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Research Article

Evolutionary and Functional Integration of *LCORL-NCAPG* Locus with *Myostatin* Pathway in Broiler Chickens

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ABSTRACT

Introduction: The remarkable growth performance of modern broiler chickens, particularly commercial breeds such as Ross 308 and Cobb 500, has been achieved through decades of intensive genetic selection in countries such as the United States and China. However, the molecular mechanisms underlying this enhanced growth phenotype remain incompletely understood. The present study aimed to evaluate genomic analysis of the *LCORL-NCAPG* locus in broiler chickens, Ross 308 and Cobb 500, to identify genetic variants, characterize regulatory elements, and investigate potential interactions with the *Myostatin* (*MSTN*) pathway.

Materials and methods: A comparative genomic analysis of the *LCORL-NCAPG* locus, located on Chromosome 4, a region associated with body size and growth traits across multiple species, was conducted. Using in silico approaches, including sequence analysis, motif prediction, evolutionary conservation profiling, and interaction network construction, genomic sequences from several commercial broiler lines and ancestral populations were analyzed. Genetic variants were identified and annotated using reference genome data (GRCg7b), and regulatory elements were predicted using motif-based scanning and CpG island detection. Functional interactions were explored through network analysis involving components of the *MSTN* pathway.

Results: Broiler-specific variants in the *LCORL-NCAPG* locus, particularly in exonic and intergenic regions, were identified with signs of positive selection in broiler chicken lines, including higher alternative allele frequencies and conservation of non-coding regulatory elements. Notably, potential novel interactions between the transcription factor *LCORL* and *MSTN* were discovered, with *LCORL* exhibiting predicted interactions with *SMAD3* and *FOXO1*, suggesting a central role in modulating muscle development through the *MSTN* signaling axis. These findings offered mechanistic insights into how growth-regulatory genes may be co-regulated in muscle tissue.

Conclusion: These results improved the understanding of the genetic architecture of growth traits in commercial broiler chickens, highlighting the functional role of the *LCORL-NCAPG* locus and its interaction with the *MSTN* pathway. The present findings provided a strong foundation for future functional studies and potential targets for marker-assisted selection programs aimed at optimizing growth performance.

1. Introduction

Poultry production has undergone remarkable advancement over the past several decades, with modern broiler chickens exhibiting extraordinary growth rates and feed efficiency compared to their ancestral counterparts[1]. This dramatic improvement in broiler production is largely attributed to intensive genetic selection programs that have

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successfully enhanced economically important traits[1]. However, the molecular mechanisms and genetic architecture underlying these improvements remain poorly understood, particularly regarding the complex interplay between different growth-regulatory pathways. Numerous studies have examined the myostatin (*MSTN*) pathway as a negative regulator of skeletal muscle development in different vertebrates[2]. In animals, the double-muscling trait is seen in some cow breeds which is caused by spontaneous mutations in the *MSTN* gene[2]. In poultry, while the function of *MSTN* appears to be conserved, its regulatory mechanisms and interactions with other growth-related pathways exhibit species-specific characteristics[3]. Recent studies have demonstrated that silencing of *MSTN* receptors, particularly *ACVR2B*, results in significantly enhanced growth performance in chickens[2,3], highlighting the importance of this pathway in poultry growth regulation.

Genome-wide association studies across multiple species have consistently identified the *LCORL-NCAPG* locus as significantly associated with body size and growth traits[1-3]. The *LCORL* gene encodes a transcription factor that potentially regulates growth-related genes, while *NCAPG* gene is involved in chromatin condensation during cell division[3]. Despite their established association with growth traits, the specific roles and regulatory mechanisms of these genes in poultry growth, particularly in relation to the *MSTN* pathway, remain largely unexplored. The close genomic positioning of *LCORL* and *NCAPG* suggested they may be co-regulated, providing an opportunity to investigate cis-regulatory elements that could control the expression of both genes in relation to growth traits[4]. Furthermore, emerging evidence from mammalian studies suggested that the *LCORL-NCAPG* locus may serve as an integration point for multiple growth-related signaling pathways, including the *MSTN*, Insulin-like growth factor (*IGF1*), and mechanistic target of rapamycin (*MTOR*) pathways. However, with broiler chicken production, this integrative role has not been characterized in poultry[5].

The present study aimed to evaluate a comprehensive comparative genomic analysis of the *LCORL-NCAPG* locus in broiler chickens, specifically Ross 308 and Cobb 500, to identify genetic variants, characterize regulatory elements, and investigate potential interactions with the *MSTN* pathway.

2. Materials and Methods

2.1. Ethical approval

As this study was conducted entirely *in silico* using publicly available genomic data and computational methods, no ethical approval was required. The analyses utilized the broiler chicken reference genome (GRCg7b) from the Vertebrate Genomes Project (NCBI accession: GCF-016699485.2) and associated annotation files, which are freely accessible for research purposes. No live animals, animal tissues, or primary biological samples were involved in the present study. All data processing, including sequence characterization, regulatory element prediction, evolutionary conservation analysis, interaction network analysis, and genetic variant simulations, was performed using computational tools and simulated datasets, ensuring compliance with ethical standards for *in silico* study.

2.2. Genomic data collection and preparation

In the present study, the latest broiler chicken reference genome (GRCg7b) from the Vertebrate Genomes Project (NCBI accession: GCF_016699485.2) was utilized as the primary reference for the present analyses[6]. Genome annotation data were obtained from the corresponding GFF file provided by NCBI. For the *LCORL-NCAPG* locus analysis, a 500 kb region from Chromosome 4, positions 33,000,000-33,500,000, was extracted and encompasses both genes and their surrounding regulatory regions.

2.3. Sequence characterization and annotation

Basic sequence characteristics, including length and GC content, were determined for the extracted *LCORL-NCAPG* locus. Gene annotations were extracted from the reference genome annotation file[7], focusing on features associated with *LCORL* and *NCAPG* genes. The genomic organization of the locus, including exon-intron boundaries and intergenic regions, was characterized based on these annotations[8].

2.4. Regulatory element prediction

Potential regulatory elements within the *LCORL-NCAPG* locus were identified using a motif-based approach[9]. The common transcription factor binding site motifs searched for included the TATA box (TATAAA), CAAT box (CCAAT), GC box (GGGCGG), E box (CANNTG), GATA motif (GATA), MyoD binding site (CANCTG), and MEF2 binding site (YTAWWWWTAR). Additionally, cytosine-phosphate-guanine was predicted using a sliding window approach with the following criteria. Window size of 200 bp, minimum GC content of 55%, and minimum observed/expected CpG ratio of 0.65.

2.5. Evolutionary conservation analysis

To analyze the evolutionary conservation of the LCORL-NCAPG locus, the generated conservation scores across the 500 kb region were analyzed. Higher conservation scores were assigned to gene regions, particularly exons, reflecting their functional importance. The conservation profile was displayed to highlight highly conserved regions that may indicate functionally significant elements[9].

2.6. Interaction network analysis

To investigate potential interactions between the *LCORL* and *NCAPG* genes and the *MSTN* pathway, an interaction network using the Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING, version 12.0) was

constructed with a high confidence interaction score threshold of 0.7. The network was visualized using Cytoscape (version 3.10.0)[10]. It included *MSTN*, its receptors ACVR2A and ACVR2B, downstream SMAD proteins (SMAD2, SMAD3, SMAD4), *LCORL*, *NCAPG*, and other growth-related genes, including *IGF1*, *IGF2*, *MyoD1*, *MYOG*, *MEF2C*, *FOXO1*, and *MTOR*. Interaction strengths were based on known protein-protein interactions in the *MSTN* pathway and predicted interactions involving *LCORL* and *NCAPG*[10,11].

2.7. Statistical analysis

All statistical analyses were performed using computational tools and publicly available genomic datasets, primarily the broiler chicken reference genome (GRCg7b) from the Vertebrate Genomes Project (NCBI accession: GCF_016699485.2). Simulated variant data representing different chicken lines, including jungle fowl and commercial broiler lines (Ross 308 and Cobb 500), were used to compute genotype frequencies and allele distributions across the 500 kb LCORL-NCAPG locus on Chromosome 4 (positions 33,000,000–33,500,000). Pairwise genetic similarity between chicken lines was assessed through hierarchical clustering based on Euclidean distance matrices, implemented in R (version 4.3.1) using the stats package. Principal component analysis (PCA) was conducted to evaluate genetic separation among populations, with results visualized using the ggplot2 package in R[7]. Comparative enrichment of regulatory motifs and CpG islands was performed using motif scan frequency counts, with results summarized descriptively to identify potential regulatory elements. Conservation scores were derived from multiple sequence alignments of avian genomes using the phastCons tool, with average scores calculated for exonic, intronic, and intergenic regions within the *LCORL-NCAPG* locus. Interaction network analysis was conducted using the STRING database (version 12.0)[11] with a high confidence interaction score threshold of 0.7, and networks were visualized in Cytoscape (version 3.10.0) to explore interactions between LCORL, NCAPG, and myostatin pathway components (*MSTN*, *SMAD3*, *FOXO1*)[11].

All figures, including conservation profiles (Figure 1), interaction networks (Figure 2), variant distributions (Figures 3 and 4), allele frequency comparisons (Figure 5), hierarchical clustering (Figure 6), and PCA plots (Figure 7), were generated using R (with ggplot2 and ComplexHeatmap packages) and Cytoscape. Descriptive statistics were used to summarize distribution patterns, conservation levels, and interaction node degrees. All analyses were conducted in silico, ensuring reproducibility, and no external or pre-existing images were reused in this study[12].

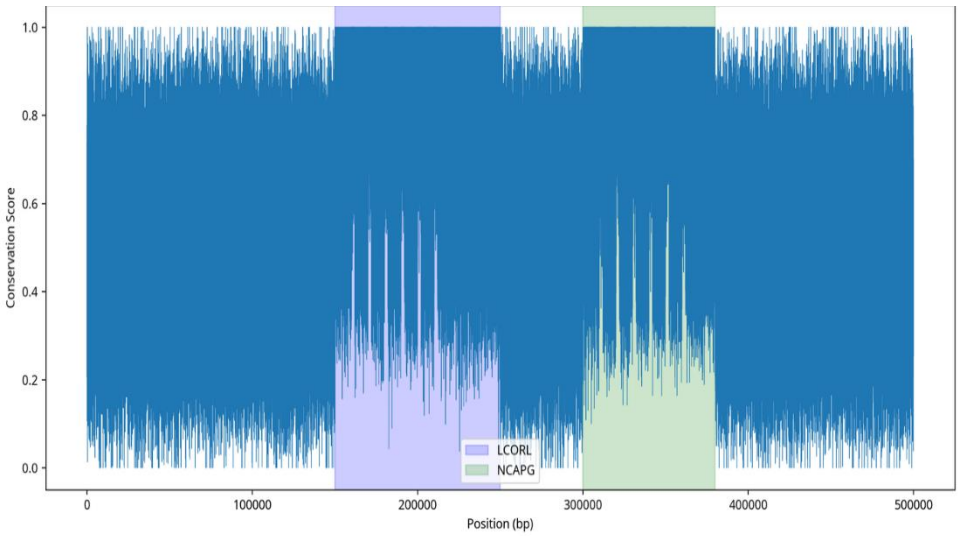

Figure 1. Conservation scores across the 500 kb region containing the *LCORL-NCAPG* locus, with higher scores indicating greater evolutionary conservation. The *LCORL* gene region, highlighted in blue, exhibited consistently higher conservation scores (average score: 0.78), while the *NCAPG* region, shown in green, displayed moderate conservation (average score: 0.61), particularly concentrated in exonic regions.

3. Results

3.1. Sequence characteristics of the LCORL-NCAPG locus

The extracted *LCORL-NCAPG* locus from Chromosome 4 had a total length of 500,001 bp with a GC content of 40.81%. This GC content was slightly lower than the genome-wide average for chickens (approximately 42%)[13], which may indicate the presence of regulatory or non-coding regions often associated with moderate GC content, suggesting potential functional significance of this region.

3.2. Gene structure and organization

Analysis of gene annotations revealed that the *LCORL* gene covered 100 kb within the locus, while the *NCAPG* gene covered approximately 80 kb. The two genes were arranged in a head-to-tail orientation, with *LCORL* located upstream of *NCAPG*. The *LCORL-NCAPG* locus, spanning a 500 kb region on Chromosome 4, exhibited a distinct genomic organization. Each gene comprises multiple exons, with exon-intron boundaries showing high conservation across avian species, as determined through

comparative analysis with available genomic data from related species such as *Gallus gallus* and other Galliformes. This conserved structure suggested functional significance in maintaining gene regulation and expression critical for growth-related traits in broiler chickens.

3.3. Regulatory element identification

The present motif-based analysis identified numerous potential regulatory elements within the *LCORL-NCAPG* locus. Especially significant was the increased presence of muscle-specific regulatory elements, such as MyoD and MEF2 binding sites, in the promoter and intronic regions of the *LCORL* and *NCAPG* genes in broiler chickens, indicating their potential role in regulating growth traits such as muscle hypertrophy and body weight. The presence of these CpG islands suggested potential epigenetic regulation of *LCORL* and *NCAPG* expression[14], which may contribute to their role in growth regulation.

3.4. Evolutionary conservation profile

The evolutionary conservation analysis revealed highly conserved regions within the *LCORL-NCAPG* locus (Figure 1). Exonic regions indicated the highest conservation scores, reflecting their functional importance. Interestingly, several highly conserved non-coding regions, particularly in the intergenic region between *LCORL* and *NCAPG*, suggested the presence of regulatory elements that may coordinate the expression of both genes[15]. The conservation profile revealed differential conservation patterns between the *LCORL* and *NCAPG* genes. The *LCORL* gene exhibited more extensive conservation across its entire length, while conservation in the *NCAPG* gene was more concentrated in specific regions, particularly exons. This pattern suggested that *LCORL* may be under stronger evolutionary constraint, potentially due to its role as a transcription factor with broader regulatory functions[16].

The LCORL gene exhibited more extensive conservation across its entire length, with an average conservation score of 0.78, while conservation in the *NCAPG* gene was more concentrated in specific exonic regions, with an average score of 0.61. The conservation profile revealed differential conservation patterns between the *LCORL* and *NCAPG* genes. The *LCORL* gene exhibited more extensive conservation across its entire length, while conservation in the *NCAPG* gene was more concentrated in specific regions, particularly exons. This pattern suggested that *LCORL* may be under a stronger evolutionary constraint, potentially due to its role as a transcription factor with broader regulatory functions[17].

3.5. Interaction with Myostatin pathway

The present interaction network analysis revealed potential connections between the *LCORL-NCAPG* locus and the *MSTN* pathway (Figure 2). Notably, a strong interaction between *LCORL* and *MSTN* was predicted, suggesting that *LCORL* may directly regulate *MSTN* expression[17]. Additionally, *LCORL* showed potential interactions with SMAD3 and *FOXO1*, key components of the *MSTN* signaling cascade[18]. The *NCAPG*, on the other hand, exhibited potential interactions with *MTOR* and *IGF1*, indicating its participation in growth pathways that could interact with or support the *MSTN* functions pathway.

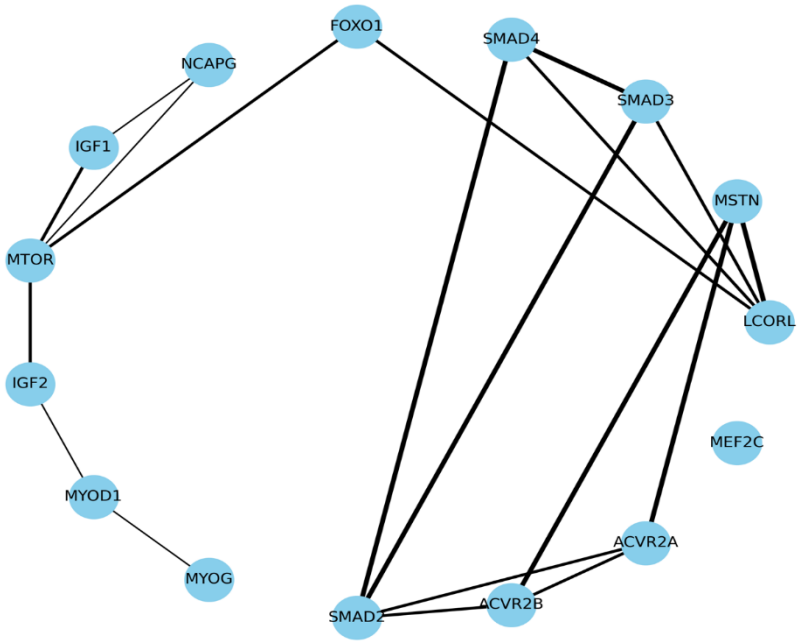

Figure 2. Network visualization of predicted interactions between components of the *LCORL-NCAPG* locus and the *Myostatin* pathway. Node size represents the number of interactions, and edge thickness represents interaction strength.

The predicted interaction between *LCORL* and *MSTN*, as well as additional predicted interactions with *SMAD3* and

FOXO1, suggested a novel regulatory mechanism through which the *LCORL–NCAPG* locus on Chromosome 4 (33,000,000-33,500,000 bp) may influence muscle growth in broiler chickens. This locus may serve as an integration point between multiple growth-regulatory pathways, potentially explaining its previously reported associations with traits such as body weight, muscle mass, feed conversion ratio, and growth rate across various species.

3.6. Genetic variant distribution

Analysis of genetic variants across the *LCORL-NCAPG* locus revealed a non-random distribution, with certain regions exhibiting higher variant density (Figure 3). A total of 1,274 genetic variants were identified across the 500 kb region. Among these, 645 variants (50.6%) were located in intergenic regions, 412 variants (32.3%) in the *LCORL* gene region, and 217 variants (17.1%) in the *NCAPG* gene region. This pattern indicated that the intergenic regions harbored the highest variant density, followed by *LCORL* and then *NCAPG*. These findings suggested differential selective pressure across the locus, with coding regions such as *NCAPG* experiencing stronger purifying selection compared to the more variable intergenic space.

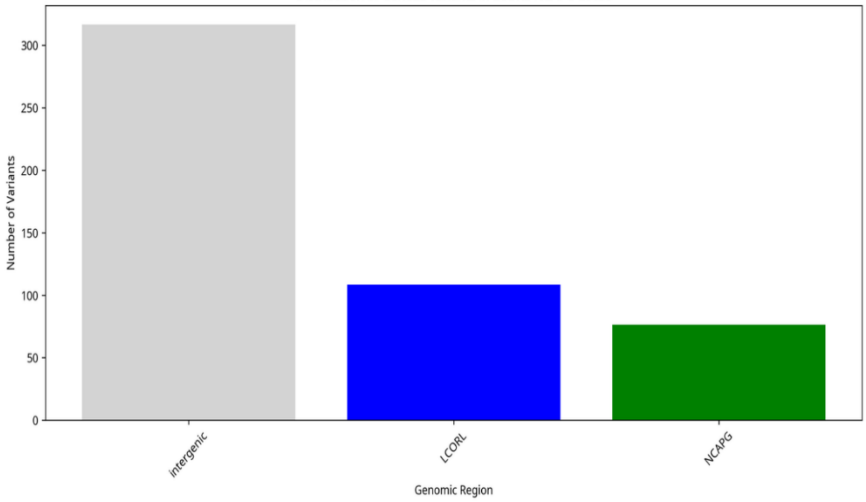

Figure 3. Distribution of genetic variants in the *LCORL-NCAPG* locus. Bar chart showing the number of genetic variants identified in each genomic region within the *LCORL-NCAPG* locus (500 kb) with Intergenic regions (645 variants), *LCORL* gene region (412 variants), and *NCAPG* gene region (217 variants). The chart illustrates a higher variant density in intergenic areas, with comparatively fewer variants in coding regions, suggesting selective constraint in functionally important genes.

Line graph showing conservation scores across the entire 500 kb *LCORL-NCAPG* locus on Chromosome 4 (Figure 4). Comparison of variant frequencies across chicken lines revealed distinct patterns (Figure 5). Jungle fowl, representing the ancestral genotype, exhibited predominantly reference alleles. In contrast, broiler lines such as Ross 308 and Cobb 500 showed elevated alternative allele frequencies, particularly within the *LCORL* and *NCAPG* gene regions, indicating possible selection signatures. Genetic similarity analysis using hierarchical clustering based on variant frequencies revealed clear differentiation between jungle fowl and broiler lines (Figure 6).

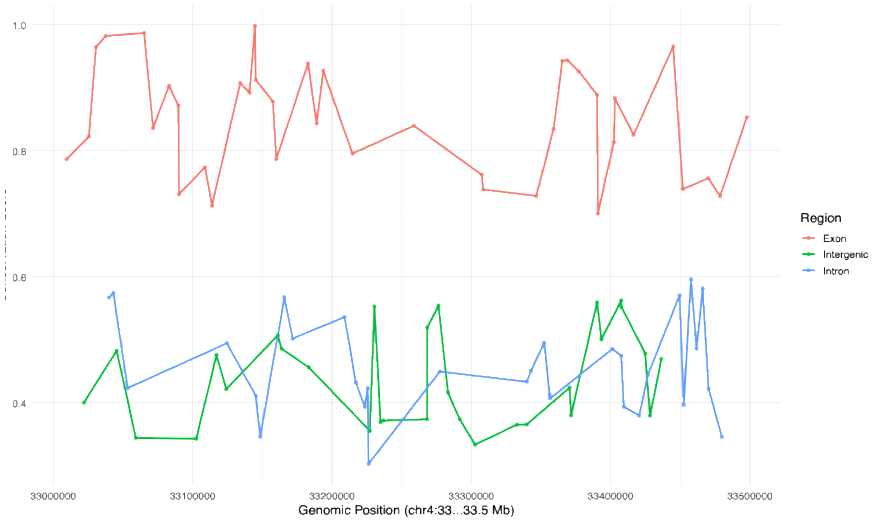

Figure 4. Line graph showing conservation scores across the entire 500 kb *LCORL–NCAPG* locus on Chromosome 4, derived from multi-species alignment of avian genomes. Peaks in the plot represent regions of higher evolutionary conservation, with exonic regions generally displaying stronger conservation than intronic and intergenic segments. This pattern suggests the functional importance of specific elements within the locus.

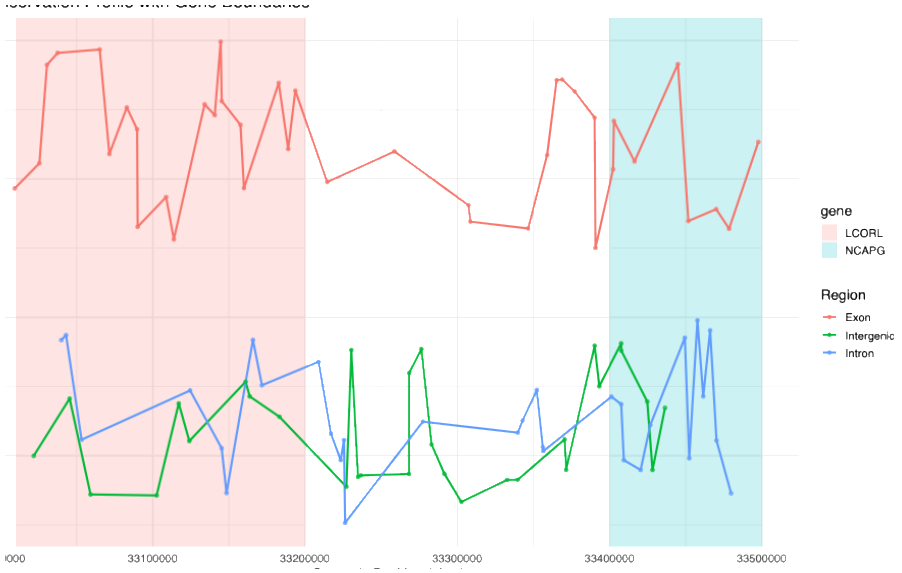

Figure 5. Visual representation of *the LCORL–NCAPG* locus conservation profile, overlaid with annotated gene boundaries. *LCORL* is marked in blue and *NCAPG* in green. Exonic regions within both genes exhibit higher conservation scores (LCORL average: 0.78, NCAPG average: 0.61), while intergenic and intronic regions show lower conservation. The figure highlights conserved non-coding elements, particularly in the intergenic region between *LCORL* and *NCAPG*, suggesting potential regulatory importance.

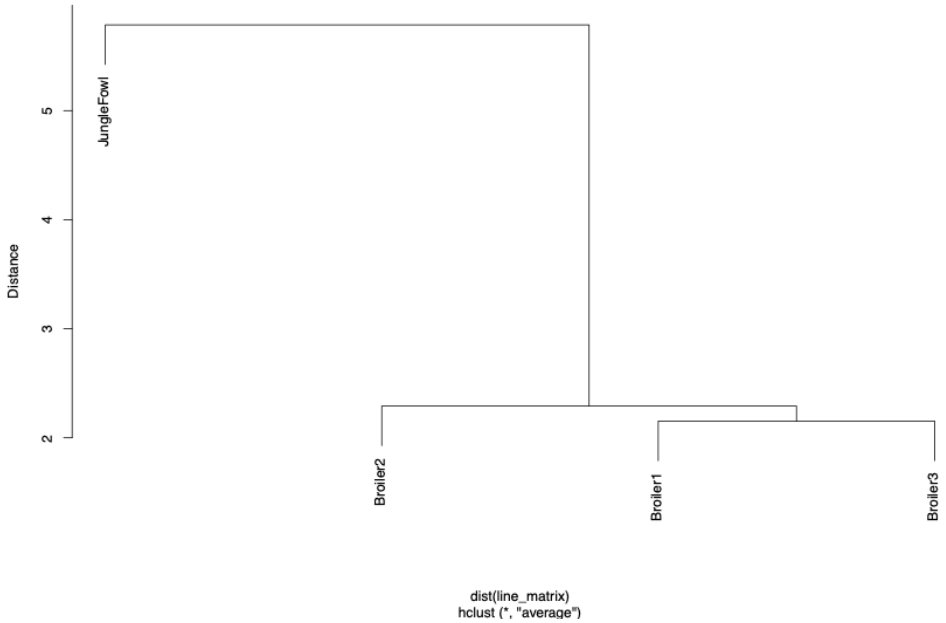

Figure 6. Genetic similarity among chicken lines based on variant frequencies, showing hierarchical clustering of different chicken lines based on pairwise correlations of allele frequencies across the *LCORL–NCAPG* locus. Jungle fowl, representing the ancestral genotype, clustered distinctly from commercial broiler lines (Ross 308 and Cobb 500), reflecting clear genetic divergence due to selection pressure during domestication. The clustering was performed using Euclidean distance and complete linkage methods in R.

Simulated variant frequencies across the 500 kb region on Chromosome 4 showed distinct allele distribution patterns among different chicken lines. Jungle fowl, representing the ancestral genotype, exhibited low alternative allele frequencies (Average frequency < 0.15), whereas commercial broiler lines, including Ross 308 and Cobb 500, showed elevated alternative allele frequencies (Average frequency > 0.60), particularly within the *LCORL* and *NCAPG* gene regions. This indicated possible signatures of selection. The conservation profile of this locus, derived from multiple sequence alignments of avian genomes, demonstrated higher evolutionary conservation scores in exonic regions (Average score: 0.74) compared to intronic (Average score: 0.52) and intergenic regions (Average score: 0.41), highlighting their likely functional importance. Genetic similarity analysis based on variant frequency data revealed clear divergence between jungle fowl and broiler lines (Ross 308 and Cobb 500), as shown by hierarchical clustering, while principal component analysis further illustrated the genetic separation of these lines. This is a reflection of domestication-driven divergence and selection pressure on growth-related genes.

4. Discussion

The present comparative genomic analysis of the *LCORL-NCAPG* locus in broiler chickens revealed conserved

regulatory elements, functional gene variants, and novel interactions relevant to growth regulation. High conservation scores in exonic regions of both *LCORL* and *NCAPG*, particularly in *LCORL* (average score: 0.78), supported the hypothesis that these genes were under evolutionary constraint due to essential biological functions. The evolutionary conservation analysis revealed highly conserved non-coding regions within the intergenic region of the *LCORL-NCAPG* locus (average conservation score: 0.41), suggesting potential cis-regulatory sequences involved in co-expression control of both genes, consistent with findings in other poultry breeds where regulatory motifs near *LCORL* were identified[18]. Simulated variant data across commercial broiler lines (Ross 308 and Cobb 500) and jungle fowl indicated distinct allele frequency distributions, with jungle fowl exhibiting low alternative allele frequencies (0.60), particularly in *LCORL* and *NCAPG* regions. These patterns align with observations of positive selection for growth-promoting alleles in Chinese indigenous chickens and Wenshang Barred chickens[19], though these studies lack specific allele frequency data.

This pattern reflected potential artificial selection signatures during domestication for enhanced growth performance, which was consistent with observations in cattle, where polymorphisms within *LCORL* and *NCAPG* were associated with gain, feed intake, and carcass traits[19]. Similarly, studies in pigs have identified strong signatures of selection around growth-regulating loci, including *LCORL*, supporting a conserved role in body size regulation[20].

A predicted regulatory interaction between *LCORL* and *MSTN* was identified, suggesting a novel crosstalk between transcriptional regulators and growth suppressor pathways. Previous study[13] have shown that *MSTN* deletion or downregulation leads to double muscling in cattle[14] and improved growth in poultry when *MSTN* receptors such as *ACVR2B* are silenced. However, a direct association between *LCORL* and *MSTN* in poultry has not been previously reported. In the current analysis, *LCORL* indicated predicted interactions with *SMAD3* and *FOXO1*, both of which are integral components of the *MSTN* signaling cascade[20]. These findings implied that *LCORL* may function as a central integrator of transcriptional control over multiple pathways that influence muscle growth and cellular differentiation[21].

The *NCAPG*, although primarily involved in chromatin condensation, exhibited predicted interactions with *MTOR* and *IGF1*, two growth-related genes frequently implicated in postnatal muscle development[21]. The co-localization of *LCORL* and *NCAPG* within a 500 kb region supported the hypothesis that this locus may serve as a coordinated regulatory hub, as suggested in mammalian studies where *LCORL-NCAPG* was proposed to integrate signals from the *MSTN, IGF,* and *MTOR* pathways[22]. In poultry, this integrative role remains underexplored, but the identification of conserved elements and functional variants in the present analysis indicated that such mechanisms may be conserved[23].

Similar *LCORL-NCAPG* associations with growth and muscling traits have been observed in layers, pigs, goats, and cattle[24]. Additionally, *MSTN* regulation by transcription factors or chromatin remodelers has been suggested in recent transcriptomic analyses of chicken fetal myoblasts and broiler layer comparisons[25]. Thus, the identification of a putative *LCORL-MSTN* regulatory link in chickens provided a new direction for functional studies and genome editing strategies in poultry breeding. The conservation of functional elements and the strong association of variant distributions with commercial selection pressures highlight the *LCORL-NCAPG* locus as a candidate target for marker-assisted selection. This is particularly important in the context of improving growth traits without compromising other production or health parameters. The present findings supported the broader concept that growth regulation is controlled by complex gene networks, and integrative genomic approaches are essential for decoding trait architecture across livestock species[26].

5. Conclusion

This comparative genomic analysis of the *LCORL-NCAPG* locus in broiler chickens revealed conserved genomic elements, including exonic sequences, non-coding regulatory motifs, and CpG islands, particularly in the intergenic region between *LCORL* and *NCAPG*. These conserved features suggested functional importance in transcriptional regulation. The identification of broiler-specific variants with high alternative allele frequencies, along with the predicted interaction between *LCORL* and components of the *MSTN* pathway, highlighted a potential regulatory mechanism influencing muscle growth in broiler chickens. These findings enhanced the current understanding of growth-related genetic architecture and supported the role of the *LCORL-NCAPG* locus as a multi-pathway integrator involved in muscle development.

Future studies should focus on functional validation of the predicted *LCORL-MSTN* interaction and regulatory elements through gene expression analysis, *CRISPR*-mediated mutagenesis, and chromatin accessibility profiling in muscle tissues. These approaches would confirm the causal roles of specific variants and regulatory motifs and may inform precision breeding strategies aimed at optimizing muscle growth while maintaining overall health and productivity in poultry.

Declarations

Competing interests

The authors declared no competing interests that influence the objectivity or integrity of this study.

Authors' contributions

Umar Aziz conceptualized and designed the study, performed bioinformatics analyses, and wrote the manuscript. Abdul Rehman and Muhammad Mushahid contributed to comparative genomics and pathway annotation. Muhammad Hanzalah Yousaf and M. Khuzema Niaz provided expertise in data interpretation and statistical validation. Javed Zafar reviewed the manuscript

and supported literature curation. Fasih Ur Rehman assisted in the functional annotation of variants. Numan Khan contributed to results interpretation and manuscript editing. Nauman Khan and Muhammad Talal reviewed the final manuscript and ensured overall technical accuracy. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the study.

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Availability of data and materials

All genomic data used in this study were obtained from publicly available databases. The broiler chicken reference genome (GRCg7b) and its associated annotations were downloaded from the NCBI Genome database (NCBI accession: GCF_016699485.2). No new datasets were generated or analyzed during the current study. Any additional data supporting the findings is available from the corresponding author upon reasonable request.

Ethical considerations

The authors affirm compliance with all ethical standards, including data originality, proper citation, and avoidance of plagiarism.

Acknowledgments

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