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Association, expression, and pathway analysis of biglycan gene with lamb quality and retail cut carcass

Muhammad Fathul Amin[1], Cece Sumantri[2], Irma Isnafia Arief[2], Anuraga Jayanegara[3], Kasita Listyarini[2], Ratna Sholatia Harahap[4] and Asep Gunawan[2*]

[1] *University of Islam Lamongan, Faculty of Fisheries and Animal Science, 62211, Lamongan, Indonesia*
[2] *IPB University, Department of Animal Production and Technology, Faculty of Animal Science, 16680, Bogor, Indonesia*
[3] *IPB University, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, 16680, Bogor, Indonesia*
[4] *University of Jambi, Faculty of Animal Science, 36122, Jambi, Indonesia*
[*] *Corresponding author:* agunawan@apps.ipb.ac.id

Abstract

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The quality of lamb meat and retail cut carcass traits are crucial determinants of meat quality, influencing consumer preference and high-quality lamb meat. The biglycan (BGN) gene, which regulates the formation and maintenance of connective tissues, including bone and cartilage, plays a crucial role in providing structural support strength and facilitating tissue healing and regeneration. This study examined the identification of polymorphism of the BGN gene in Indonesian sheep and its association, expression, and pathway analysis with lamb quality and retail cut carcass traits. Samples were collected from the longissimus dorsi muscle of 100 male animals (JTTS, BCS, CAS, GS, and JS), aged between 10 and 12 months, and analyzed for polymorphisms of the BGN gene using PCR-RFLP methods. The data were analyzed using the General Linear Model (GLM) test to investigate the association between genotype and phenotype characteristics. The expression levels of BGN mRNA were estimated using qRT-PCR, pathway, and network analysis using KEGG PATHWAY analysis. The BGN gene was polymorphic in different Indonesian sheep breeds. The BGN gene (g.77807325 G > C) exhibited two genotypes, GG and CC. The BGN polymorphism was significantly associated with ($P < 0.05$) lamb quality characteristics, such as pH value and retail cut carcass traits, including leg, muscle in the leg, muscle in the rack, shank, and muscle in the shank. The GG genotype demonstrated superior values for these traits compared to the CC genotype. The analysis of the BGN gene expression showed no significant difference ($P > 0.05$) between the GG and CC genotypes. The pathway analysis showed that the BGN gene is part of the small leucine-rich proteoglycans (SLRP) family, which plays a role in the muscle cell cytoskeleton that affects the meat quality and retail cut carcass. Sheep with the GG genotype showed improved values for several desirable traits. Thus, the G>C SNP of the BGN gene holds promise as a genetic marker for selection to improve the lamb meat quality and retail cut carcass of Indonesian sheep.

Keywords: biglycan; BGN; lamb quality; PCR RFLP; retail cut carcass; sheep

Introduction

Indonesia's lamb trade balance has been consistently in deficit over the years. Specifically, the deficit has increased by 18.77% annually over the last decade (Sujarwanta et al., 2024). This trend is largely influenced by consumer preferences for lamb quality. Choosing a quality meat product depends not only on the taste, but also on the right carcass cut. The quality of the meat, which is determined by its texture, tenderness, and fat content, greatly affects the pleasure of eating it. High-quality meat offers a great eating experience, with a more delicious taste and a more tender texture. Meanwhile, carcass cuts such as rib-eye or tenderloin, with their perfect layer of fat, provide a richer and more delicate taste. However, cuts such as sirloin or brisket, although more affordable, can be a great choice if prepared in the right way. The balance between quality and the chosen cut is the key for consumers to enjoy meat dishes. Therefore, to enhance both the quantity and quality of lamb meat, it is crucial to improve its consumption through selective breeding. Conventional selection, while effective, is time-consuming. Alternatively, molecular-based selection offers higher accuracy and a shorter selection time (Hasan et al., 2021). One such molecular technique, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), is known for its high yield, fast processing speed, and cost-effectiveness in identifying species and genes in sheep (Kayan et al., 2013). PCR-RFLP is widely used in various countries for single nucleotide polymorphism (SNP) detection, which is essential for analyzing genes affecting meat quality traits, their associations, linkage analyses, and gene expression (Listyarini et al., 2023).

The biglycan (BGN) gene is among 68 functional and related genes overexpressed in lamb liver, correlating with high tenderness levels. In this instance, a study by Listyarini et al. (2023) included RNA sequence analysis and discovered that the quality of lamb meat and the market value of carcass cuts may be significantly affected by the BGN gene. Located on the X chromosome and composed of eight exons, the BGN gene contains an SNP at position g.77807325 (G>C) in the three prime UTR (Untranslated region). Previous studies have demonstrated the role of BGN in regulating marbling in cattle (Jeong et al., 2017) and its influence on Angus, Brangus, and Simmental cattle carcasses (Oswalt et al., 2021). The BGN gene has been extensively studied in various species, including cattle (Heegaard et al., 2007) (Tripathi et al., 2012), pigs (Neufeld et al., 2014), rabbits (Fuller et al., 2016), horses (Alberi et al., 2018), mice (Ward et al., 2012), and humans (Zhao et al., 2022). The research is still limited to these animals and has not been conducted on sheep.

The BGN gene plays a crucial role in livestock lamb quality through its involvement in the structure and functionality of collagen fibrils, which are essential for tendon strength and flexibility. BGN and decorin, two proteoglycan categories, are key connective tissue components that influence collagen fibril composition and mechanical properties (Robinson et al., 2017). Studies have shown that genetic polymorphisms in genes, such as BGN, can significantly affect meat quality traits, as observed in cattle (Hsieh et al., 2014). Understanding genetic polymorphisms and their associations with the expression of the BGN gene, is essential to improve breeding programs, increase meat production efficiency, and ensure the health and resilience of livestock populations. The aim of the study was to determine the genetic polymorphism in the BGN gene and to evaluate its association, expression, and pathway analysis with lamb quality and retail cut carcasses in Indonesian sheep.

Materials and Methods

Ethical approval

The Animal Ethics Commission of IPB University, under permission number 117-2018 IPB, granted authorization for all animal procedures conducted in this study.

Animals

Association analyses of SNP in the BGN gene with meat quality of lamb used a total of 100 male lambs consisting of 60 Javanese thin-tail sheep [JTTS], 15 Jonggol Sheep [JS], 12 Garut Sheep [GS], 6 Composite Agrinak Sheep [CAS], 7 Barbados Cross Sheep [BCS]. The entire muscle longissimus dorsi sample was obtained to identify polymorphisms in the BGN gene. Additionally, liver tissues are used for gene expression, and biceps femoris is used for lamb quality assessment. The samples were placed in an ice flask and stored at a temperature of -20°C.

Study period and location

This study was conducted from June 2023 to May 2024. All the lambs were under similar control and management in the group pen system. This measure helps ensure that uniform care and handling are provided to the male lambs. Slaughtering was conducted at PT Pramana Pangan Utama (PPU), a halal-certified animal welfare-compliant abattoir. The sheep were sourced from different farms with similar animal husbandry practices.

Lamb quality analysis

Tenderness, pH, cooking loss, and water-holding capacity (WHC) were among the factors evaluated for lamb qual-

ity. Meat tenderness was measured using the Warner-Bratzler shear force (WBSF) technique. The WBSF technique is a method used to measure the force required to cut meat, which is then used as an indicator of meat tenderness. The carcasses were stored for 24 hours following slaughter, and the final pH was determined using a pH meter. This technique was applied in research conducted by Harahap et al. (2021). Cooking loss was assessed by measuring the weight of the meat samples both before and after cooking. This technique provides a straightforward approach to quantifying the amount of water lost during beef cooking.

Essentially, this technique is used to determine the extent of weight reduction in meat during cooking, mostly attributable to moisture evaporation during the cooking procedure. This approach is valuable for assessing the quality of meat and quantifying its ability to maintain moisture and texture throughout the cooking process. The sample was inserted into a bimetal thermometer, which is a temperature-measuring device that uses two different types of metals to detect changes in temperature. Subsequently, the sample was cooked in a water bath, which was a container filled with water that was heated to reach the desired temperature. The cooking process was performed until the internal temperature of the sample reached 80–81°C, ensuring that the sample had been cooked well and reached the desired temperature for research or testing purposes. The meat was then cooled to ambient temperature and reevaluated in order to determine the amount of weight lost throughout the cooking process (Wahyuni et al., 2018). The measurement of water holding capacity (WHC) was conducted using the Filter Paper Press Method (FPPM), as described by Hunt et al. (2011). In this method, a 0.3-gram sample was placed on a filter paper that had been pre-weighed. The sample was then pressed with a 10 kg weight for a duration of 5 min. Afterwards, the filter paper containing the sample was reweighed to calculate the WHC, following the procedure outlined by Harahap et al. (2021).

Retail cut analysis

The procedure of cutting an animal carcass into smaller parts was performed for analysis. Animal carcasses were divided into eight retail cuts: leg, loin, flank, shoulder, rack, breast, shank, and neck. Each cut was then further separated into meat, bones, and fat (subcutaneous fat under the skin and intermuscular fat between the muscles). After the separation, each component was weighed. The cutting and weighing procedure followed the method described by Amri et al. (2023).

DNA extraction, PCR-RFLP and genotyping

The muscle samples were extracted using the Geneaid gSYNC DNA Isolation Kit GS050/100/300, which was exactly in accordance with the usage instructions provided by the company (Geneaid, New Taipei City, Taiwan). The BGN gene fragment amplification process was carried out using GeneAmp® PCR equipment, which is a product of Applied Biosystems, Inc. of the USA. The PCR stages refer to the My Taq HS Redmix PCR kit guide, namely preheating at 95°C for 1 min for denaturation, followed by 35 cycles, which include denaturation at 95°C for 15 s, annealing at 65 °C for 15 s, and DNA extension for 10 s at 72 °C. The procedure includes a final extension step at 72° C for a duration of 1 min. This series of steps effectively allows for amplification of the specific region of the DNA of interest during the PCR analysis. Meanwhile, the electrophoresis method was utilized to trace the PCR products using 1.5% agarose gel. The outputs of this electrophoresis process were visualized using Thermo Fisher UV trans-illuminator EU Lithuanian GmbH, as reported by Lee et al. (2012). This process is commonly employed to visualize the PCR products and demonstrate the successful amplification of the desired DNA region.

The polymerase chain reaction (PCR) products were then digested using the *HinfI* enzyme from Thermo Fisher Scientific, EU, Lithuania, which was carried out at 37°C for a period of 4 hours. The digested product was separated on a 2.0% agarose gel that had been stained with FloroSafe DNA from 1st BASE, Singapore, and then observed using a UV transilluminator from Alpha Imager, Alpha Innotech, Santa Clara, USA to determine the specific genotype: GG (491 bp), CC (155, 336 bp), and GC (491, 336, and 155 bp) as shown in Table 1. We designed the BGN gene primers in cDNA and mRNA ourselves with reference to the SNP points from previous RNA sequencing research (Listyarini et al., 2023), for the GAPDH and B-Actin genes referring to Harahap et al. (2021) research.

RNA extraction and reversed transcription PCR

RNA was extracted from the liver tissue. The isolated tissue was extracted with the RNeasy Mini Kit (Qiagen) as per the producer's instructions. Quantitative real-time PCR (qRT-PCR) reverse transcribes to cDNA (First Strand cDNA, Thermo Scientific, Lithuania, EU).

Analysis of expression with quantitative real-time PCR

The BGN mRNA expression was evaluated using qRT-PCR, as it was found to have a significant association with one of the sheep in the association study on lamb tenderness. A quantitative real-time PCR study was performed to determine BGN gene expression based on genotype. Liver tissue samples for gene expression study were obtained from 100 rams: GG (n = 96) and CC (n = 4).

Table 1. Primer sequence and size of PCR of gene BGN

Gene	Accession number	Primer sequence	Application	TA (°C)	Size of PCR (bp)	Restriction Enzyme	SNP	Digest fragments length	Methodology Used
BGN	NC_019484.2	F:5'-GCT GAG GAG GGA TGT GTG TC-3' R:5' -CTT CAA CAA CCC CGT TCC CT-3'	Genotyping	62	491	*Hinf* I	g.77807325 G > C	GG = 491 GC= 491, 336 and 155 CC = 155 and 336	Designed with Primer3 https://primer3.ut.ee
BGN	NM_001009201.1	F:5'-CTT GGT CCT CGT GAA CAA CA -3' R:5'-GGA TGC GGT TGT CAT GGA TG-3'	qRT-PCR	65	161				Designed with Primer3 https://primer3.ut.ee
GADPH	NC_019460.2	F:5'-GAG AAA CCT GCC AAG TAT GA-3' R:5'-TAC CAG GAA ATG AGC TTG AC-3'	qRT-PCR	60	203				Harahap et al., (2021)
β-Actin	NC_019471.2	F:5'GAA AAC GAG ATG AGA TTG GC-3' F:5'CCA TCA TAG AGT GGA GTT CG-3'	qRT-PCR	60	194				Harahap et al., (2021)

Source: Authors' own elaboration

A total of six samples were chosen to represent two genotypes, GG and CC, each with three individuals. All primers were specifically designed to target ovine BGN, GAPDH, and β−actin sequences (Table 1). The SYBR Green Premix kit was used to conduct the qRT PCR. The reaction mixture of the PCR was 10 µL, which contained 5 µL of SYBR green premix, 0.5 µL of each of the forward and reverse primers, 2 µL of cDNA, and 2 µL of RNase-free ddH_2O. The pre-denaturation step was set at 95°C for 30 seconds, RT-PCR was performed at 95°C for 5 seconds, and 65°C for 30 seconds for 35 cycles of TaqGold polymerase. For melting, the temperature was increased to 95°C and held for 5 seconds and then 60˚C for 1 minute, and then the temperature was cooled down to 50˚C for 30 seconds. The housekeeping genes GAPDH and β-actin were utilized to normalize the target genes. The delta cycle threshold (ΔCt) was the final result, which was determined by the difference between the target's Ct value and the gene housekeeping's Ct value. The common t-test in Minitab Software was applied to analyze the differences in BGN mRNA expression levels. Statistically significant differences were defined as confidence interval values of 95% ($P < 0.05$).

Statistical analysis

The method described by Nei and Kumar (2000) and Furqon et al. (2017) was used to calculate genotype and allele frequencies. This method is a procedure used in genetic analysis to determine how often a particular genotype or allele variation occurs in a particular population.

$$Y_{ij} = \mu + \text{genotype}_i + E_{ij} \quad \text{and} \quad x_{ii} = \frac{n_{ii}}{N}$$

where: $X(i)$ = I allele frequency, x_{ii} = *ii* genotype frequency, N = total number of samples, n_{ii} = number of individuals with genotype *ii*, n_{ij} = number of individuals with genotype *ij*.

The Hardy-Weinberg equilibrium was determined using Wigginton et al. (2005). We used a fixed model (ANOVA) using PROC GLM, as reported by Esenbuğa et al. (2009), to assess how genotype affects lamb quality and retail cut the carcass in lambs. Data processing was carried out using Minitab® 18 software, as described by Christensen (2016). A limit of $P < 0.05$ is one of the general standards used to determine whether a result is considered statistically significant or not. When the results of an analysis have a P value of less than 0.05, this indicates that the possibility of the results appearing due to chance or measurement error is very small, so the results are considered statistically significant. In the context of research or data analysis, significance is often interpreted as the existence of a real relationship or difference between the observed variables. The genotype effects were compared using Tukey's model, which examined the pairwise differences. In this model, the variable Y_{ij} represents the quality of meat in the sample or individual, while the symbol µ describes the population average of the variable. Genotype, which is a fixed effect of the i^{th} genotype, is calculated to show how certain genetic variations may influence the observed lamb quality. Meanwhile, E_{ij} refers to residual errors in the model, which reflect variability or differences that cannot be explained by the genetic factors under study.

Expression analysis

Evaluation of the transcription of genes associated with the lamb quality and with the retail cut carcass. The expression levels of BGN mRNA were estimated by a ratio of the target gene to the geometric mean of the reference genes (ΔCT) by the formula given by Silver et al. (2006) as follows:

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

Pathways and network analysis

We conducted a biological pathway and network analysis of the BGN gene using KEGG PATHWAY analysis with the genome website (https://www.kegg.jp/pathway/oas04820), to identify and visualize the biological pathways associated with the BGN gene.

Results

Polymorphism of BGN gene

An SNP was found in the BGN gene at position g.77807325 G > C (Figure 1) in the sheep populations under investigation, indicating the presence of two genotypes, GG and CC (Figure 2). The GG genotype was characterized by a 491 bp band, while the CC genotype showed 155 bp and 336 bp bands. Table 2 shows that there were polymorphisms detected in Composite Agrinak Sheep (CAS), Barbados Cross Sheep (BCS), Garut Sheep (GS), Jonggol Sheep (JS), and Javanese thin-tailed Sheep (JTTS). Genotype frequencies indicated a higher occurrence of the GG genotype (0.96) with 96 individuals, compared to the CC genotype (0.04) with four individuals.

Association of BGN gene polymorphism with lamb quality

A BGN gene (g.77807325 G > C) polymorphism substantial ($P < 0.05$) associated with lamb quality parameters, especially pH value. The BGN gene polymorphism was not significantly associated with characteristics such as water-holding capacity (WHC), cooking loss, or tenderness. Sheep with the homozygous GG genotype had better pH values compared to those with the CC genotype (Table 3).

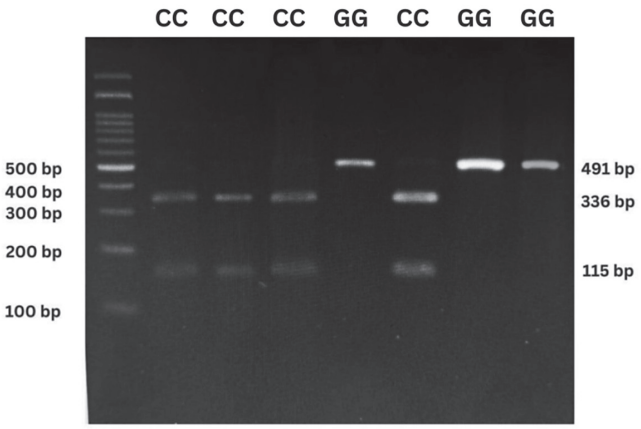


Fig. 2. PCR-RFLP result of BGN gene; M = 100 bp ladder size standard; GG (491) dan CC (155, 336 bp); genotype; bp = base pair

Source: Authors' own elaboration

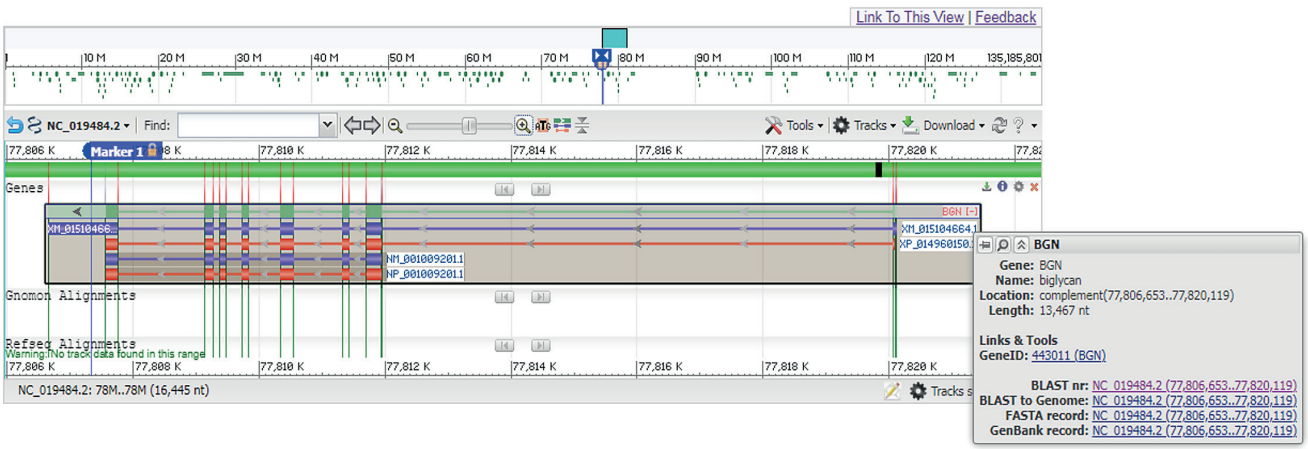


Fig. 1. Primer position (underline) and the target sequence (498 bp) in ovine BGN (GenBank: NC_019484.2) belonging to 3'prime UTR position: 77807325 and *Hinf* I restriction site (ATGG)

Source: Authors' own elaboration

Table 2. The number of animals per genotype and allele frequency of each sheep breed

Sheep Breed	N	Genotype frequency		Allele frequency		Chi-square (χ^2)
		GG (n = 96)	CC (n = 4)	G	C	
JTTS	60	0.99 (60)	0.00 (0)	1.00	0.00	–
JS	15	0.87 (13)	0.13 (2)	0.87	0.13	15.00
GS	12	1.00 (12)	0.00 (0)	1.00	0.00	–
CAS	6	1.00 (6)	0.00 (0)	1.00	0.00	–
BCS	7	0.71 (5)	0.29 (2)	0.86	0.14	7.00
	100	0.96 (96)	0.04 (4)	0.98	0.02	100.00

N, number of samples, (100) = number of samples which GG and CC genotype, χ^2 table = 3,84, JTTS (Javanese Thin Tails Sheep), JS (Jonggol Sheep), GS (Garut Sheep), CAS (Compass Agrinak Sheep), BCS (Barbados Cross Sheep).
Source: Authors' own elaboration

Table 3. Association of BGN gene with lamb quality

Parameters	N	Genotype (μ±SE)		P Value
		GG (n = 96)	CC (n = 4)	
pH	100	5.77±0.24[b]	6.34±0.03[a]	0.001
Tenderness (kg/cm^2)	100	3.76±0.07	4.38±0.49	0.09 [ns]
Cooking loss (%)	100	45.68±0.81	51.36±1.32	0.16 [ns]
Water Holding capacity (mgH_2O)	100	83.67±1.05	91.00±5.73	0.16[ns]
Water Holding Capacity % (mgH_2O)	100	27.94±0.36	29.58±1.34	0.16 [ns]

μ = means of lamb quality; SE = standard error; ns = not significant; [a, b] Mean in the same row with different superscripts differ significantly (P<0.05). The numbers shown in parentheses are the number of individuals with the specified genotype.
Source: Authors' own elaboration

Association of BGN gene polymorphism with retail cut carcass

The investigation revealed a noteworthy association (P ≤ 0.05) between the BGN gene polymorphism (g.77807325 G > C) and many retail cut carcass features, such as the leg, leg muscle, rack muscle, shank, and shank muscle. The GG genotype was associated with favorable measurements in the leg, leg muscle, rack muscle, shank, and shank muscle traits compared to the CC genotype (Table 4).

Table 4. Association of BGN gene with the composition of the retail cut carcass in the Indonesian sheep population

Variable / g	Genotype (μ±SE)		P Value
	GG (n = 96)	CC (n = 4)	
Leg	1450.00±0.03[a]	1140.00±0.17[b]	0.04
Muscle	962.50±22.50[a]	724±131[b]	0.04
Bone	400.36±7.75	344.70±36.90	0.17 [ns]
Subcutaneous fats	53.60±4.11	47.10±22.60	0.75 [ns]
Intramuscular fats	30.27±3.48	24.48±6.60	0.75 [ns]
Pelvic fats	13.73±1.26	5.45±3.19	0.19 [ns]
Loin	340.00±0.011	260.00±0.07	0.15 [ns]
Muscle	99.12±4.57	82.60±19.30	0.50 [ns]
Bone	189.22±5.73	139.30±40.30	0.09 [ns]
Subcutaneous fats	24.54±2.34	26.50±17.70	0.87 [ns]
Intramuscular fats	9.53±1.64	3.90±3.90	0.50 [ns]
Pelvic fats	27.73±2.75	29.9±18.70	0.81 [ns]
Flank	120.00±0.01	90.00±0.03	0.34 [ns]
Muscle	94.73±5.57	58.50±17.70	0.19 [ns]
Bone	0.00±0.00	0.00±0.00	[ns]
Subcutaneous fats	21.58±1.66	27.60±10.40	0.47 [ns]

Table 4. Continued

Intramuscular fats	9.81±2.49	6.03±3.80	0.77 ns
Pelvic fats	0.00±0.00	0.00±0.00	ns
Shoulder	760.00±0.02	650.00±0.10	0.26 ns
Muscle	482.20±12.9	357.6±64.00	0.05ns
Bone	195.08±6.16	207.0±19.40	0.66 ns
Subcutaneous fats	33.30±2.67	46.7±28.90	0.36 ns
Intramuscular fats	38.19±3.23	24.85±7.25	0.44 ns
Pelvic fats	0.00±0.00	0.00±0.00	0.82 ns
Rack	340.00±0.01	300.00±0.01	0.45 ns
Muscle	194.88±6.50[a]	119.4±41.80[b]	0.02
Bone	120.26±3.42	119.1±22.80	0.97 ns
Subcutaneous fats	19.88±2.02	19.9±12.10	0.90 ns
Intramuscular fats	10.74±1.24	7.65±4.24	0.64 ns
Pelvic fats	0.03±0.02	0.00±0.00	0.78 ns
Breast	400.00±0.01	400.00±0.105	0.10 ns
Muscle	205.35±6.01	151.60±29.80	0.08 ns
Bone	125.16±3.10	111.40±15.60	0.37 ns
Subcutaneous fats	32.11±2.36	23.80±13.80	0.51 ns
Intramuscular fats	30.39±2.65	14.20±6.13	0.22 ns
Pelvic fats	0.00±0.00	0.00±0.00	ns
Shank	390.00±0.01[a]	320.00±0.057[b]	0.02
Muscle	241.28±6.64[a]	161.60±21.70[b]	0.02
Bone	119.48±2.90	95.08±5.94	0.10 ns
Subcutaneous fats	14.98±1.17	12.60±4.61	0.71 ns
Intramuscular fats	7.53±0.68	2.05±1.19	0.10 ns
Pelvic fats	0.00±0.00	0.00±0.00	ns
Neck	380.00±0.01	390.00±0.085	0.54 ns
Muscle	227.50±11.1	179.8±45.80	0.39 ns
Bone	115.99±6.77	93.5±11.20	0.55 ns
Subcutaneous fats	18.16±2.81	23.70±6.72	0.68 ns
Intramuscular fats	12.04±1.73	11.6±11.60	0.98 ns
Pelvic fats	0.00±0.00	0.00±0.00	ns
Recover	4186±103	3306±622	0.11 ns

μ = means of retail cut carcass; SE = standard error; ns = not significant; [a, b] Mean in the same row with different superscripts differ significantly (P<0.05). The numbers shown in parentheses are the number of individuals with the specified genotype.

Note = designed using primer3; F = forward; R = reverse; GADPH = glyceraldehyde-3-phosphate dehydrogenase

Source: Authors' own elaboration

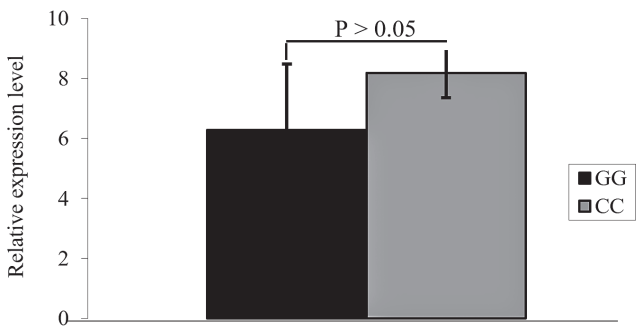


Fig. 3. mRNA expression of BGN gene in animals with divergent genotypes for lamb quality traits using qRT-PCR

The genotypes GG (black) and CC (dark grey) were placed in X-axis and Y-axis represents mRNA expression level of gene expression quantified with qRT-PCR. The mRNA expression was not significantly different between GG and CC genotypes (P > 0.05). BGN = biglycan; qRT-PCR = quantitative real-time polymerase chain reaction.

Source: Authors' own elaboration

Expression of BGN gene

There were no significant differences ($P > 0.05$) in BGN gene expression levels among the populations studied (Figure 3). However, the GG genotype showed lower total leg, leg muscle, rack muscle, calf muscle, and shank muscle and optimal total pH, indicating that lambs raised from GG the BGN genotype lambs would be better in terms of optimal pH content and retail cut carcass. The results of the study on gene expression showed that the BGN gene was downregulated.

Pathway analysis and network of BGN gene

The analysis of KEGG pathways revealed that the BGN gene was found to be part of the cytoskeleton during the signaling pathway of muscle cells, which influences lamb meat quality and the metabolic activities of retail carcass cuts (Figure 4). There are 17 members of the SLRP (small leucine-rich proteoglycan) family, which includes BGN, DCN, ECM2, ASPN, FMOD, LUM, KERA, OMD, PRELP, OGN, EPYC, OPTC, CHAD, NYX, TSKU, PODN, and PODNL1, which exhibit varying expression patterns for mean muscle cytoskeleton (Figure 4). More broadly, members of SLRP family are regarded as belonging to the class of extracellular matrix proteins along with: members of Versican family (ACAN, VCAN, BCAN), the basement membrane component family (HSPG2, AGRN, COL15A1, COL18A1, SMC3, P3H) and others (SRGN, SPOCK2, SPOCK1, PRG4, COL9A2, COL9A1, COL9A3, COL12A1, COL14A1), which are present within muscle cytoskeleton.

Discussion

The PCR-RFLP method effectively amplified the BGN gene fragment for polymorphism analysis at SNP g.77807325 G > C in Indonesian sheep samples. Two genotypes – GG and CC were identified, with the GC genotype being comparatively uncommon in the populations (JTTS, BCS, CAS, GS, and JS). The BGN gene SNPs did not follow Hardy-Weinberg equilibrium (HWE), likely due to factors affecting genotype distribution, such as mutation, natural selection, gene flow, non-random mating, heterozygous deficiency, and genetic drift (Febriana et al., 2022; Saputra et al., 2020). According to Hardy-Weinberg equilibrium, sexual reproduction maintains a constant level of genetic diversity across generations in the absence of other influencing factors (Griffiths et al., 2015). The deviations from HWE observed in this study could be attributed to random chance within the population.

Regarding lamb meat quality, the BGN gene SNP (g.77807325 G > C) was significantly associated with pH levels, with the GG genotype showing a favorable pH value of 5.7 in lamb meat (Table 4). This finding is consistent with previous studies reporting an optimal pH value of 5.7 in various sheep populations, such as Romney, Romney Cross, Perendale, and Mixed Sheep (Hernández-Castellano et al., 2013; Petersen et al., 1984). The association between the BGN gene and meat pH may be related to its role in gelatinase and collagenase activity, which influence lamb quality and texture (Schönherr et al., 1995). The mechanism of pH reduction in frozen meat involves the loss of minerals and small protein compounds during freezing and thawing, leading to changes in ion balance and a subsequent decrease in pH (Ernawati et al., 2022). Additionally, protein denaturation during freezing can result in the release of hydrogen ions, further contributing to the pH decrease (Leiphart et al., 2021).

The BGN gene SNP also exhibited a noteworthy association with other retail carcass cuts, such as the leg, leg muscle, rack muscle, shank, and shank muscle. The GG genotype exhibited superior values for these traits: leg (1450.00 g), leg muscle (962.5 g), rack muscle (194.88 g), shank (390.00 g), and shank muscle (241.28 g). The BGN gene plays an important role in the collagen filament assembly for the development of connective tissues, which influences the strength, structure, and tenderness of the meat (Leiphart et al., 2021). Other studies have explored the role of BGN in the embryonic development of muscles and tendons, thus confirming its role in muscle and connective tissues (Bowe et al., 2000). The BGN gene also influences the amount of intramuscular fat (IMF), and it is well-known that IMF influences meat quality in terms of taste, juiciness, and tenderness (Jager et al., 2013). According to the IFI study, this gene could influence the deposition of intramuscular fat and, thus, the quality of meat in livestock (Han et al., 2020). Fat deposition and collagen regulation are the two most important components of meat quality, and so is the BGN gene for both of these (Han et al., 2020). The BGN gene regulating lamb meat quality is all about the interactions of genetic determinants affecting the characteristics of the meat. Genes such as BGN are necessary for coordinating muscle development and parameters of meat quality, and the functional characteristics of meat are not compromised (Salimi et al., 2021). The enhanced proliferation of cells has been associated with the expression of BGN, and this suggests that this protein may be involved in tissue development, which may impact meat quality attributes (Salimi et al., 2021).

The BGN is associated with multiple biological processes such as bone growth, muscle specification, and tissue regeneration (Wang, 2023). Forty, this describes BGN like genes concentrated in muscle regeneration, fibrosis, and calcification regions, leading to speculation about BGN role

in those regions (Heezen et al., 2022). BGN knockout: for instance, better growth and more plasma levels of growth factor-β, to mention some of the signaling pathways, BMP4 etc., have been discovered in normal body fat can illustrate the importance of BGN (Tang et al., 2012). Additionally, BGN may play key roles in muscle growth and repair due to its interaction with actin in skeletal muscle (Jørgensen et al., 2017). In particular, multiple studies highlighting the role of BGN expression and function in different human tissues explain its role in the pathophysiology of many conditions. In the anterior vertebral ligament, hypertrophy in BGN expression is mediated through a mechanism involving cell proliferation and differentiation (Salimi et al., 2021). This may be due in part to studies in animals, particularly mice, which go a long way toward establishing a role for BGN in musculoskeletal health (Ameye et al., 2002). The BGN gene is associated with the COL3A1 gene and has an impact on lamb meat quality by regulating type III collagen. A notable example is this regulation, which plays a role in the critical quality traits of lamb, such as tenderness, water-holding capacity, and intramuscular fat content (Chuan-hao et al., 2016). Furthermore, the BGN gene is also merged with the DCN gene, which is involved in the regulation of collagen fibrils as well as the biomechanical properties of muscle tissue (Zhang et al., 2016) (Sevane et al., 2013). A potential application of these genetic interactions can be used as markers in selective breeding programs aiming to enhance lamb meat quality. In this research, we noticed a significant correlation between BGN and COL3A1, which is consistent with the previous finding, indicating the value of these genes as genetic markers. This association of BGN with the DCN gene strengthens the association between collagen regulation and lamb meat quality.

The BGN gene, a member of the small-leucine-rich proteoglycan family, is a core component of the extracellular matrix that is involved in many important physiological processes, such as skeletal development, metabolic regulation, and tissue repair. The extracellular matrix is a dynamic macromolecular network that provides a three-dimensional infrastructure and regulates cell behavior in tissues. As an example, proteoglycans, including BGN, are critical components of the extracellular matrix, regulating its assembly, organization, and signaling capacity (Nallanthighal et al., 2019). BGN interacts with several growth factors, cytokines, and other extracellular matrix molecules, functioning as a co-receptor to alter downstream signaling pathways. Recent evidence implies that BGN is critical for the regulation of muscle mass and metabolism. Skeletal muscle is an essential tissue for movement and plays a significant role in the regulation of whole-body metabolism and glucose homeostasis, according to Zhao et al. (2022). Signaling pathways like IGF1-Akt/PKB have been extensively investigated for their role in muscle growth and adaptation (Schiaffino and Mammucari, 2011). Interestingly, BGN is known to modulate these pivotal signaling pathways, which may play a role in the development, maintenance, and plasticity of skeletal muscle. In the new study, the researchers describe the potential role of BGN in muscle wasting, a common occurrence in disease and aging.

The BGN gene is an important regulator of muscle mass, suggests a potential therapeutic target for muscle-wasting diseases. In addition to its function on the skeletal muscle, BGN is implicated in lipid metabolism regulation and the accumulation of fatty acids in tissues. Proteoglycans such as BGN bind lipid-associated molecules, modulating lipid transport, deposition, and/or oxidation, which affects fatty acid composition in meat and other tissues. Studies have shown contradictory findings in BGN gene expression in skeletal and liver tissue. Inconsistent theory: For example, while some studies reported downregulation of BGN (Nastase et al., 2012), others up regulation (Bianco et al., 1990). The BGN is a small leucine-rich proteoglycan involved in the regulation of inflammation and the musculoskeletal system.

The BGN is expressed in multiple cell types, including both connective tissue and epithelial cells (Nastase et al., 2012). According to Bianco et al. (1990), the skeletal system uses BGN as a structural and signaling regulator in processes that control bone and cartilage homeostasis (Kram et al., 2020). In the liver, 70 - 85% of the volume of the liver is made up of hepatocytes, which are also capable of expressing and secreting various proteins, including those playing a role in innate immunity (Kram et al., 2020). Zhou et al. (2016) reported that BGN might also contribute to immune function in the liver. Some data describe its expression and localization in human tissues during development, where they could find spatially limited expression and localization of the matrix in close association with skeletal tissues and specific developmental events (Bianco et al., 1990). The BGN distribution in these tissues also indicates that this protein has cell-regulatory functions and a role in structural biology. The differences observed in expression patterns of BGN in both muscle and liver tissues may be attributed to several variables, including both differences in the types of cells and the stages of development analyzed, as well as variations in experimental conditions. The BGN in the tissues remains to be fully elucidated through further study (Nastase et al., 2012) (Bianco et al., 1990). These lead to the conclusion the gene BGN could influence carcass quality in lamb and retail-cut meat.

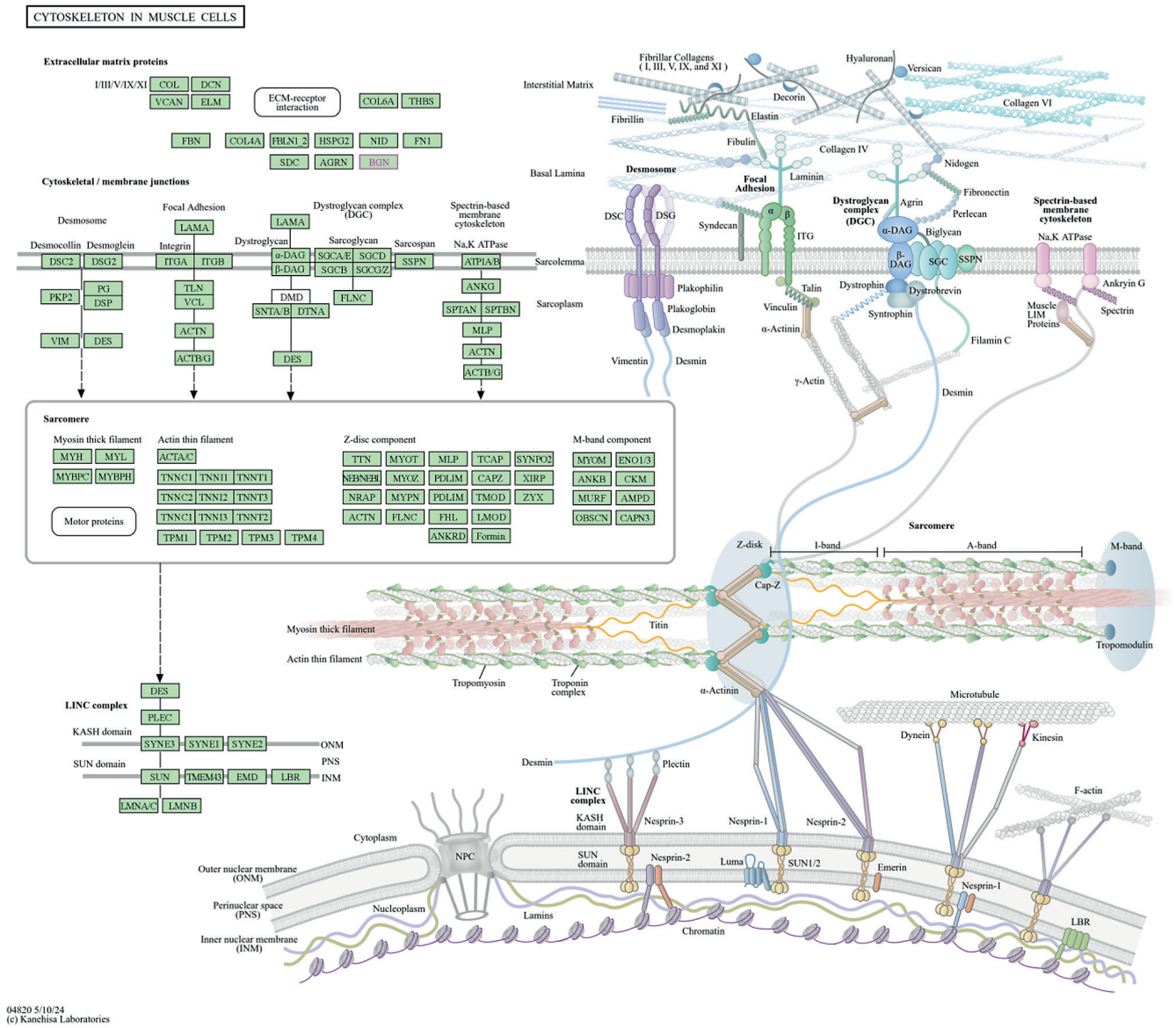


Fig. 4. Metabolic pathway of BGN gene (KEGG pathway, 2024)
Molecular interaction or relation = indirect link or unknown reaction
Source: Authors' own elaboration

KEGG pathway analysis confirmed and visualized the involvement of the enriched BGN gene in the cytoskeleton-activated receptor signaling pathway (Figure 4). The BGN is a muscle-device protein involved in protecting cardiac cells. The diagram it provides illustrates that BGN binds to multiple elements of the extracellular matrix (collagen, decorin, and other matrix molecules). These interactions are vital for muscle cells and skeletal muscle tissue to become strong. Moreover, BGN plays a major role in cytoskeleton-targeting within muscle cells as well. It aids in stabilizing cell-matrix adhesion sites like focal adhesions, integrins, and the dystroglycan complex (DGC). DGC transfers the mechanical characteristics of the ECM into the cell responsible for muscle contraction. The BGN also assists in the agglomeration of DGC and the CMC matrix protein, which guarantees automatic contraction of various tissues, transmission of im-

portant signals, and rapid detection and repair of damaged tissues. Defects and mutations of the BGN gene result in abnormal muscle contractility as this important interaction fails. The connective tissue's role in skeletal muscle function is described through these features. Muscle cytoskeleton describes the structure and function of myocyte's cytoskeleton (a system of protein filaments that organize elements in cells and dictate their physiology) in myocyte's (muscle's) cytoplasm. Its role is in a wide range of cellular functions, from muscle contraction to signaling and maintenance of cell shape. Actin filaments (or microfilaments), myosin filaments, intermediate filaments, microtubules, and dystrophin-glycoprotein complexes are the predominant elements of the cytoskeleton in muscle cells. The cytoskeleton forms structural support, modulates cell morphology, and affects muscular tissue integrity and durability that can, in case of favourable ECM interactions, lead to improved meat quality (Zhou et al., 2016; Stromer, 1998).

Conclusions

The BGN gene was polymorphic in different Indonesian sheep breeds of JTTS, BCS, CAS, GS, and JS. The SNP g.77807325 of the G > C BGN gene was significantly associated with pH value, leg, muscle in the leg, muscle in the rack, shank, and muscle in the shank. For sheep with GG genotypes, the values of some profitable traits were higher. For this reason, the GG genotype is desirable for some desirable traits. The expression of the BGN gene showed no significant difference in the expression level of the BGN gene among the populations studied. Analysis of pathways revealed was found to be part of the cytoskeleton during the signaling pathway of muscle cells, which influences lamb meat quality and the metabolic activities of retail carcass cuts. The BGN gene SNP of G > C is a potential marker for selecting sheep meat with appropriate lamb quality and retail cut carcass traits.

Conflict of interest

The authors have no conflict of interest with any personal, financial, or other relationship with people or other organizations regarding the material discussed in the manuscript.

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