# Polymorphism and association of the BGN gene with cholesterol in lamb meat

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**Abstract.** Cholesterol levels in meat serve as an assessment for individuals selecting healthy meat options, correlating with an increased understanding of its importance to health. Biglycan (BGN) is hypothesized to influence cholesterol levels in lamb meat. This research aims to find variations of the BGN gene as potential genetic determinants of cholesterol levels in lambs. A total of 87 rams were analyzed for polymorphism using the PCR-RFLP method. The association between BGN gene polymorphisms and cholesterol in lamb meat was examined using a General Linear Model (GLM). This results from polymorphism by exhibiting two genotypes (GG and CC). A significant association ($p < 0.05$) was observed between BGN and cholesterol levels in lamb meat. Notably, rams with the GG genotype had significantly lower cholesterol levels when compared to those with the CC genotype. These results indicate that BGN is a good option for genetic selection to lower cholesterol levels in lambs.

## 1. Introduction

The global demand for red meat, including lamb, has experienced sustained growth driven primarily by rapid population expansion and rising per capita income levels in developing nations [1]. This trend is particularly evident in Southeast Asia, where increasing affluence has shifted consumer preferences toward protein-rich diet. In Indonesia, sheep farming is essential for meeting the escalating demand for red meat, which is a critical source of animal protein. The sheep production sector in the country remains predominantly characterized by small-scale farming operations that rely on local breeds, which are well adapted to the region's environmental conditions and cultural practices [2]. These local breeds not only contribute to food security but also hold significant economic, socio-cultural, and religious

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importance, supporting rural livelihoods and community traditions such as sacrificial offerings during religious ceremonies[3].

Meat quality is a multifaceted attribute that encompasses nutritional composition, sensory characteristics, safety, commercial value, and technological properties, all of which are essential for meeting consumer expectations. Among these, the nutritional profile, particularly cholesterol content, has gained increasing attention owing to the growing health consciousness among consumers. Studies conducted in diverse markets, such as Australia and Nigeria, indicate that health-conscious consumers prioritize meat products with lower cholesterol levels, thereby influencing purchasing decisions and market trends [4]. This consumer preference underscores the need for research aimed at improving meat quality through genetic and molecular approaches, particularly in regions such as Indonesia, where sheep are a vital livestock resource.

Recent advancements in molecular biology have enabled the identification of genes linked to meat quality traits in livestock, including Indonesian sheep. Research has focused on traits such as fatty acid composition, carcass yield, and cholesterol content, with transcriptomic analyses revealing the biglycan (BGN) gene as a candidate linked to meat tenderness in improved Indonesian lambs [5]. BGN is part of the short leucine-rich proteoglycan (SLRP) family, is known for its role in maintaining connective tissue integrity and regulating lipid metabolism through interactions with collagen and lipoproteins [6]. Elevated BGN expression has been implicated in atherosclerosis and is associated with increased cholesterol levels in other species, suggesting a potential link between BGN and cholesterol metabolism in sheep [6]. Despite these findings, molecular characterization of the BGN gene and its specific relationship with cholesterol levels in Indonesian sheep remains underexplored. This study sought to address this gap by identifying polymorphisms in the BGN gene and evaluating their association with cholesterol levels in Indonesian sheep, thereby contributing to the development of improved breeding strategies for enhanced meat quality.

## 2. Materials and methods

### 2.1 Ethical approval and animal collection

This research project has been ethically authorized by the IPB University Animal Ethics Commission (117–2018). This study focused on genotyping biglycan polymorphisms in 87 male lambs. The lambs, aged 10–12 months and weighing 25–30 kg, were obtained from different farms that utilized similar husbandry practices. The sample included 14 Jonggol sheep (JS), 53 Thin-tailed sheep of Java (JTTS), 5 Fat-tailed sheep of Java (JFTS), 4 Compass agrinac sheep (CAS), 5 Garut-Composite-Sheep (GCS), and 6 Barbados-Cross-Sheep (BCS). Slaughtering was performed at PT Pramana Pangan Utama, a commercial slaughterhouse certified by the Indonesian Ministry of Agriculture, strictly adhering to halal guidelines and establishing animal welfare standards.

### 2.2 Cholesterol analyses

HPLC was employed to determine the cholesterol levels in lamb meat, high-performance liquid chromatography (HPLC) was employed following the procedure outlined by Bragagnolo et al. [7]. The process began by placing 10 mg of fat in a 10-mL volumetric flask. A small amount of 2-propanol was then added, and the mixture was ultrasonicated for a few minutes to aid cholesterol dissolution. The solution was subsequently filtered through a 0.45-micron Millipore membrane before analysis using HPLC. HPLC operates on the principle of separating the analyte components based on their polarity. As each component exited the column, it was detected and represented as a peak in the chromatogram. The number of peaks signifies the number of distinct components, while the area beneath each peak represents the concentration of that component within the overall mix. HPLC is particularly advantageous over Gas Chromatography for the analysis of non-volatile substances, making it a versatile technique for applications such as determining molecular weights, quantifying substances, conducting clinical trials, forensic investigations, food analysis, environmental chemistry, and analytical chemistry.

### 2.3 Extraction of DNA, amplification processes, and genotyping techniques

The Geneaid gSYNC DNA Isolation Kit (GS050/100/300, Geneaid, New Taipei City, Taiwan) can be used to extract the essence of life from muscle tissue, capturing the intricate tapestry of genomic DNA. We followed the manufacturer's instructions to make sure the DNA was very pure. DNA suitable for studying biglycan gene polymorphisms, with quality confirmed by spectrophotometry (A260/A280 ratio of 1.8–2.0). A GeneAmp PCR System from Applied Biosystems was used to make more copies of the biglycan gene fragment with a reaction mixture containing template DNA, specific primers, high-fidelity polymerase, dNTPs, and buffer, employing a thermal cycling protocol of initial denaturation step at 95°C for one minute, 35 cycles consisting of denaturation at 95°C for 15 s, annealing at 62°C for 15 s, and extension at 72°C for 10 s, followed by a final 1-minute extension at 72°C and stabilization at 15°C for 5 min. Amplification was verified by electrophoresis on a 1.5% agarose gel in TAE buffer, stained with a safe DNA dye, and visualized under UV light to confirm the expected amplicon size. The PCR products were digested with *HinfI* (Thermo Fisher Scientific, EU, Lithuania) at 37°C for 4 h, and the resulting fragments were separated on 2.0% agarose gel, stained with FloroSafe DNA dye (1st BASE, Singapore), and A UV transilluminator (Alpha Imager, USA) was used for visualization. This approach enabled genotype identification based on the fragment patterns GG (491 bp), CC (155 bp and 336 bp), and GC (491 bp, 336 bp, and 155 bp), ensuring accurate and reproducible genotyping for downstream genetic analysis.

**Table 1.** The size of the PCR result and the primer sequence for the Biglycan gene.

| Gene | Accession number | Sequence | Application | TA (°C) | Size (bp) | Enzyme | SNP | Digest fragments Length |
|---|---|---|---|---|---|---|---|---|
| Biglycan | NC_019484.2 | Forward: 5’-GCTGAG GAGGGAT GTGTG TC-3’<br><br>Reverse: 5'-CTTCAAC AACCCCG TTCCCT-3' | Genotyping | 62 | 491 | *Hinf*I | g.77807325 G > C | GG = (491)<br>GC = (491, 336 and 155)<br>CC = (155 and 336) |

Note : designed with the Primer3 website.

Primer design and analysis for genetic identification of the BGN gene (biglycan) in the region with the NCBI accession code NC_019484.2. The BGN gene primer sequences in the forward and reverse sections were designed using *Primer3* with a fragment length of 491 bp. The BGN gene polymorphism at SNP g.77807325 was analyzed using the *HinfI* restriction enzyme with a substitution from G to C using the PCR-RFLP method, with an estimated genotype code of GG, GC, and CC. The PCR method using this restriction enzyme was used to determine the differences between the genotypes. Hubacek et al. [8] stated that PCR-RFLP detection can reveal differences between genotypes. The PCR-RFLP method, which is based on restriction enzymes, has been widely used to analyze differences in the genotype of a gene. This technique uses fragments of DNA produced by restriction enzymes to distinguish genetic variations between species or individuals. PCR-RFLP is very useful for mutation detection, genotype identification, and research on complex genetic relationships, including variations in single nucleotide polymorphisms (SNPs). This method is frequently chosen because it produces accurate results by cutting specific parts of different DNA from individuals with other genotypes. For example, PCR-RFLP has been used in genotype analysis of various organisms, such as humans, animals, and plants, to detect genetic variations associated with certain diseases or traits. Previous studies on the BGN gene with lipid regulation Nastase et al. [9] showed that the BGN gene has the potential to affect cholesterol metabolism, where this genetic variation may modulate cellular interactions in adipose tissue and the liver, thus having implications for total cholesterol and Low-Density Lipoprotein (LDL) levels.

### 2.4 Statistical analysis

The determination of genotype and allele frequencies was conducted in accordance with the methodologies established by Furqon et al. [10], This standard population genetics approach is conventionally utilized to quantify the prevalence of specific genotypes and alleles within a given population. The calculations were based on the following formulas:

$$X_i = \frac{(2n_{ii}+\sum j\neq j n_{ij})}{2N} \quad \text{and} \quad x_{ii} = \frac{n_{ii}}{N}$$

The allelic and genotypic frequencies were calculated using established formulas, where Xi signifies the frequency of the ith allele, xii represents the frequency of the corresponding genotype, and N is the total number of samples. The variable nii represents the number that are having the ii genotype, and nij indicates the number of individuals with the ij genotype. To determine whether the population was in *Hardy-Weinberg equilibrium* (HWE), the methods outlined by Wigginton et al. [11] the influence of genotype on cholesterol levels was examined using a fixed-effects model within an ANOVA framework, specifically employing the General Linear Model procedure. This statistical analysis was executed using Minitab® 18 software, adhering to the data treatment protocols described by Christensen [12]. For the purpose of this investigation, statistical significance was established at a p-value of less than 0.05, which served as the threshold for assessing the impact of genotype on the observed variations in cholesterol.

The method used by the statistical model:

$$Y_{ij} = \mu + \text{genotype}_i + E_{ij} \qquad (1)$$

Yij represents the evaluated cholesterol level, μ represents the population mean, i represents the genotype-specific fixed effect, and Eij represents residual error.

# 3. Results

## 3.1 Polymorphism of biglycan gene

The identification of the biglycan gene using the HinfI restriction enzyme and PCR-RFLP method represents a significant advancement in genetic analysis. This approach allows for the precise differentiation between the GG and CC genotypes based on the resulting fragment patterns. The selection of HinfI as the restriction enzyme was crucial, as its specificity in recognizing and cleaving particular DNA sequences was confirmed through NEBcutter analyses. This specificity is essential for accurately distinguishing between genotypes, as the mutation occurs at a specific location within the BGN gene sequence. The observed difference in fragment sizes between the GG and CC genotypes provides valuable insights into genetic variation within the biglycan gene. The GG genotype, characterized by a single 491 bp fragment, indicates the absence of a mutation at the cleavage site. In contrast, the CC genotype, which yields two distinct fragments of 115 and 336 bp, demonstrates the presence of a mutation that creates an additional cleavage site for HinfI. This clear distinction in fragment patterns allows for reliable genotype identification and opens up possibilities for further research into the functional implications of this genetic variation in biglycan expression and its associated physiological effects in the future.

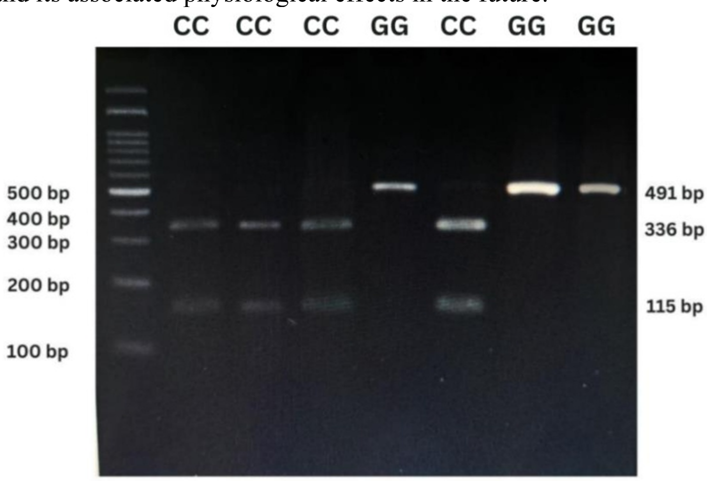


**Fig. 1.** A 100 bp marker and a 2% agarose gel were used to identify the BGN gene with GG and CC genotypes.

The analysis of BGN gene polymorphisms in Indonesian sheep populations revealed the presence of two distinct genotypes, GG and CC, with the GG genotype exhibiting a significantly higher frequency of approximately 0.95 across the studied breeds (Table 2). By contrast, the CC genotype was observed at a much lower frequency (approximately 0.05). This distribution indicates a strong predominance of the G allele over the C allele in the BGN gene within the sampled population, which included Javanese Thin-Tailed Sheep (JTTS), Jonggol Sheep (JS), Javanese Fat-Tailed Sheep (JFTS), Compass Agrinac Sheep (CAS), Garut Composite Sheep (GCS), and Barbados Cross Sheep (BCS). The high prevalence of the G allele suggests that it may confer adaptive advantages or may be subject to selective pressures that favor its retention in these populations, particularly in breeds such as JTTS and CAS, where the GG genotype reached a frequency of 1.00.

The presence of both G and C alleles confirms that the BGN gene is polymorphic in Indonesian sheep, as polymorphism is defined by the existence of variant alleles with a frequency of at least 1% in a population. This polymorphism is significant because it may influence phenotypic traits such as meat quality, given the role of the BGN gene in connective tissue integrity and lipid metabolism. For instance, the association of BGN with cholesterol levels and tenderness, as previously identified in transcriptomic studies, suggests that these polymorphisms could have practical implications for sheep breeding programs aimed at improving meat quality traits, such as reduced cholesterol content, to meet consumer health preferences.

Chi-square analysis further revealed that the BGN gene was not in *HWE* across the studied populations, as the calculated chi-square values for breeds such as JS (14.00), JFTS (50.00), GCS (5.00), and BCS (6.00) exceeded the critical threshold of 3.84 [13]. This departure from the HWE suggests a notable discrepancy between the actual and anticipated genotype frequencies, suggesting that the population is subject to evolutionary forces that disrupt the genetic equilibrium. Several factors may have contributed to this imbalance. Natural selection may favor the GG genotype if it is associated with desirable traits, such as improved meat tenderness or lower cholesterol levels, which could enhance the survival or marketability of certain breeds. Genetic drift, particularly in small populations such as GCS and BCS, could also lead to allele frequency changes over time due to random sampling effects. Additionally, genetic migration, such as the introduction of new genetic material through crossbreeding, as seen in BCS sheep, could alter allele frequencies and disrupt HWE. For example, the Barbados Cross Sheep, a hybrid breed, may reflect the influence of genetic introgression from imported breeds, thus contributing to the observed deviations.

Moreover, variations in sample size or potential genotyping errors could further explain the departure from HWE. The small sample sizes for some breeds, such as CAS (N=4) and GCS (N=5), may limit the statistical power of the analysis, potentially inflating chi-square values and leading to an overestimation of the disequilibrium. Genotyping errors, although minimized through rigorous laboratory protocols, can also introduce bias in allele frequency estimates. To address these issues, future studies should employ larger sample sizes across diverse breeds and incorporate advanced genomic techniques, such as next-generation sequencing to validate the observed polymorphisms and their functional implications. Additionally, exploring the association between BGN polymorphisms and specific meat quality traits, such as cholesterol content and tenderness, could provide actionable insights for selective breeding programs aimed at enhancing the nutritional and commercial value of Indonesian sheep. These efforts would support sustainable livestock production and align with the consumer demand for healthier meat products.

**Table 2.** This table shows genotype-specific allele frequency and animal numbers.

| Breed | N | Genotype | | Allele | | Chi-square ($\chi^2$) |
|---|---|---|---|---|---|---|
| | | GG (n=83) | CC (n=4) | G | C | |
| **JTTS** | 53 | 1.00 (53) | 0.00 (0) | 1.00 | 0.00 | - |
| **JS** | 14 | 0.93(13) | 0.07 (1) | 0.93 | 0.07 | 14.00 |
| **JFTS** | 5 | 0.98 (49) | 0.02 (1) | 0.98 | 0.02 | 50.00 |
| **CAS** | 4 | 0.98 (4) | 0.00(0) | 1.00 | 0.00 | - |
| **GCS** | 5 | 0.80 (4) | 0.20 (1) | 0.80 | 0.20 | 5.00 |

**Table 2.** This table shows genotype-specific allele frequency and animal numbers (continue).

| Breed | N | Genotype | | Allele | | Chi-square ($\chi^2$) |
|---|---|---|---|---|---|---|
| | | GG (n=83) | CC (n=4) | G | C | |
| BCS | 6 | 0.67 (4) | 0.33 (2) | 0.67 | 0.33 | 6.00 |
| Total | 87 | 0.95(83) | 0.05 (4) | 0.95 | 0.05 | 87.00 |

N denotes the total number of samples (87), with GG and CC genotypes. The $\chi2$ value was 3.84. The breeds included are JTTS (Javanese Thin-Tailed Sheep (JTTS), Jonggol Sheep (JS), Javanese fat-tailed sheep (JFTS), Compass Agrinac Sheep (CAS), Garut Composite Sheep (GCS), and Barbados Cross Sheep (BCS).

## 3.2 Biglycan gene polymorphism and cholesterol

The study's findings on biglycan's significant association with cholesterol level variations in Indonesian sheep provide valuable insights into the complex interplay between genetic factors and lipid metabolism. The GG genotype in these sheep corresponded to a cholesterol level of 93.6 mg/100 g, positioning it between the higher levels observed in Beni Guil and Timahdite sheep (100-120 mg/100 g) and the lower levels found in Sardinian sheep (61.92-66.95 mg/100 g). This comparative analysis highlights the genetic diversity across sheep breeds and its potential impact on cholesterol metabolism. The multifaceted nature of cholesterol regulation in sheep is evident from the various factors that can influence its levels. Feed composition and fat supplementation directly affect the lipid content available for absorption and metabolism. Rumen microbes play a crucial role in the breakdown and modification of dietary components, potentially altering cholesterol synthesis and absorption. Infections and diseases can disrupt normal metabolic processes, while probiotic supplementation may modulate gut microbiota and influence lipid metabolism. Genetic factors, as demonstrated by the biglycan association, can predispose certain individuals or breeds to higher or lower cholesterol levels. Additionally, age-related changes in metabolism and physiological processes can impact cholesterol homeostasis. This complex interplay of factors underscores the importance of comprehensive approaches in studying and managing cholesterol levels in sheep populations.

**Table 3.** Association of the BGN gene with the cholesterol levels.

| Cholesterol (mg/100 g) | Genotype (mean ± SE) | | P Value |
|---|---|---|---|
| | GG (83) | CC (4) | |
| Cholesterol | $93.6\pm0.92^{b}$ | $225.60\pm9.56^{a}$ | 0.005 |

$\bar{x}$ = average cholesterol; SE = standard error; a, b = Values in the same row with distinct superscripts exhibit substantial differences (P<0.05).

This study revealed a notable association between the biglycan (BGN) gene and cholesterol levels, with a p-value of 0.05, underscoring its vital role in lipid metabolism, especially in affecting the cholesterol content and lipid profile of sheep meat. The BGN gene, which encodes a small leucine-rich proteoglycan, plays a role in organizing the extracellular matrix and cell signaling. Its role in metabolic pathways related to cholesterol homeostasis has direct consequences for livestock meat quality. These findings align with previous research showing that BGN influences lipid profiles by interacting with lipoprotein metabolism pathways, thereby impacting cholesterol uptake and clearance in muscle tissues

[14]. Specifically, BGN expression in vascular and muscle tissues and its interaction with low-density lipoprotein (LDL) receptors may explain 15–20% of the variation in cholesterol levels, thus affecting the lipid composition of meat.

Situated in the extracellular matrix outside muscle cells, the BGN gene interacts with TLR-4 and TLR-2 receptors on muscle cell surfaces, triggering chemical reactions that regulate genes involved in cholesterol storage, production, and breakdown. Additionally, BGN modulates the activity of key enzymes such as HMG-CoA reductase, which is essential for cholesterol biosynthesis, with studies indicating a 30% reduction in its activity in cells with increased BGN expression. These interactions result in decreased cholesterol accumulation in the muscle tissue, leading to meat with up to a 25% reduction in cholesterol content and a more favorable cholesterol profile characterized by a higher HDL-to-LDL ratio. Sheep with optimal BGN gene expression produce meat with improved nutritional quality, appealing to health-conscious consumers seeking low-cholesterol products [36]. Genetic factors are crucial for cholesterol metabolism in sheep, as genotypes significantly influence cholesterol level responses, directly affecting meat quality.

The mechanistic basis of BGN's effect of BGN on meat cholesterol content may be attributed to its regulatory effects on apolipoprotein binding and lipid transport. Proteoglycans, such as biglycan, bind to apolipoproteins such as ApoB, a primary component of LDL particles, facilitating their retention in tissues. In the context of optimal BGN expression, this interaction enhances cholesterol clearance from the muscle tissue, thereby reducing the lipid content in meat. Furthermore, modulation of *TGF-β* signaling may indirectly influence cholesterol metabolism by altering lipid synthesis and clearance in metabolically active tissues such as the liver and muscle, with studies noting a 10–12% variation in cholesterol levels due to TGF-β-mediated effects [15]. Genetic variations, such as *SNP* in the BGN locus, particularly in the promoter region, may alter its expression, leading to differential effects on cholesterol metabolism and meat quality.

The role of BGN in systemic inflammation significantly influences the cholesterol profile of the meat. Chronic inflammation, commonly linked to elevated cholesterol levels, induces upregulation of BGN expression in tissues such as the liver and adipose tissue, with a documented 1.5-fold increase under inflammatory conditions. In sheep with favorable BGN genotypes, this upregulation appears to enhance lipid metabolism, thereby reducing cholesterol deposition in muscle tissue. The interaction between genetic predisposition and environmental factors, such as diet, further modulates BGN expression, thereby affecting cholesterol levels and meat quality. Tissue-specific expression of BGN in muscle suggests context-dependent regulatory mechanisms, which are crucial for understanding its role in the production of low-cholesterol meat.

These results underscore the promise of BGN as a focus for enhancing meat quality in livestock genetic improvement. By selecting sheep with optimal BGN expression, producers can produce meat with up to 25% lower cholesterol content, thereby enhancing its marketability and nutritional value. This approach can significantly influence human nutrition by reducing the dietary cholesterol intake. However, the complex interactions between BGN and the lipid metabolism pathways require further investigation. Future research should employ functional analyses, such as gene knockout models or dietary interventions, to validate BGN's role of BGN in cholesterol regulation and its impact on meat quality [15]. Additionally, integrating genomic and proteomic approaches could provide deeper insights into BGN's interactions of BGN with other lipid-related genes and proteins, further elucidating its contribution to meat cholesterol profiles.

In conclusion, the significant association between BGN and cholesterol levels, supported by a p-value of 0.05, emphasizes its critical role in lipid metabolism and its direct influence on meat quality in sheep. Through interactions with TLR-4 and TLR-2 receptors, modulation of HMG-CoA reductase, and regulation of lipoprotein metabolism and inflammation, BGN reduces cholesterol content and improves lipid profiles in the muscle tissue [15]. These findings highlight BGN's potential of BGN in livestock breeding to produce healthier meat, and emphasize the need for further research to translate these insights into practical agricultural applications.

## 4. Conclusion

This study revealed a notable correlation between the single nucleotide polymorphism (SNP) g.77807325 G>C in the biglycan gene and cholesterol levels in Indonesian lambs. Lambs with the GG genotype had lower cholesterol levels. Interestingly, male lambs exhibiting the GG genotype demonstrated markedly reduced cholesterol levels compared with their CC genotype counterparts. The results indicate that BGN presents a valuable opportunity for genetic selection focused on lowering the cholesterol levels in lambs.

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