

Article

Association Between InDel and CNV Variation in the *FBLN1* Gene and Slaughter Traits in Cattle

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Abstract: The *FBLN1* gene encodes the fibulin-1 protein, the first member of the ECM glycoprotein family, and is crucial for embryonic development and organ tissue formation in mammals. Our previous transcriptome analysis identified the *FBLN1* gene and suggested its potential role in influencing slaughter traits by regulating ECM function. This study aims to uncover key genetic variants (InDel and CNV) within the *FBLN1* gene and examine its relationship with slaughter traits in beef cattle. In this study, the beef cattle genetic resources population Gaoqing black cattle were selected (n = 641), leading to the identification of three polymorphic InDel loci (13 bp insertion, 28 bp insertion, and 24 bp insertion) and two CNVs. Association analysis revealed that InDel polymorphisms in Gaoqing black cattle were significantly correlated with certain slaughter traits ($p < 0.05$), such as left limb weight and right limb weight. In addition, the CNV loci were significantly correlated with traits such as skirt steak and round small intestine ($p < 0.05$), and reached extremely significant levels ($p < 0.01$) in certain traits such as chunky II and high rib. In summary, the identified InDel and CNV polymorphisms in the *FBLN1* gene represent potential molecular markers associated with slaughter traits in Gaoqing black cattle. These findings provide valuable insights for marker-assisted selection to enhance genetic improvement in beef cattle breeding.

Keywords: bovine; *FBLN1*; InDel; CNV; slaughter traits



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1. Introduction

In recent years, the beef cattle industry has grown rapidly within the agricultural sector, making the improvement of slaughter traits a key factor for enhancing economic benefits. Consequently, research on livestock slaughter characteristics and the genetic regulation of growth and development has received increasing attention [1]. Combining the exploration of regulatory genes with molecular-marker-assisted selection (MAS) is now central to modern animal breeding. For instance, selecting DNA markers associated with growth, slaughter, and meat quality traits can accelerate genetic improvement, thereby contributing to the sustainable development of livestock breeding. Since body size influences reproductive performance, growth, and slaughter traits, it remains a primary consideration

in beef cattle breeding programs [2]. In addition, slaughter traits, which are often measured using techniques such as ultrasonic inspection, have a direct impact on carcass composition and meat quality [3].

Our previous work involved the transcriptome analysis of experimental cattle from the Huaxi cattle in Urgai (Mongolia, China) to identify key candidate genes. In that study, cattle were divided into three feeding groups: the ad libitum feeding (ALF) group, the 75% restricted feeding (RF75) group, and the group under a 50% feed restriction (RF50). The detailed methodology can be found in our previous studies [4]. Results indicated that the *FBLN1* gene was significantly up-regulated in the RF50 group. Gene set enrichment analysis (GSEA) further demonstrated that *FBLN1* played a major role in extracellular matrix (ECM) remodeling, suggesting its involvement in adaptive physiological regulation under restricted feeding conditions. These findings hint at a potential link between *FBLN1* expression and the development of slaughter traits in beef cattle.

FBLN1 encodes fibulin-1, an extracellular glycoprotein essential for maintaining ECM structure and function [5,6]. Studies in human and mouse embryos have shown that *FBLN1* is critical for organogenesis and embryonic development, and its dysregulation is associated with various human diseases [7–9]. For example, the *FBLN1*-D splicing variant, as a special isoform of the *FBLN1* gene, has been found at significantly lower levels in fibroblast extracts from synpolydactyly patients compared to normal fibroblasts. Moreover, *FBLN1* mutations have been linked to impaired cell proliferation, reduced cell viability, and increased apoptosis [10]. As the ECM plays a central role in regulating cellular functions such as growth and differentiation, the proper expression of the *FBLN1* gene is crucial for normal development, including traits affecting carcass composition.

Transcriptome analysis, as a high-throughput technology, enables the comprehensive profiling of RNA transcripts in specific cells or tissues under particular conditions. It provides deep insights into gene expression changes and helps identify transcriptional variations associated with biological processes or experimental treatments [11]. With the rapid advancement of next-generation sequencing (NGS) technologies, such as RNA sequencing (RNA-seq), researchers can not only accurately quantify gene expression levels, but also identify novel transcripts. Due to its high sensitivity and low bias, RNA-seq has become a mainstream tool in molecular biology research [12]. In our previous study, a combination of transcriptome analysis and bioinformatics approaches was used to identify *FBLN1* as a significantly differentially expressed gene (DEG) in the RF50 group, where it was enriched in the ECM signaling pathway [4]. Additionally, compared to the RF75 and the ALF groups, the RF50 group exhibited superior meat quality traits, including higher pH values and lower cooking loss. Therefore, *FBLN1* may serve as an important candidate gene influencing cattle production efficiency and meat quality by regulating the structure and function of the ECM.

The above research indicates the impact of this gene on animals and humans. Its research in human diseases has been relatively in-depth, but its research in livestock and poultry is still relatively scarce. Based on our previous work, we hypothesize that insertion/deletion (InDel) and copy number variation (CNV) polymorphisms in the *FBLN1* gene have a significant impact on key slaughter traits in beef cattle. To test this hypothesis, we analyzed data from 641 Gaoqing black cattle. By examining the molecular markers in *FBLN1*, our study aims to provide essential genetic insights that can support breeding improvements in Gaoqing black cattle and promote long-term sustainability within the livestock industry.

2. Materials and Methods

2.1. Ethical Statement

The collection of samples adhered to the Chinese national guidelines on “Guidelines for Laboratory Animal Welfare and Ethical Review” (GB/T 35892-2018 [13]). The experiment was conducted in accordance with the animal management regulations of Northwest A&F University (NWAUFU-314020038).

2.2. Collection of Animal Samples

There were 641 Gaoqing black cattle from the beef cattle genetic resource population used in this study. They came from two farms with similar breeding conditions (Shandong Yangxin Yiliyuan Halal Meat Company and Shandong Kaiyuan Animal Husbandry Company). All cattle in the population were healthy adult individuals with similar physiques and dietary levels (including feed allocation, living environment, and health management). The cattle began their fattening period at 10 to 11 months of age and were fattened until centralized slaughter at 26 to 28 months of age. Neck muscle tissue samples of each individual were collected and quickly frozen and stored at -80°C for subsequent whole genome extraction. After slaughter, a total of 19 slaughter traits, including three-rib S-cut abdomen, weight of left limbs, weight of right limbs, entry weight, beef neck edge, beef slices, triangular brisket, high rib, sirloin, short rib, meat tendon, carcass fat, skirt steak, chunky II, beef plate, beef short plate, oxtail, knuckle tendon, and ribeye, were measured and collected for this study. During the measurement of these traits, the entire slaughter and carcass segmentation process was carried out strictly in accordance with the national standard GB/T 27643-2011 [14]. Measurements were conducted with precision by the same trained technician following standardized protocols. A calibrated electronic scale was utilized to assess the weight of various parts, and the data were recorded in a spreadsheet.

2.3. Extraction of Genomic DNA

The genomic DNA from Gaoqing black cattle was extracted through the high-salt extraction technique [15]. The neck tissues were mechanically homogenized in SE buffer and subjected to overnight digestion with proteinase K (Merck, Germany) ($5\text{ }\mu\text{L}$, 20 mg/mL) at 65°C . Following digestion, proteins were precipitated by adding 6 mol/L NaCl, and the lysate was treated with chloroform (TaKaRa Biotech Co. Ltd., Beijing, China) to separate organic phases. After centrifugation ($12,000\times g$, 10 min , 4°C), the aqueous layer containing DNA was collected. DNA was then precipitated by adding chilled absolute ethanol (-20°C) and washed twice with 70% ethanol to remove residual contaminants. The purified DNA pellet was air-dried and redissolved in double-distilled H_2O (ddH₂O). The concentration and purity of the extracted DNA were determined using NanoDrop™ p2000 (Thermo Scientific, Waltham, MA, USA) [16]. DNA samples with OD_{260/280} ratios between 1.6 and 1.8 were considered suitable for further analysis. The qualified DNA was uniformly diluted to $20\text{ ng}/\mu\text{L}$ with ddH₂O and preserved at -20°C for subsequent genetic evaluations.

2.4. Primer Design

On the basis of the genetic variation information of *FBLN1* gene in bovines (Gene ID: 514588), the Ensembl database (<https://asia.ensembl.org/>, accessed on 17 May 2024) was used to identify five potential InDel variants in the bovine *FBLN1* gene, selecting these with lengths greater than 5 bp and considering their positional information. Subsequently, primers were designed using the NCBI database (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>, accessed on 23 May 2024) and Primer Premier 5 software. Ultimately, three InDel variants were found to be polymorphic (Table 1). Two CNV loci, CNV1 and CNV2, were also investigated. These loci were selected from the Animal Omics Database

(<http://animal.nwsuaf.edu.cn/>, accessed on 3 June 2024), and the *BTF3* gene was used as the reference gene (Tables 2 and 3). The *BTF3* gene is considered an ideal internal control gene in cattle CNV analysis because of its conserved structure, stable expression, and lack of interference under experimental conditions [17]. The synthesis of these primers was undertaken by Sangon Biotech (Shanghai) Co., Ltd. (China).

Table 1. The primer information of PCR amplification of the *FBLN1* gene.

Primer Names	Primer Sequences(5'-3')	Region	Product Sizes (bp)	Polymorphisms
P1	F:TGAGAGTAAGCTCAGAAACGGA R:TGCTGCTAACCTCTGAGTTCC	Intron1	145/158	Yes
P2	F:GCTTCAGTTTCCAAAGGCCG R:CCCTGAGTAGGTGACGAGA	Intron1	196/224	Yes
P3	F:CTGCAATTGAAGCACCTGGAT R:GGGCTCAGAGACGGTTTGTG	Intron1	265/289	Yes
P4	F:CGGATGCGCTAACAAGAAGTC R:CAGTATTATGGCCCCCTGCC	Intron1	95/107	No
P5	F:AGTGACCTCTCAGCAAGGGT R:AGGGAGGGACAGCCTAGTTT	Intron4	234/252	No

Table 2. Information of copy number variations in the bovine *FBLN1* gene.

Primers	Chromosome	Start	End	Length	Location
CNV1	5	115,918,196	115,920,996	2800	Exon7
CNV2	5	115,937,723	115,940,523	2800	Intron14

Table 3. Primers for detecting CNV polymorphisms and the their relative expression of the *FBLN1* gene. Note: *BTF3*, reference gene.

Primers	Primer Sequences (5'-3')	Sizes (bp)
CNV1	F:CGCATGTGCTTTCTAGTCCC R:TCATGCTTTTTACGCAGCGG	127
CNV2	F:CGAACCTTGTTTGCTGACG R:CTTGAGAGGCACATTGGGGG	140
BTF3	F:AACCAGGAGAAACTCGCCAA R:TTCGGTGAAATGCCCTCTCG	166

2.5. Identification and Genotyping of InDel Variants

The amplification of polymorphic fragments was carried out through polymerase chain reaction (PCR), and the corresponding reaction procedures were carried out (pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 68 °C with a 1 °C decrease stepwise for 30 s, extension at 72 °C for 20 s for 18 cycles; then, denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 20 s for 30 cycles; finally, extension at 72 °C for 5 min and then cooled to 4 °C). A total reaction volume of 13 µL was employed for PCR amplification. The mixture comprised 0.5 µL each of forward and reverse primers, 6.5 µL of 2× Eco Taq PCR Super Mix (Bioteke Co. Ltd., Shanghai, China), 0.8 µL of genomic DNA, and 4.7 µL of ddH₂O. Agarose gel electrophoresis with a mass concentration of 3.0% was used to identify the genotypes of different individuals, and representative genotypes were sequenced by Sangon Biotech (Shanghai) Co., Ltd. to verify the variations.

Each sample was subjected to qPCR amplification in triplicate for each primer pair using the Quant Studio 5 cycler (Thermo Fisher Scientific Inc., Shanghai, China). The reaction parameters and protocols were identical to those described in previous work [18]. Subsequently, the copy numbers of the *FBLN1* gene were determined using the $2^{-\Delta\Delta C_t}$ method, where $\Delta C_t = C_{t_{\text{target}}} - C_{t_{\text{internal}}}$. Here, $C_{t_{\text{target}}}$ denotes the number of cycles at which the amplification of the target gene reaches the threshold, while $C_{t_{\text{internal}}}$ refers to the cycle count when the amplification of the reference gene reaches the threshold. The CNVs

were subsequently categorized into three distinct types based on the $2^{-\Delta\Delta C_t}$ values: Gain ($2^{-\Delta\Delta C_t} \geq 3$), Loss ($2^{-\Delta\Delta C_t} < 2$), and Medium ($2^{-\Delta\Delta C_t} = 2$) [18,19].

2.6. Statistical Analysis

The SHEsis program (<http://analysis.bio-x.cn>, accessed on 10 October 2024) was used to evaluate Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) at InDel loci. Genetic parameters, such as polymorphic information content (PIC), were estimated using an online genetic diversity analysis tool (<http://www.msncall.com/Gdical.aspx>, accessed on 11 October 2024), following Nei's method [20]. IBM SPSS Statistics 27.0 software was used to analyze the relationship between genetic variants and cattle slaughter traits. If a variant had three different genotypes with each genotype present in more than three instances, a one-way analysis of variance (ANOVA) was conducted. In cases where any genotype was observed in fewer than three samples, an independent sample *t*-test was conducted. A *p*-value below 0.05 was regarded as statistically significant. The general linear model is given by $Y_{ijk} = \mu + G_j + S_i + E_{ijk}$, where Y_{ijk} is the observed trait, μ represents the mean, G_j denotes the genotype effect, S_i refers to the sex effect, and E_{ijk} accounts for random error.

3. Results

3.1. Identification and Analysis of Bovine *FBLN1* Gene

As shown in Figure 1, in our previous studies, the *FBLN1* gene has been typed as an important candidate gene affecting the production efficiency and quality of beef cattle [4]. Using a pooled DNA approach to identify genotypes within five insertion and deletion loci, it was determined that three loci, namely P1 (5:115894272, 13 bp-ins), P2 (5:115896195, 28 bp-ins), and P3 (5:115900875, 24 bp-ins), exhibited polymorphism. Loci P1 and P2 displayed three distinct genotypes: homozygous for the insertion (II), homozygous for the deletion (DD), and heterozygous (ID). Locus P3 showed two genotypic variants: homozygous for the insertion (II) and heterozygous (ID). As shown in Figure 1D, the P1, P2, and P3 loci are all located in the first intron of the *FBLN1* gene. Two CNVs were identified within the *FBLN1* gene, designated as CNV1 (NC_037332.1:115918196-115920996) and CNV2 (NC_037332.1:115937723-115940523). The CNV1 and CNV2 loci are located in the seventh exon and the fourteenth intron, respectively.

We obtained the electrophoretic and sequencing maps of P1, P2, and P3 through 3.0% agarose gel electrophoresis and sequencing. As shown in Figure 2, the electrophoretic map clearly demonstrated the length differences among individuals with different genotypes in the Gaoqing black cattle population, providing a visual confirmation of genotype variation. The subsequent sequencing results further detailed the sequence composition and precise chromosomal locations of the inserted fragments corresponding to the three InDels in the bovine genome. In addition, as shown in Figure 3, CNV primers were detected via qPCR, and the melting curve analysis confirmed that these primers generated a single target-specific product with high amplification efficiency.

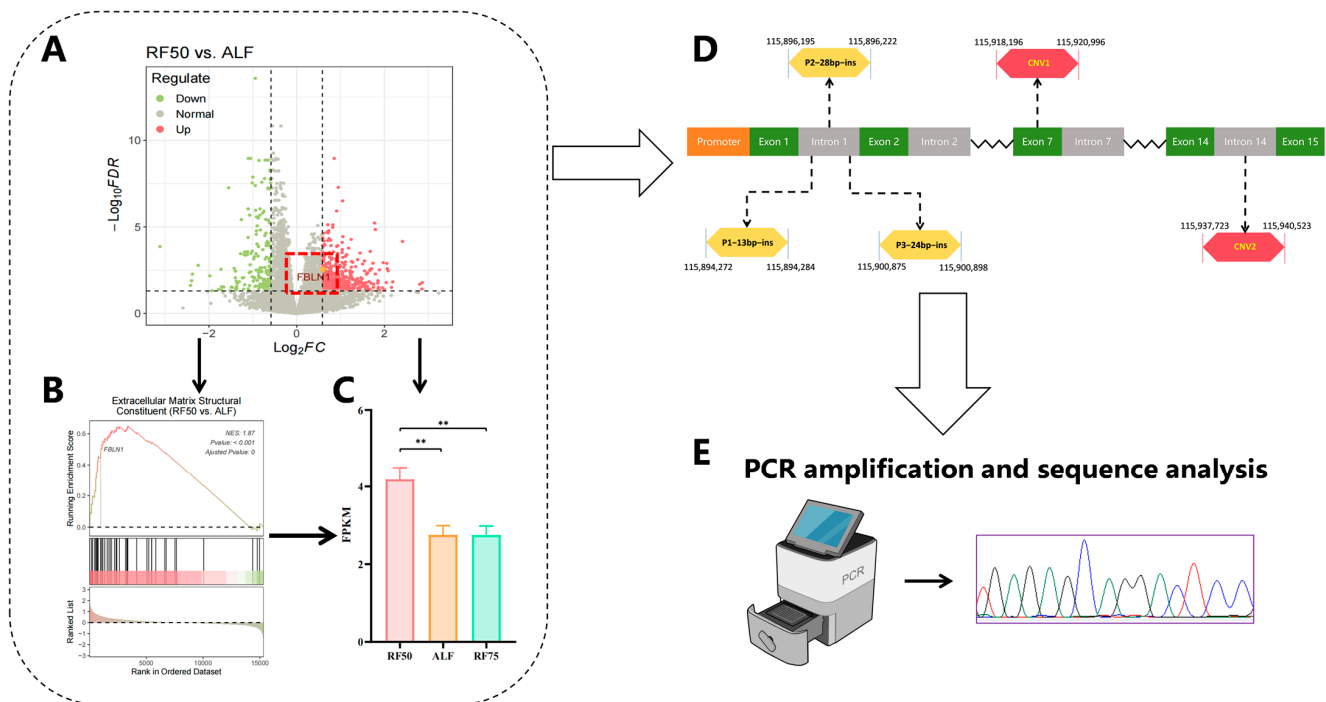


Figure 1. Research strategies for examining *FBLN1* genetic variations and slaughter traits (created with bioart.niaid.nih.gov, accessed on 27 January 2025). **(A)** Volcano plot showing DEG distribution for RF50 vs. ALF, with data points colored according to expression changes: red for up-regulated genes, green for down-regulated genes, and grey for genes without significant change. **(B)** GSEA plot showing enrichment of extracellular matrix structural constituent pathways for RF50 vs. ALF. **(C)** Bar plot showing the expression levels of the *FBLN1* gene across three experimental groups (RF50, ALF, RF75) measured in FPKM, ** indicates significant differences with $p < 0.01$. **(D)** The positions of analyzed *FBLN1* gene variants. **(E)** PCR amplification and sequencing.

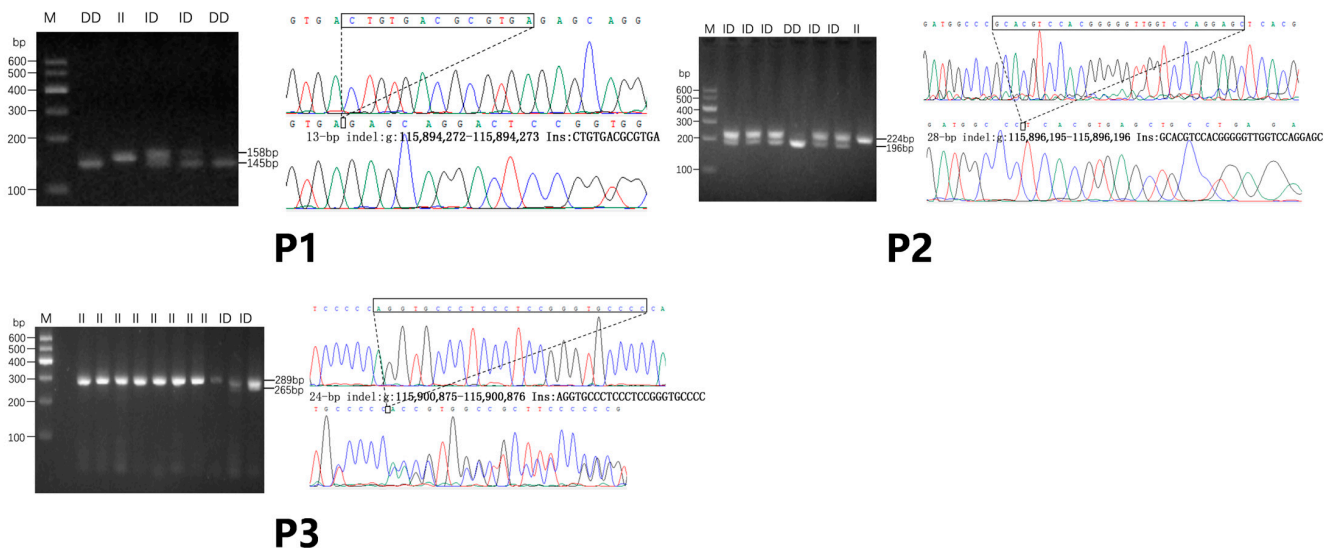


Figure 2. Agarose gel electrophoresis and DNA sequence analysis of bovine *FBLN1* gene loci. Note: M, DNA marker.

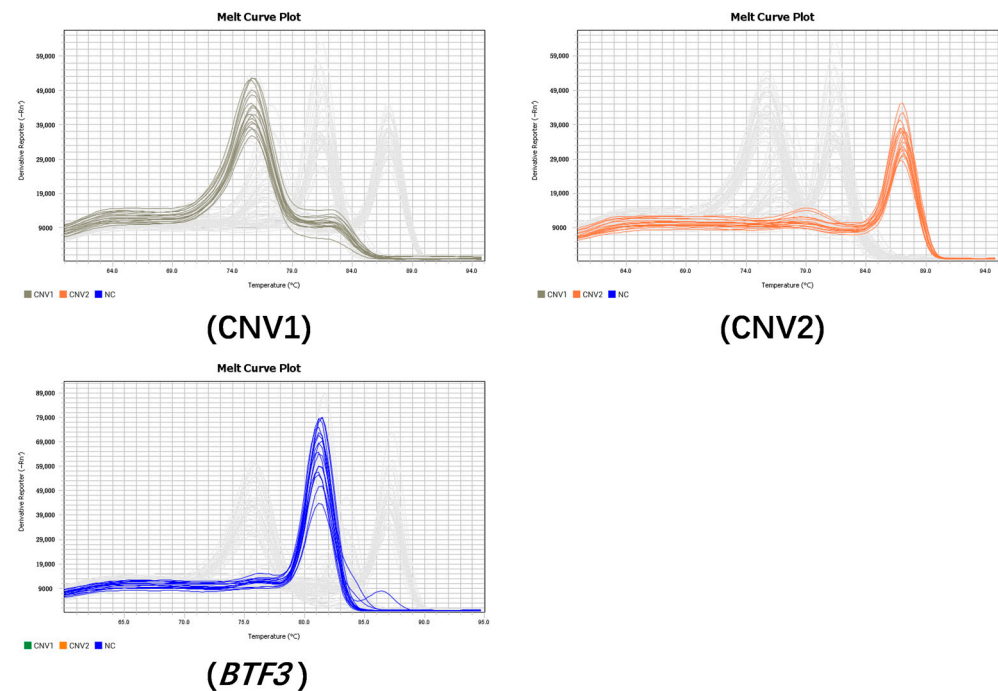


Figure 3. Melting curve of CNV1, CNV2, and *BTF3*. Note: *BTF3*, reference gene.

3.2. Estimation of InDel (P1, P2, P3) Polymorphism Parameters of *FBLN1*

Using the genetic variations found at three InDel loci in the *FBLN1* gene, we calculated the distribution of genotypic and allelic frequencies, along with additional population genetic parameters (Table 4). At these three loci, we found that loci P1 and P2 did not conform to the Hardy–Weinberg equilibrium ($p < 0.05$) and had medium genetic polymorphism ($0.25 < \text{PIC} < 0.5$), while locus P3 had low genetic polymorphism ($\text{PIC} < 0.25$) and was in the Hardy–Weinberg equilibrium ($p > 0.05$), which suggests that the multiallelic polymorphism of locus P3 will maintain its equilibrium state [21].

Table 4. Estimation of polymorphism parameters of P1, P2, and P3 of bovine *FBLN1* gene.

Loci	Sample Sizes	Genotypic Frequencies			Allelic Frequencies		HWE p -Value	Population Parameter Estimates			
		II	ID	DD	I	D		Ho	He	Ne	PIC
P1	641	0.168 (n = 108)	0.557 (n = 357)	0.275 (n = 176)	0.447	0.553	0.0136	0.506	0.494	1.978	0.372
P2	638	0.404 (n = 258)	0.528 (n = 337)	0.067 (n = 43)	0.668	0.332	1.27×10^{-6}	0.557	0.443	1.796	0.345
P3	417	0.866 (n = 361)	0.134 (n = 56)	0 (n = 0)	0.933	0.067	0.142	0.875	0.125	1.143	0.117

Note: HWE represents Hardy–Weinberg equilibrium; Ho denotes homozygosity; He indicates heterozygosity; Ne stands for the effective number of alleles; PIC refers to polymorphism information content.

3.3. Analysis of Linkage Disequilibrium (LD)

Given that the three InDel polymorphisms are all located within the same gene, we hypothesized their possible associations. To explore the potential connections between the variants of *FBLN1*, we carried out a linkage disequilibrium (LD) analysis using the SHESIS online tool. The results indicated that the association values between loci P1, P2, and P3 within the *FBLN1* gene for D' and r^2 are 0.704, 0.172, and 0.562, and 0.314, 0.003, and 0.011, respectively, indicating that there is no significant correlation among these three variants (Figure 4).

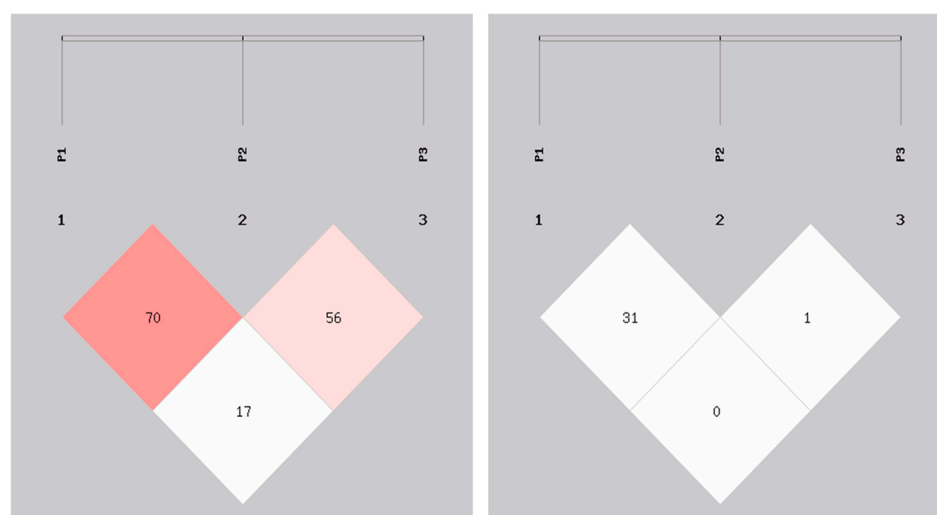


Figure 4. LD assessment of InDel variation in the bovine *FBLN1* gene. P1, P2, and P3 represent P1-13bp-ins, P2-28bp-ins, and P3-24bp-ins.

3.4. CNV Identification: Distribution of Genotypes in the Bovine *FBLN1* Gene

To determine the distribution of *FBLN1* gene copy numbers in bovine *FBLN1*, we selected 115 cattle. According to the $2^{-\Delta\Delta C_t}$ method, we divided the CNV types into three classes, including Loss type (0~2), Medium type (2), and Gain type (>2). As shown in Table 5, CNV1 and CNV2 displayed three types (Loss, Medium, and Gain) in *FBLN1* bovines.

Table 5. Typical frequencies of copy number variations within the *FBLN1* gene in bovines.

Loci	Sizes (bp)	Genotypic Frequencies		
		Loss	Medium	Gain
CNV1	127	0.031	0.055	0.913
CNV2	140	0.362	0.378	0.260

3.5. Association Analysis of *FBLN1* with Slaughter Traits

To explore the association between genetic variants and slaughter traits, we analyzed the correlation of InDel locus and CNV locus using SPSS 27 (Tables 6 and 7). Specifically, one-way ANOVA analysis revealed that, in both male and female populations, individuals with the II and ID genotypes at the P1 locus showed higher phenotypic values for several slaughter traits than those with the DD genotype ($p < 0.05$). In certain specific traits, such as triangular brisket and entry weight, the differences were highly significant ($p < 0.01$). Unlike the situation at the P1 locus, for the P2 locus, individuals with the ID and DD genotypes exhibited higher phenotypic values in slaughter traits relative to those with the II genotype ($p < 0.05$), and for certain traits, including entry weight, the differences were highly significant ($p < 0.01$). Furthermore, the independent sample *t*-test analysis of the P3 locus indicates that individuals with the ID genotype exhibited higher phenotypic values for the beef short ribs trait in comparison with those carrying the II genotype ($p < 0.05$), while the difference in meat tendon traits was highly significant ($p < 0.01$).

Statistical analysis revealed a strong association between CNV1 and the carcass fat trait ($p < 0.01$). The CNV2 locus showed a highly significant correlation with chunky II and high rib traits ($p < 0.01$). For the CNV1 locus, the Gain type is the most prevalent and outperforms the Loss and moderate types in traits. For the CNV2 locus, the Medium and Gain genotype showed higher phenotypic values in slaughter traits compared to the Loss genotype.

Through association analysis, we found that the three-rib S-cut abdomen and high rib are susceptible to the influence of genetic variations (Indel and CNV), so they have relatively large selection potential and improvement potential, which can be considered in the selection process.

Table 6. Statistics of slaughter traits significantly associated with different genotypes of three InDel variants.

Loci	Gender	Traits (kg)	Sample Size	Observed Genotypes (Mean $\pm$ SE)			p-Value
				II	ID	DD	
P1	Male	Three-rib S-cut abdomen	94	1.38 $\pm$ 0.08 a (n = 19)	1.35 $\pm$ 0.04 b (n = 59)	1.12 $\pm$ 0.07 a (n = 16)	0.040
	Female	Three-rib S-cut abdomen	382	1.26 $\pm$ 0.04 ^A (n = 64)	1.27 $\pm$ 0.03 ^A (n = 204)	1.13 $\pm$ 0.03 ^B (n = 114)	0.001
		Left limbs weight	388	213.36 $\pm$ 3.90 ^A (n = 65)	210.15 $\pm$ 2.25 ^A (n = 207)	199.75 $\pm$ 3.13 ^B (n = 116)	0.007
		Right limbs weight	388	214.32 $\pm$ 4.04 ^A (n = 65)	210.68 $\pm$ 2.31 ^A (n = 207)	199.71 $\pm$ 3.19 ^B (n = 116)	0.005
		Entry weight	388	329.14 $\pm$ 5.80 ^A (n = 65)	329.39 $\pm$ 3.47 ^A (n = 207)	306.09 $\pm$ 4.53 ^B (n = 116)	0.000134
		Beef neck edge	386	1.28 $\pm$ 0.03 ^A (n = 65)	1.26 $\pm$ 0.02 ^A (n = 206)	1.15 $\pm$ 0.03 ^B (n = 115)	0.000450
		Short rib	379	3.21 $\pm$ 0.08 ab (n = 62)	3.25 $\pm$ 0.05 ^a (n = 204)	3.01 $\pm$ 0.06 ^b (n = 113)	0.012
		Beef slices	384	2.04 $\pm$ 0.05 ^A (n = 65)	2.05 $\pm$ 0.03 ^A (n = 205)	1.87 $\pm$ 0.04 ^B (n = 114)	0.001
		Triangular brisket	395	6.47 $\pm$ 0.14 ^A (n = 65)	6.19 $\pm$ 0.09 ^A (n = 213)	5.80 $\pm$ 0.13 ^B (n = 117)	0.003
		High rib	397	16.65 $\pm$ 0.36 ^A (n = 67)	16.41 $\pm$ 0.22 ^A (n = 214)	15.38 $\pm$ 0.30 ^B (n = 116)	0.007
		Sirloin	393	13.04 $\pm$ 0.26 ^A (n = 67)	12.78 $\pm$ 0.16 ^A (n = 210)	11.73 $\pm$ 0.24 ^B (n = 116)	0.000139
P2	Male	Right limbs weight	94	223.15 $\pm$ 5.20 ^b (n = 31)	230.57 $\pm$ 3.79 ^b (n = 59)	262.00 $\pm$ 21.83 ^a (n = 4)	0.048
		Triangular brisket	106	6.72 $\pm$ 0.19 ^B (n = 42)	7.35 $\pm$ 0.16 ^A (n = 60)	8.63 $\pm$ 0.62 ^A (n = 4)	0.003
		Three-rib S-cut abdomen	97	1.21 $\pm$ 0.07 ^b (n = 33)	1.37 $\pm$ 0.04 ab (n = 60)	1.56 $\pm$ 0.29 ^a (n = 4)	0.047
		High rib	107	16.88 $\pm$ 0.84 ^b (n = 42)	19.78 $\pm$ 0.52 ab (n = 61)	22.60 $\pm$ 1.98 ^a (n = 4)	0.012
		Sirloin	103	12.43 $\pm$ 0.36 ^b (n = 38)	13.51 $\pm$ 0.30 ab (n = 61)	15.50 $\pm$ 1.34 ^a (n = 4)	0.012
	Female	Entry weight	385	313.34 $\pm$ 3.83 ^B (n = 161)	329.91 $\pm$ 3.60 ^A (n = 204)	315.75 $\pm$ 7.82 ^{AB} (n = 20)	0.006
		Left limbs weight	385	201.98 $\pm$ 2.76 ^b (n = 161)	211.02 $\pm$ 2.16 ^a (n = 204)	213.03 $\pm$ 8.17 ^a (n = 20)	0.026
		Right limbs weight	385	202.27 $\pm$ 2.82 ^b (n = 161)	211.38 $\pm$ 2.22 ^a (n = 204)	215.10 $\pm$ 8.43 ^a (n = 20)	0.024
		Beef neck edge	383	1.18 $\pm$ 0.02 ^b (n = 159)	1.25 $\pm$ 0.02 ^a (n = 204)	1.26 $\pm$ 0.08 ^a (n = 20)	0.047
		Beef slices	382	1.93 $\pm$ 0.03 ^b (n = 159)	2.05 $\pm$ 0.03 ^a (n = 203)	1.94 $\pm$ 0.09 ab (n = 20)	0.026
		Triangular brisket	393	5.93 $\pm$ 0.12 ^b (n = 164)	6.28 $\pm$ 0.09 ^a (n = 208)	6.05 $\pm$ 0.22 ab (n = 21)	0.041
		High rib	394	15.79 $\pm$ 0.26 ^b (n = 164)	16.52 $\pm$ 0.21 ^a (n = 208)	15.27 $\pm$ 0.79 ^b (n = 22)	0.038
		Sirloin	389	12.16 $\pm$ 0.21 ^b (n = 161)	12.82 $\pm$ 0.15 ^a (n = 206)	11.89 $\pm$ 0.52 ^b (n = 22)	0.017
P3	Female	Short rib	291	3.16 $\pm$ 0.05 ^b (n = 251)	3.50 $\pm$ 0.18 ^a (n = 40)		0.012
		Meat tendon	218	4.88 $\pm$ 0.36 ^b (n = 188)	7.80 $\pm$ 1.01 ^a (n = 30)		0.01

Note: Values with different letters (^a, ^b / ^A, ^B) within the same row differ significantly at $p < 0.05$ / $p < 0.01$.

Table 7. Statistics of slaughter traits significantly associated with different genotypes of two CNVs of *FBLN1*.

Variation	Gender	Traits (kg)	Sample Size	Observed Genotypes (Mean $\pm$ SE)			p-Value
				Loss	Medium	Gain	
CNV1	Female	Carcass fat	113		84.10 $\pm$ 1.11 ^B (n = 5)	91.45 $\pm$ 1.55 ^A (n = 108)	0.001
CNV2	Female	Skirt steak	115	1.95 $\pm$ 0.06 ^b (n = 42)	2.18 $\pm$ 0.06 ^a (n = 41)	2.14 $\pm$ 0.06 ^{ab} (n = 32)	0.019
		Chunky II	115	38.46 $\pm$ 1.36 ^B (n = 42)	43.71 $\pm$ 1.21 ^A (n = 41)	44.70 $\pm$ 1.36 ^A (n = 32)	0.002
		Beef plate	113	2.70 $\pm$ 0.07 ^b (n = 41)	2.92 $\pm$ 0.06 ^a (n = 40)	2.73 $\pm$ 0.08 ^{ab} (n = 32)	0.049
		Beef short plate	114	6.12 $\pm$ 0.17 (n = 41)	6.48 $\pm$ 0.19 (n = 41)	6.77 $\pm$ 0.19 (n = 32)	0.05
		Three-rib S-cut abdomen	113	1.29 $\pm$ 0.05 ^b (n = 41)	1.46 $\pm$ 0.05 ^a (n = 41)	1.30 $\pm$ 0.04 ^b (n = 31)	0.02
		High rib	115	16.19 $\pm$ 0.42 ^B (n = 42)	17.85 $\pm$ 0.36 ^A (n = 41)	17.51 $\pm$ 0.45 ^A (n = 32)	0.009
		Oxtail	111	0.68 $\pm$ 0.04 ^b (n = 41)	0.75 $\pm$ 0.03 ^{ab} (n = 39)	0.83 $\pm$ 0.04 ^a (n = 31)	0.013
		Knuckle tendon	109	1.18 $\pm$ 0.05 ^b (n = 41)	1.25 $\pm$ 0.05 ^{ab} (n = 39)	1.39 $\pm$ 0.08 ^a (n = 29)	0.043
		Ribeye	115	11.20 $\pm$ 0.33 ^b (n = 42)	12.23 $\pm$ 0.21 ^a (n = 41)	12.10 $\pm$ 0.35 ^a (n = 32)	0.026

Note: Values with different letters (^a, ^b, ^A, ^B) within the same row differ significantly at $p < 0.05$ / $p < 0.01$.

4. Discussion

In our recent study, we established a nutritional intervention model using restricted feeding and conducted a grouped study, which led to the identification of the differentially expressed *FBLN1* gene. Restricted feeding, as an important nutritional regulatory strategy, can alter the balance of energy metabolism, thereby inducing the tissue-specific reprogramming of gene expression. By comparing the transcriptomic profiles among the ALF, RF75, and RF50 groups, we systematically identified key regulatory genes under various nutritional conditions. This approach is consistent with that of Ma et al. [22], who identified circRNAs associated with longissimus dorsi development in cattle under different feeding regimes. Similarly, Ding et al. [23] performed the transcriptome sequencing of adipose tissue under different nutritional conditions and identified tens of thousands of differentially expressed genes. Collectively, these studies confirm that nutritional intervention is an effective means for exploring the genetic regulatory mechanisms underlying economically important traits. Our analysis indicates that the *FBLN1* gene may be involved in extracellular matrix remodeling, thereby playing a crucial role in the development of beef cattle slaughter traits. Therefore, this study selected *FBLN1* as the target gene to investigate the relationship between its genetic variations and beef cattle slaughter traits. Further investigation identified three polymorphic InDel loci and two CNV loci within the introns and exons of the *FBLN1* gene. Experimental results demonstrated that these variants are associated with 19 beef cattle carcass traits ($p < 0.05$). In addition, we found that the three-rib s-cut abdomen and high rib were significantly correlated not only with InDel loci, but also with CNV2 locus. These findings corroborate the report by Li et al. [24], who observed a significant association between variations in the *CRY2* gene and the high rib trait, suggesting that genetic variations in different genes may influence corresponding slaughter traits through similar regulatory mechanisms. Such insights are of considerable value in beef cattle breeding, as marker-assisted selection targeting these mutation-sensitive traits could substantially enhance the economic efficiency of beef cattle production. Notably, this study is the first to reveal a significant association between the InDel and CNV polymorphisms of the *FBLN1* gene and slaughter traits in beef cattle.

Slaughter traits in beef cattle are important economic traits and serve as key indicators for evaluating growth performance and economic value, thus further assessing the breeding potential of individuals [25]. At the same time, slaughter traits in beef cattle have relatively high heritability, and associating gene polymorphisms with slaughter traits and its application in marker-assisted selection can stabilize the inheritance of these traits to the next generation through early selection. This has significant research implications for cattle genetic resource protection, subsequent breeding selection, and genetic improvement [26]. Additionally, recent studies have shown that the ultrasonic measurement of carcass traits is accurate, fast, and convenient [2], and can be used to evaluate animals with potential for muscle development and fat deposition, thereby estimating the economic value of beef cattle [27,28]. Therefore, selecting for superior genotypes of slaughter traits may have potential significance for maximizing the production potential of beef cattle and improving industry development.

The *FBLN1* gene, as a member of the extracellular matrix (ECM) protein family, encodes a protein that interacts with other ECM proteins to maintain the structural integrity of the ECM [29,30]. It plays a crucial role during the early stages of embryonic development in animals and in the construction of various organs and tissues. Moreover, when mutations or variations occur in this gene, a series of limb defects are often observed [8,10,31], suggesting that it may have an as yet unclear influence on the growth and development of limbs. To date, most studies on the association between *FBLN1* and animals have mainly focused on molecular-level research, including associations with disorders such as syndromes, tumors, and HPV [9,32,33]. Tang et al. [32] suggested, through single-cell sequencing and other methods, that the abnormal methylation of *FBLN1* is linked to the pathological development of Alzheimer's disease (AD). Xu et al. [33] found that *FBLN1* promotes chondrocyte proliferation in the knee joints of elderly patients by phosphorylating Smad2. McCoy et al. [9] identified *FBLN1* as a strong candidate gene for kidney disease in Phelan-McDermid Syndrome (PMS). In terms of organismal growth and development, Yang et al. [7] demonstrated that *FBLN1* plays a key role in regulating osteogenic differentiation and bone regeneration in umbilical cord mesenchymal stem cells (WJCMSC). Similarly, Raman et al. [34] cleverly used zebrafish models to reveal that *FBLN1* helps maintain skeletal integrity by encoding extracellular matrix proteins.

Through linkage disequilibrium (LD) analysis, we found that, although the three InDel loci are located within the same intron of the *FBLN1* gene, no significant LD was detected between them. The close proximity of these variations within the gene did not lead to significant LD. These results suggest that the three indel loci may have independent effects on the *FBLN1* gene, making them valuable as independent markers in association studies. Future studies should focus on elucidating the functional impacts of these independent InDel variants on *FBLN1* expression and their potential associations with economically important traits in Gaoqing black cattle.

Moreover, although the variations of InDel and CNV2 are located in the introns of the gene, many previous studies have demonstrated that non-coding regions, such as introns and the 5' UTR, can influence traits by regulating gene expression [35,36]. For example, one study reported that a 168-bp deletion in the 5' UTR of the *HOXB13* gene, a non-coding region, was strongly correlated with hip width in Qiaoxi black-headed sheep [37]. Similarly, another study identified a novel InDel locus in an intron of the *HMGA2* gene and showed that it affects the estrous cycle and ovarian size in Holstein cows [35]. Combined with our findings, this further supports the idea that intronic variations can modulate traits through the regulation of gene expression. In our study, although three insertion InDel loci and CNV2 locus are located in the intronic regions and do not directly affect the protein sequence, they may be associated with specific transcription factors that affect gene

expression [38]. In conclusion, our study highlights the relationship between the three newly discovered insertion–deletion loci and two CNVs in the *FBLN1* gene with slaughter traits in cattle. These genetic variations hold promise for use in marker-assisted selection (MAS) in cattle breeding, providing theoretical and experimental support for accelerating the breeding of high-quality black cattle and further advancing the beef cattle industry.

5. Conclusions

In conclusion, our study highlights the identification of three polymorphic InDel loci and two CNV loci in the *FBLN1* gene of Gaoqing black cattle and demonstrates significant associations between these genetic variants and key slaughter traits. These findings support the potential use of these variants as DNA molecular markers in marker-assisted selection for beef cattle improvement. However, several limitations should be acknowledged. The sample size, while adequate for this initial investigation, may not fully represent the genetic diversity across broader populations, and additional independent validations are warranted. Furthermore, the functional mechanisms underlying the effects of these variations on slaughter traits remain to be elucidated. Future research should aim to expand the sample size, include diverse cattle populations, and integrate functional and multi-omics studies to further clarify the regulatory networks involved. These future directions will not only enhance our understanding of the genetic architecture of economically important traits, but also support more effective breeding strategies, ultimately contributing to the sustainable development of the beef industry.

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Abbreviations

The following abbreviations are used in this manuscript:

InDel	Insertion–deletion
CNV	Copy number variation
ECM	Extracellular matrix
NGS	Next-generation sequencing

RNA-seq	RNA sequencing
DEG	Differentially expressed gene
ALF	Ad libitum feeding
RF	Restricted feeding
GSEA	Gene set enrichment analysis
LD	Linkage disequilibrium
5' UTR	5' Untranslated region

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