysis

Regarding the expression of mRNA, this analysis will provide information regarding which flavor and odour compounds gene transcription was evaluated concerning PCYOX1. Gene expression was quantitatively compared to a reference gene s*et al*ready written by Gunawan *et al*., (2018).

$$\Delta CT = Ct_{\text{target gene}} - Ct_{\text{reference gene}}$$

ΔCT — ΔCT is used to test the expression and hence will be a way equivalent of gene on or off. This technique uses more than one reference gene to determine the geometric mean for normalizing each targeted sample and determines whether there is a difference between specific samples or conditions in their expression of genes. T-test post-doc analysis shows a significant difference in gene expression between groups.

STRING and KEGG analysis of molecular pathways

The specific information about the biological roles functionally annotated with PANTHER 19.0 and UniProt (UniProt, 2025) for these genes is shown in the functional analysis section. Protein analysis through evolutionary relationships (PANTHER) is another online gene over-representation categorization tool with added perspective on the regulatory connection to known gene families and pathways in terms of three general GO ontologies: molecular function, biological process, and cellular component. This is combined with the resource from UniProt, which provides complete protein sequence information that includes sequences along with protein structures and functions, in addition to post-translational modifications. For gene–gene interaction analysis, we used STRING version 12.0, which offers protein-protein interactions with experimental data as well as some machine learning and text mining information. Data integration improves transcriptomics over multiple spatio-temporal scales: This acts as an atlas to delineate major interactions, co-expression patterns, and potential shared biological pathways, which adds a layer of functional significance to the genes (Maulana *et al*., 2025).

Results

Polymorphism of the PCYOX1 gene

Primers 5′-ACG GTG CAA ACT AAA GTA AG-3′ (forward) and 5 ′ -TAC GTG AT G CT GA A GG TSG (reverse) were designed to amplify genomic DNA for the PCYOX1 gene fragment at SNP g.37912033 with NC_019460.2, producing a PCR product of 384 bp, respectively (Table 1). PCR was conducted at 62°C, and genotyping was performed with PCR-RFLP. In order to supplement the evidence, representative samples of the relevant genotype were sequenced to confirm whether the SNP mutations existed. The GG genotype produced a 384 bp band, the AG genotype demonstrated 384 bp, 267 bp, and 117 bp bands, and we observed from the sequenced data that the AA genotype showed the 267 bp and 117 bp bands, which confirmed that our amplified products were consistent with the mutations identified in GenBank (Figure 1). Gene expression was analyzed by qRT-PCR using cDNA and primers targeting PCYOX1, with GAPDH and β-actin used for normalization. Polymorphisms in PCYOX1 were assessed using the BspHI enzyme (Table 2), which revealed AA, AG, and GG genotypes. The AA genotype frequencies were 0.76 and 0.28, whereas the GG genotype was less frequent (0.08). The A allele (0.75) was more prevalent than the G allele (0.25), and PCYOX1 was considered polymorphic, with a combined genotype frequency of less than 0.99 in the JTTS and JS populations. The gene was in Hardy-Weinberg equilibrium in these populations.

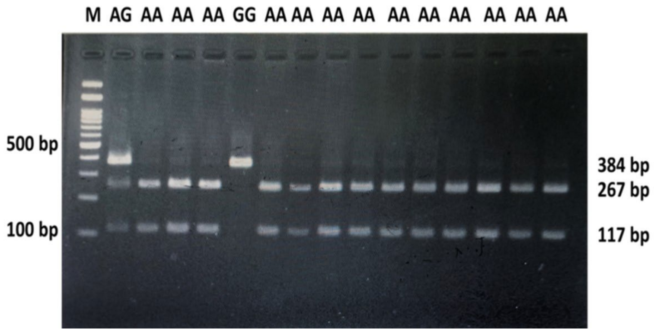

Figure 1. PCR-RFLP result of PCYOX1 gene; M= 100 bp ladder size standard; GG (384); AA (117 and 267 bp); AG (117, 267, and 384 bp) genotype; bp= base pair.

Table 2. The quantity of animals per genotype and the allele frequency.

Breed	N	Genotype frequency			Allele frequency		Chi-square (χ^2)
		AA (n=48)	AG (n=31)	GG (n=6)	A	G	
JTTS	71	0.55 (39)	0.39 (28)	0.06 (4)	0.75	0.25	0.12
JS	14	0.64 (9)	0.21 (3)	0.14 (2)	0.75	0.25	2.57
	85	0.56 (48)	0.36 (31)	0.07 (6)	0.75	0.25	1.89

N represents the number of samples, (85) indicates the count of samples with AA, AG, and GG genotypes, and χ2 table = 3.84. JTTS : Javanese Thin-tailed sheep, and JS : Jonggol Sheep.

Association of the PCYOX1 Gene with Flavor and Odor

PCYOX1, which contains a SNP (g.37912033 G>A), was positively associated with the perception of flavor and odor compounds, particularly 4-methyl-octanoic acid (MOA) and 4-methyl-nonanoic acid (MNA) (Table 3). The effect was most pronounced in individuals who were GG homozygotes, followed by those who were AG and AA homozygotes, respectively. Individuals with the AG genotype demonstrated a milder flavor and odor, accompanied by a significantly diminished mutton character in the MOA parameter compared to those with the AA and GG genotypes. Individuals with the AA genotype exhibited lower MOA values than those with AG and GG genotypes.

Table 3. Association of the PCYOX1 gene with flavor odor compounds.

Breed	N	Genotype frequency			Allele frequency		Chi-square (χ^2)
		AA (n=48)	AG (n=31)	GG (n=6)	A	G	
JTTS	71	0.55 (39)	0.39 (28)	0.06 (4)	0.75	0.25	0.12
JS	14	0.64 (9)	0.21 (3)	0.14 (2)	0.75	0.25	2.57
	85	0.56 (48)	0.36 (31)	0.07 (6)	0.75	0.25	1.89

μ represents the means of flavor odor; SE stands for standard error; ns indicates not significant

The mRNA expression level of the PCYOX1 gene

Significant differences were found in PCYOX1 mRNA expression levels (P<0.05) between the GG and AG genotypes. The AG genotype exhibited elevated expression levels compared to those of the AA and GG genotypes (Figure 2). The qRT-PCR mRNA expression analysis results corresponded with the association study findings, revealing that sheep with the AG genotype exhibited reduced levels of 4-methyl-octanoic acid and 4-methyl-nonanoic acid.

Analysis PCYOX1 Interaction and Pathway Enrichment

STRING analysis of proteins related to oxidoreductase activity (GO:0071704) and organonitrogen compound metabolism (GO:0022414) (Figure 3) revealed that PCYOX1 interacts with various proteins, including ART4, W5PY81_SHEEP, PIGQ, FADS1, and ELOVL5. The network diagram displays a complex graph with nodes representing genes or proteins

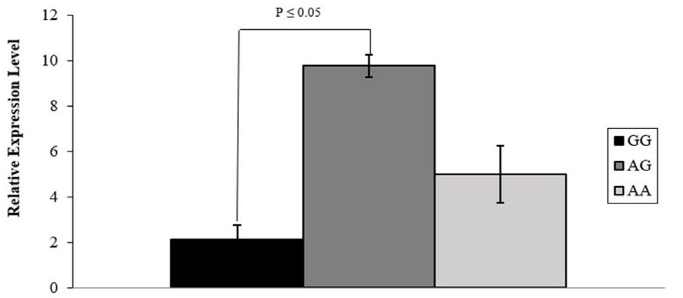

Figure 2. PCYOX1 gene mRNA expression w ith divergent genotypes for flavor odor traits using qRT-PCR.

connected by edges and organized into clusters. Nodes are color-coded (pink and white) and sized according to their connectivity and expression levels. Key genes such as "FADS1," "SCD," and "ELOVL5" are involved in fatty acid metabolism, with FADS1 encoding a desaturase, SCD converting saturated to monounsaturated fatty acids, and ELOVL5 extending long-chain fatty acids.

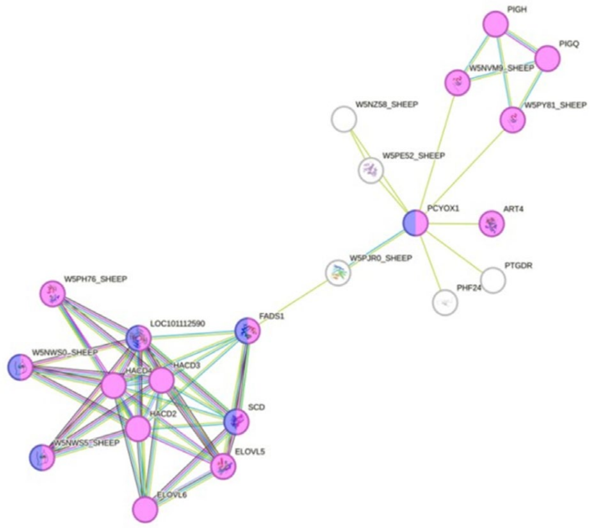

Figure 3. Gene interaction of PCYOX1 (STRING platform: https://string-db.org).

KEGG pathway analysis revealed that PCYOX1 (hsa:51449) encodes Prenylcysteine Oxidase 1, an FAD-dependent flavoprotein involved in prenylated protein degradation (Figure 4). This process produces cysteine, hydrogen peroxide (H_2O_2), and prenals such as farnesal. PCYOX1 plays a role in isoprenoid metabolic homeostasis, particularly through its involvement in terpenoid backbone biosynthesis (hsa00900). This pathway generates IPP and DMAPP, which are building blocks for terpenoid synthesis, leading to the formation of geranyl pyrophosphate, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate. Although primarily involved in catabolism, PCYOX1 plays an integral role in isoprenoid metabolism. KEGG Mapper (Figure 4) helps visualize PCYOX1's connection to the terpenoid pathway.

Discussion

The PCYOX1 gene, involved in fatty acid metabolism, plays a key role in determining the flavor and odor of sheep meat. Our data demonstrate that the g.37912033 G>A SNP in PCYOX1 is associated with the perception of important flavor and odor compounds, in particular 4-methyl-octanoic acid (MOA) and 4-methyl-nonanoic acid (MNA), which are both important constituents of mutton aroma. In Table 3, we demonstrate that the effect is greatest in GG homozygotes, followed by AG and AA homozygotes, suggesting GG homozygotes have the strongest mutton aroma, whilst AG and AA have softer flavors and aromas. Considering our result, the AG and AA genotypes may be more favorably viewed by consumers, particularly in Europe, North America, and Australia, where milder flavors are preferred, especially as consumers are unfamiliar with flavors that are exceptionally strong, which sheep meat usually possesses. The AG genotype was identified as having the least strong flavor; thus, marketing to targeted regions with the AG genotype should produce lower desired masses.

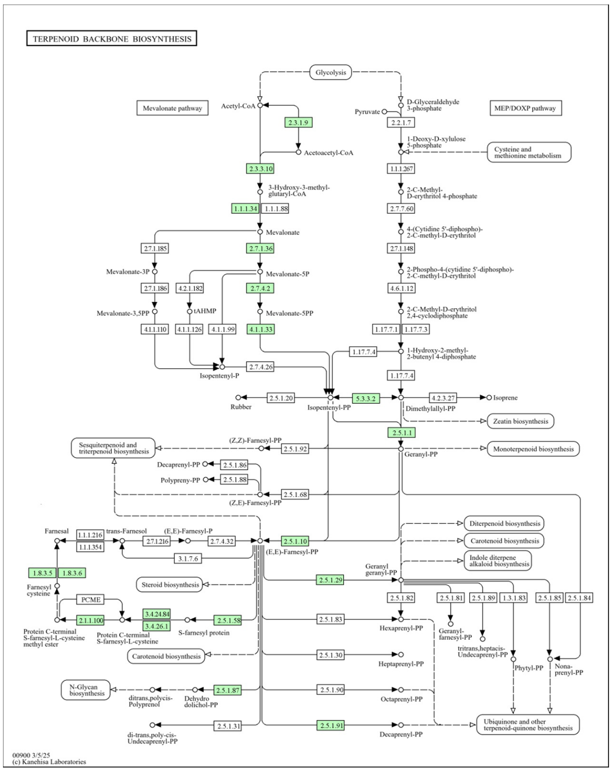

Figure 4. Analysis of pathways using the KEGG.

The PCYOX1 gene is polymorphic since the AA, AG, and GG genotypes were identified among sheep populations tested. The fact that the PCYOX1 gene is also polymorphic strengthens claims for a genetic basis of meat quality, because some variation in this gene seems to be at hand, given that genotype frequencies are less than 0.99 (Table 3). Polymorphism is a word that has been defined by law as an indication of polymorphism when it comes to a gene, which means the existence of two or more alleles at frequencies greater than 1% expressed in the population (Zelenskaya *et al*., 2020). Considering that AG and AA genotypes of both MOA and MNA groups had significantly lower values, this is in agreement with consumer liking of milder flavoured drinks. The results suggested that the PCYOX1 gene might affect meat quality traits and sensory characteristics. To increase consumer value in markets where a mild-tasting product is sought, only AG and AA genotypes would need to be selected for sheep meat.

PCYOX1 metabolizes fatty acids, thereby linking genetic variation in this gene by virtue of its ability to form MOA and MNA with meat sensory traits. The consideration of sheep meat as a potential food resource is largely due to its aroma, with the variability in production of compounds related to the expression level of depidides (depending on genotype), which provides support for an inherent functional involvement of PCYOX1 in flavor and odor. PCYOX1 is also related to fatty acid metabolism in some species (Banfi *et al*., 2023); this gene seems to participate biologically in any sensory trait linked with meat quality. On the contrary, there are several competitive advantages for the sheep meat industry in the genetic variability of PCYOX1. For example, breeding programs with sheep that have an A over G and also AA could produce meat to meet the global market's expectations regarding aroma and taste. The current study does not offer experimental validation of sheep meat quality traits, and further studies will be needed to experimentally validate the significant trait associations reported here in genetically heterogeneous animal populations using functional genomics approaches (such as gene editing) for comprehensive genomic description/detailing/regulation characterisation and annotation of PCYOX1 which could also lead toward developing recommended allele tests for genetic improvement programs aimed at providing better-quality meats that would meet consumer demand.

Consistent with the genetic analyses is the fact that, as shown in Figure 2, PCYOX1 expression was greater in AG than in AA ($P \leq 0.05$), which underscores the relationship between allelic variation within this gene and lipid oxidation. Although the present study supports an indirect association and does not prove a direct cause-and-effect relationship, increased expression of PCYOX1 in AG animals might favor lipids among metabolic substrates to form aromatic MOA (3-methylindole) and MNA (4-methylnonanol). The present results demonstrate the regulatory role of PCYOX1 in modulating sheep meat sensory traits. The expression levels of PCYOX1 may indeed differ amongst samples due to sample heterogeneity, incomplete datasets, and genetic differences (De Leeuw *et al*., 2023; Gamazon *et al*., 2015; Mancuso *et al*., 2017; Uygun *et al*., 2016). PCYOX1—It forms an intrinsic part of the regulation of degradation of prenylated proteins, crucial for redox balance and cell metabolism; It affects blood clotting (Banfi *et al*., 2022), inflammation, and adipogenesis (Banfi *et al*., 2023, 2022), among other cellular processes unfolded in mitochondria. Furthermore, increased activity of PCYOX1 results in the production of higher levels of H2O2, which could affect LDL oxidation and thus lead to loss of flavor or aroma during meat storage (Herpina *et al*., 2023; Herrera-Marcos *et al*., 2018). The favored taste associated with AG sheep meat was also related to preferred odor in our study (Figure 2).

STRING (Figure 3) and KEGG pathway analyses of the PCYOX1 gene suggest that FADS1, SCD, as well as ELOVL5 are associated with fatty-acid desaturation/elongation and thus represent hub genes together influenced by a single-collaborating event on some levels according to their structure/function relationships on network architecture (KEGG maps). This correlation suggests that the PCYOX1 protein affects the structural composition of fatty acids, thus affecting flavor characteristics due to its lipid peroxidation nature when associated with burning under heating. Additionally, the enzymatic activity of PCYOX1 in prenylcysteine degradation generates hydrogen peroxide (H_2O_2), which has been reported to promote lipid oxidation, leading to volatile aldehydes and ketones that are essential for mutton aroma formation. STRING and KEGG analyses of the PPI network indicate that PCYOX1 is a hub gene with interactions to FADS1, SCD, and ELOVL5, which are major factors in the biosynthesis as well as desaturation of fatty acids (Figure 3). Conclusions and significance taken together, we consider that the connectivity between PCYOX1 and lipid results supports a relevant key-regulatory role of this expression on meat flavor/odor traits through lipid oxidation. Prenylcysteine Oxidase 1 (PCYOX1), a protein catalyst of prenylcysteine degradation, provides cysteinyl building blocks for isoprenoids and H2O2 generation, which appears to be associated with lipid metabolism pathways. PCYOX1 could interact with lipoproteins and directly affect meat quality due to its influence on adipogenesis of muscle tissue development and lipid oxidation. The role of PCYOX1 in adipogenesis and its influence on fat storage further links it to meat flavor, as adipose tissue composition, particularly fatty acid profiles, determines the sensory characteristics (Figure 4). Oxidative processes, influenced by PCYOX1 enzymatic activity, generate compounds such as 2-alkylfuran, which contribute to desirable meat aroma. While direct studies on PCYOX1's impact on sheep meat quality are limited, the STRING and KEGG Pathway evidence strongly suggests its involvement in lipid metabolism, shaping the flavor and odor of meat.

The fatty acid composition of sheep meat, which is influenced by genes such as FADS1 and SCD, directly affects meat aroma (Clark *et al*., 2017). PCYOX1's role in producing hydrogen peroxide (H2O2) may enhance lipid oxidation, leading to aldehyde and ketone production, thereby influencing the flavor profile (Figure 4). The findings from bioinformatics resources (STRING, KEGG Pathway), relating to genetic diversity and possible PCYOX1 association with meat characteristics, support the analysis of potential genetic relationships. Bioinformatics resources help support the hypothesis and identify potential genetic interactions. The network diagram provides a conceptual framework for understanding these interactions.

PCYOX1 has a significant regulatory effect on lipid metabolism and important implications for the fatty acid composition of mutton sheep meat. This enzymatic activity is responsible for the taste and odor, which result from lipid oxidation. In addition, more experimental verification is needed to elucidate the underlying mechanisms of PCYOX1 on meat quality due to the limited current studies. Altered PCYOX1 expression in multiple tissues, including the brain and liver (Grabež *et al*., 2019), reinforces an expanded metabolic relevance. It could be expected that since sheep and other ruminants are phylogenetically related, a PCYOX1 ortholog should also exist in sheep. Sheep meat is also characterised by some volatile compounds (which in turn can make the raw and cooked odour properties), including terpenoids biosynthesised through lipid metabolism. In accordance with Grabež *et al*., (2019), slashing-like terpenoids, including β-caryophyllene, are positively related to grassy flavors in sheep meat. While some aldehydes, such as farnesal, may be associated with PCYOX1 products, direct evidence in sheep meat is limited. The terpenoid pathway and genes such as PCYOX1 modulate the flavor profile through volatile compounds. Although PCYOX1 primarily functions in degradation, its products, such as farnesal, may contribute to the sensory characteristics of sheep meat.

Conclusion

The PCYOX1 gene exhibits polymorphism in local sheep breeds, particularly within the JTTS and JS groups. A specific SNP (g.37912033 G>A) in the PCYOX1 gene has been found to have a significant association with the levels of 4-methyl-octanoic acid (MOA) and 4-methyl-nonanoic acid (MNA) in sheep. Sheep possessing the AA and AG genotypes showed favorable concentrations of these flavor compounds. As a result, the AA and AG genotypes are preferred over the GG genotype. Notable differences in the expression levels of the PCYOX1 gene were detected among the populations studied. Investigations using STRINGdb and KEGG pathways revealed that the PCYOX1 gene is crucial for flavor and odor characteristics. Thus, PCYOX1 can serve as a genetic marker for selecting sheep with reduced flavor odors.

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Conflict of interest

The authors have no conflict of interest to declare.

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