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A 31-bp Indel in the 5' UTR region of the GNB1L gene is significantly associated with body weight and carcass traits in chickens

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Abstract

Background

G-protein subunit beta 1 like (*GNB1L*) can encode a G-protein beta-subunit-like polypeptide, the chicken *GNB1L* gene is up-regulated in the breast muscle of high-feed efficiency chickens, and its expression is 1.52-fold that of low-feed efficiency chickens. However, there are no reports describing the effects of *GNB1L* gene Indel on the growth and carcass traits of chickens.

Results

This study identified a 31-bp Indel in 5' UTR of the *GNB1L* gene and elucidated the effect of this gene mutation on the growth and carcass traits in chickens. The results indicated that the 31-bp Indel was highly significant associations with body weight at 8 different stages, and also significantly correlated with daily gain of 0 to 4 weeks and 4 to 8 weeks. Similarly, the mutation was significantly associated with small intestine length, breast width, breast deep and breast muscle weight in carcass traits. Moreover, *DD* and *ID* are inferior genotypes for the growth and carcass traits of chickens.

Conclusions

In a word, these findings suggest that the 31-bp Indel of *GNB1L* gene is significantly affected body weight and carcass traits in chickens, and can serve as a potential molecular marker for chicken genetics and breeding programs.

Background

Compared to pigs and cattle, chickens have high-feed efficiency and short growth period, chicken meat is already the second largest meat product after pork in China [1]. Therefore, chicken breeds play an indispensable role in husbandry. The body weight of animals as an economic trait can directly reflect the balance of nutrients, animals through digestive absorption and energy metabolism lead to skeletal growth, lean or fat deposition [2, 3].

G-protein subunit beta 1 like (*GNB1L*) can encode a G-protein beta-subunit-like polypeptide, which contains six WD40 repeats, but which lacks homology with known proteins [4]. In humans, the hemizygous deletion of *GNB1L* can cause sensory motor gating defects, which are related to schizophrenia and other serious mental diseases [5, 6]. Changes in *GNB1L* expression are also associated with markers related to psychosis [7]. In the study of chickens, a candidate gene *GNB1L* for the ear-tufted trait was verified by GWAS and haplotype analysis [8], and a study also showed that *GNB1L* gene is related to higher feed efficiency, the *GNB1L* gene is up-regulated in the breast muscle of high-feed efficiency chickens, and its expression is 1.52-fold that of low-feed efficiency chickens [9]. However, there

are no any reports describing the effects of *GNB1L* gene Indel on the growth and carcass traits of chickens.

Gene variants such as insertion/deletion (Indel) and single-nucleotide polymorphism (SNP) are widely distributed in animal's genome, and there are many research reports in humans and livestock animals [10, 11]. Compared with SNP, the genotyping of large fragments Indel has a higher efficiency [12]. Indel mutations also play crucial roles in many aspects of animal economic traits. There is a 10-bp Indel in the *PAX7* promoter, which is located at the binding site of *ZNF219*. This Indel affects the promoter activity and expression of the *PAX7* gene, which in turn affects the early growth traits of cattle [13]. A19-bp Indel in the intron region of the *PLAGI* gene affects the growth traits of the Chinese cattle [14]. The 16-bp Indel in 5' untranslated regions (UTR) of the *ZNF132* gene was significantly affected the body length of the Hainan black goat [3]. Recent studies revealed that 11-bp Indel in the intron 22 of *DNMT3B* was significantly associated with the first-born litter size of goat [15]; two Indels (P2-16 bp and P14-15 bp) of *DSCAML1* were markedly related to sperm quality in male goat, and three Indels of *DSCAML*1 were significantly associated with the first-birth litter size in female goat [16]. A study has shown that 13-bp Indel polymorphism in the 3' UTR of the *DGAT2* gene affects its expression and fat deposition in porcine [17]. In poultry research, two novel Indels in the promoter region of the chicken *QPCTL* gene significantly affected body weight at the age of 0, 4, 6, 8, and 12 weeks and carcass traits[18]; the multiallelic Indel in the promoter region of the chicken *CDKN3* gene was significantly associated with growth and carcass traits [19]; a 22-bp Indel in the chicken *ZNF764L* gene was markedly related to birth weight, body slanting length, chest breadth and subcutaneous fat weight [20]. A 65-bp Indel in the fifth intron region of the *GOLGB1* gene was associated with chicken body weight and Carcass Traits [21]. The 80-bp Indel polymorphism within the *PRLR* gene was significantly associated with chicken body weight, leg weight, and shank length [22].

In this study we verified a 31-bp Indel polymorphism in the *GNB1L* gene from 10x whole-genome resequencing data of ten XH and ten RW chickens (data unpublished) (EVA accession number: PRJEB36864). The chicken *GNB1L* gene is located on chromosome 15, comprising 15 exons and encodes a protein of 328 amino acids. Furthermore, a total of 80 Indels were found in the chicken *GNB1L gene* in the Ensembl database (http://asia.ensembl.org/Gallus_gallus/Gene/ Variation_Gene/Table?db = core;g = ENSGALG00000001925;r = 15:1232691–1273276;t = ENSGALT 00000002979). However, there is no report and verify about the Indel of chicken *GNB1L gene*. The aim of this research is to verify the Indel mutation of the *GNB1L* gene, to clarify the effect of the *GNB1L* Indel on chicken economic traits, and to analyze the expression of *GNB1L* in different tissues, leg muscles and chest muscle tissues at different embryonic development stages. In addition, we examined the distribution of 31-bp Indel mutations in different populations. These results indicate that the 31-bp Indel of the *GNB1L* gene can be used as a potential molecular marker for chicken growth traits, and provide a reference for molecular breeding of chickens.

Results

Genotyping and sequencing confirmation

A novel 31-bp Indel in the 5' UTR region of the *GNB1L* gene was observed by whole genome resequencing and DNA sequencing (Figure S1) (TSINGKE, Guangzhou, China). All PCR products were detected using 3.0% agarose gel electrophoresis, we found three genotypes including the 301 bp homozygous *DD* genotype, the heterozygous *ID* genotype (332 bp and 301 bp) and 332 bp homozygous *II* genotype (Figure S2).

Genetic Diversity of the 31-bp Indel in different populations

The genotype frequencies, allele frequencies and genetic parameters of in seven different breeds and F2 population were analyzed (Table 1). The results suggest that the *I* allele frequency was higher than that of *D* in all breeds, except for LS chicken. Meanwhile, we counted different genotype distribution among the dual-purpose chickens (ND, GX, WC, QY and LS), F2 population, commercial broilers (RW) and commercial layers (ISA). The percentage of the *DD* genotype was the lowest in all breeds (Figure S3). The results of a χ^2 test showed that the genotype frequencies of F2, ND and RW were not in HWE ($P < 0.05$), and ISA, WC, QY and LS were in HWE ($P > 0.05$). The values of He is from 0.46 to 0.50, and the values of Ne is from 1.85–1.99. The smallest and largest values of PIC are 0.35 and 0.37, respectively. The results revealed that the 31-bp Indel of *GNB1L* represents intermediate polymorphism, and lack of high genetic diversity in all populations (Table 1).

Differential selection of the 31-bp Indel locus

Results of differential selection suggested that between the LS and QY, LS and GX with moderate genetic differentiation ($0.05 < Fst < 0.15$). Moreover, we observed between the other breeds with little genetic differentiation ($Fst < 0.05$) (Table 2).

Association between the GNB1L gene 31-bp Indel and economic traits

Mixed Model were used to analysis the relationship between genotypes and economic traits. As shown in Table 2, the three genotypes showed significant correlation with 11 chicken growth traits, and greatly significantly associated with 9 growth traits. Especially, different genotypes were very significantly related to body weight at 7, 14, 21, 28, 35, 42, 49 and 56 weeks, Daily gain of 0 to 4 weeks ($P < 0.01$), and were significantly associated with Daily gain of 4 to 8 weeks and shank length of 49 weeks ($P < 0.05$) (Fig. 1a, 1b) (Table 2). Importantly, the *DD* and *ID* genotypes were greater than the *II* genotype in all related growth traits.

Notably, the 31-bp Indel displayed highly significantly associated with breast width, breast deep, breast muscle weight and small intestine length in carcass traits, and were significant correlation with fat cingula width (Table 3). Interestingly, the *DD* and *ID* genotypes were greater than the *II* genotype in all related carcass traits. In the association analysis of 31-bp Indel and meat quality traits, the different

genotypes showed significant correlation with dry matter content of leg muscle, and critical correlation with crude fat content of leg muscle in Table S3.

Relative expression of the GNB1L gene

The expression of *GNB1L* gene in 12 tissues of 20 weeks QY spotted-brown chickens was detected by qPCR. Based on qPCR, *GNB1L* was relatively highly abundant in heart, breast muscle, leg muscle, kidney and ovary, and in liver, spleen, lung, small intestine and abdominal fat had relatively low expression levels (Fig. 2). Furthermore, the *GNB1L* gene expression level increases first and then decrease in breast muscle at different embryonic stages, and expression level decreases first and then increases in leg muscle at different embryonic stages (Fig. 3a, 3b).

Transcription Factor Prediction in the 31-bp Indel of the GNB1L gene

The transcriptional binding sites in the 31-bp Indel of the 5' UTR region of *GNB1L* gene were analyzed by online prediction website, and the results revealed five potential transcription factors (NF-1, SP1, T3R, RAR-α and GR) (Figure S4).

Discussion

The allelic frequency of genes can reflect the genetic diversity between different groups, which means that new mutations are introduced to some extent [2, 23]. In recent decades, the breeding of commercial broilers and layers focuses on growth and reproductive traits, respectively. In these commercial breeds, dominant genotypes for specific traits may be selected for breeding. Moreover, manual selection also determines the number and distribution of genetic variation during domestication [23]. In this study, I was the predominant allele in the ND, GX, WC, QY, F2 population, RW and ISA, except for LS chicken. The results show that the LS chickens may undergo different selection pressure during evolutionary processes than other chickens. Interestingly, LS chicken is the only breed that can produce blue eggs in these breeds [24].

Body weight of chickens is a heritable trait with about 0.24%-0.47% heritability during growth [25]. Compared with commercial broilers, Chinese domestic broilers have a relatively low growth rate and body weight. Therefore, we studied the association between the 31-bp Indel polymorphism in the 5' UTR region of the *GNB1L* and F2 population growth and carcass traits. As shown in Table 2, the 31-bp Indel highly significant associations with body weight at 8 different stages. Moreover, the three different genotypes were also significantly correlated with daily gain of 0 to 4 weeks and 4 to 8 weeks, and shank length of 49 weeks (Table 2). Significantly, the *DD* and *ID* genotypes were greater than the *II* genotype in all related growth traits, the *DD* genotype has the greatest weight at 7, 14, 21, 28, 35, 42 and 49 weeks, except for 56 weeks. Interestingly, the *DD* is the dominant genotype in daily gain of 0 to 4 weeks, and the *ID* is the

dominant genotype in daily gain of 4 to 8 weeks. We hypothesize that *DD* and *ID* genotypes may have a higher feed conversion ratio during chicken development. In summary, the *II* genotype is a disadvantaged genotype in all growth traits.

Chinese domestic chickens have a good carcass yield, with breast muscles accounting for about 30% of the carcass weight, and the weight of muscles accounts for about 40% of the weight of the carcass [26]. Therefore, individuals with larger breast width, breast depth and breast weight are also the breeding direction of local yellow-feathered broilers. As shown in Table 3, the mutation was significantly associated with breast width, breast deep, breast muscle weight and small intestine length of carcass traits. Similarly, the *DD* and *ID* genotypes were greater than the *II* genotype in all related carcass traits. Growing evidence suggests that the small intestine mainly responsible for the efficient absorption and metabolic processing of nutrients, and the small intestinal villi are the main parts for absorbing nutrients [27]. Perhaps the longer length of the small intestine is helpful to improve the efficiency of animal absorption of food. We speculate that *GNB1L* 31-bp Indel may affect the conversion efficiency of feed by affecting the length of the small intestine, which ultimately leads to differences in growth and carcass traits of individuals with different genotypes. Previous research results also indicate that *GNB1L* is related to higher feed efficiency [9].

Studies have demonstrated that mutations in 5' UTR of some genes can affect gene expression [28, 29]. Furthermore, TFs are also essential factors that regulation gene expression, prediction results of TFs showed that there are five potential TFs such as NF-1, SP1, T3R, RAR-α and GR in 31-bp of *GNB1L*. We guess these TFs may be involved in the transcription of *GNB1L* gene, which in turn lead to differences of phenotype in three genotypes. In this research, the expression of the *GNB1L* gene was relatively highly abundant in heart, breast muscle, leg muscle, kidney and ovary, and other tissues had relatively low expression levels. Moreover, the expression of *GNB1L* increases first and then decreases in breast muscle at different embryonic stages, and decreases first and then increases in leg muscle. These results showed that the *GNB1L* may be related to the embryonic muscle development.

Conclusion

In conclusion, we first time found that the *GNB1L*, a candidate gene for high-feed efficiency, has a 31-bp Indel in its 5' UTR that is significantly related to chicken growth and carcass traits. Moreover, *DD* and *ID* are inferior genotypes for the growth and carcass traits of chickens. In addition, our research once again proved that *GNB1L* gene may be a candidate gene for higher feed conversion rate. In summary, this study showed that the *GNB1L* gene may be involved in the embryonic development and growth of chickens, and the 31-bp Indel of *GNB1L* gene can be used as a molecular marker for chicken genetics and breeding programs.

Methods

Animal samples and trait measurement

DNA samples of 766 chickens from eight populations, Lushi chickens (LS, n = 39, 6 weeks), Ningdu chickens (ND, n = 95, 12 weeks), Wenchang chickens (WC, n = 65, 7 weeks), Qingyuan Partridge chickens (QY, n = 70, 7 weeks), Recessive White Rock chickens (RW, n = 55, 7 weeks), ISA Brown laying hen (ISA, n = 54, 20 weeks), Guangxi chickens (GX, n = 71, 12 weeks) and F2 population (F2, n = 360, 13 weeks) were used. These DNA samples are all from the chicken breed resource library kept in our laboratory. In eight different breeds, LS, ND, WC, QY and GX are domestic chicken breeds in China, RW and ISA are commercial broilers and layer hens, respectively. And the F2 resource population is a hybrid strain of RW and Xinghua (XH) chickens, XH chickens represent a slow-growing Chinese domestic chicken. In the laboratory of South China Agricultural University, 5% Pentobarbital 2 mL (No. 57–33 – 0 of Chinese Academy of Sciences, Beijing Siyuan Technology Co., Ltd.) was injected intraperitoneally into chickens. After 2–3 min of no spontaneous respiration, the chicken was sacrificed by bleeding through the carotid artery. All F2 population had data records about economic traits, and detailed information on measuring methods is as previously described [30].

12 different tissues were obtained from four QY chickens. Moreover, breast muscle of six embryonic periods (E10-15) and leg muscle of four embryonic periods (E12-15) was used to detect the relative expression of *GNB1L* gene.

cDNA synthesis and qRT-PCR

RNA extraction using the TRIzol (Takara, Dalian, China) method, reverse transcription using the cDNA reverse transcription kit (Takara, Dalian, China) followed by PCR. Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method, and significance using ANOVA followed by Duncan's test. All reactions using three biological and technical repetitions, and the PCR reactions involved: 95 °C for 3 min, 35 cycles at 95◦C for 30 s, 60◦C for 30 s, 72◦C for 30 s, and a final melting curve. The relative expression of *GNB1L* in different tissues and embryo ages were analyzed by qRT-PCR. Primers of *GNB1L* qRT-PCR and internal control *β-actin* are listed in Table S1.

Polymorphism detection and diversity analysis of different breeds

A 31-bp Indel in the *GNB1L* gene from whole-genome re-sequencing data of ten XH and ten RW chickens (unpublished data). Genotyping of *GNB1L* 31-bp Indel by PCR amplification and gel electrophoresis in eight diverse populations. Blood samples were used for the extraction of DNA, using the phenol-chloroform method, and the final concentration of DNA used for amplification was diluted to 50 ng/μL. *GNB1L* PCR Primers based on the genome is listed in Table S1. Each 15-μL PCR amplification volume contained 1 μL DNA, 0.75 μL of each primer, 7.5 μL of 2 × Taq Master mix (TSINGKE, Beijing, China), and 5 μL double-distilled water. PCR procedure included at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The PCR products after amplification were separated by 3.0% gel electrophoresis.

The genotypes and allele frequencies of the 31-bp Indel were calculated directly in different breeds. Hardy-Weinberg equilibrium (HWE) was calculated using the SHEsis online website (http://analysis.biox.cn). Moreover, genetic indices of heterozygosity (He), allele numbers (Ne) and effective polymorphism information content (PIC) were analyzed using PopGene software (Version 1.3.1) [31, 32].

Transcription Factor Prediction

The transcription factors (TFs) in the 31-bp Indel of 5' UTR regions of the *GNB1L* gene were predicted using online AliBaba software (Version 2.1) [24].

Statistics

Association analysis of F2 population by SPSS 22.0 software, and was used two different models in the analysis. All growth traits use Model Ⅰ (Yijkl = μ + Gi + Sj + Hk + fl + eijkl), and all carcass traits use Model II (Yijkl = μ + Gi + Sj + Hk + fl + b (Wijkl - $\bar{W}$) + eijkl), and carcass weight as a concomitant variable in Model II. Yijkl represents the observed value, μ is the overall population mean, Gi is the fixed effect of genotype, fl is the fixed effect of family, Sj is the fixed effect of sex, Hk is the fixed effect of hatch, b is the regression coefficient for carcass weight, $\bar{W}$ is average slaughter weight, Wijkl represents the individual slaughter weight, and eijkl represents the random error in Model Ⅰ and Model II. *P*-value < 0.05 is considered significant, and the Bonferroni's test serve as multiple comparisons [18].

Abbreviations

GNB1L: G protein subunit beta 1 like; Indel: insertion/deletion; SNP: single-nucleotide polymorphism; LS: Lushi chickens; ND: Ningdu chickens; WC: Wenchang chickens; QY: Qingyuan Partridge chickens; RW: Recessive White Rock chickens; ISA: ISA Brown laying hen; GX: Guangxi chickens; F2: F2 population; XH: Xinghua chickens; HWE: Hardy-Weinberg equilibrium; He: genetic indices of heterozygosity; Ne: allele numbers; PIC: effective polymorphism information content; TFs: The transcription factors; UTR: untranslated regions; SE: Standard error of the mean; BW: Body weight; SL: Shank length; SD: shank diameter; DG: daily gain; LWS: Live weight before slaughter; BWH: Breast width; BP: Breast deep; BSL: Body slanting length; BAW: Breast angle width; CW: Carcass weight; SFT: Subcutaneous fat thickness; FCW: Fat cingula width; SEW: Semi-Eviscerated weight; EW: Eviscerated weight; BMW: Breast meat weight; LMW: Leg meat weight; WW: Wing weight; AFW: Abdominal fat weight; SIL: Small intestine length.

Declarations

Ethics approval and consent to participate

We followed the guidelines of Institutional Animal Care and Use Committee for use and care of laboratory animals, and approved by the South China Agricultural University (approval ID: SCAU#0014). All efforts were made to minimize damage to the animal.

Consent for publication

Not applicable.

Availability of data and materials

All the data and materials supporting the conclusions of the study are included in the manuscript and Additional file 1.

Competing interests

The authors declare that this article has no conflict of interest.

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Authors' contributions

Tuanhui Ren performed the experiments, analyzed the data, prepared figures and tables, and wrote the manuscript. Ying Yang and Wujian Lin collected the samples and performed the experiments, Wangyu Li and Mingjian Xian analyzed the data. Rong Fu and Zihao Zhang and performed the additional experiments. Guodong Mo and Wen Luo revised the manuscript. Xiquan Zhang designed the study and reviewed the manuscript. All authors have read and approved the final manuscript.

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References

1. Ren T, Li Z, Zhou Y, Liu X, Han R, Wang Y, Yan FB, Sun GR, Li H, Kang X. Sequencing and characterization of lncRNAs in the breast muscle of Gushi and Arbor Acres chickens. *Genome* 2018; 61(7414).
2. Jia X, Lin H, Nie Q, Zhang X, Lamont SJ. A short insertion mutation disrupts genesis of miR-16 and causes increased body weight in domesticated chicken. Sci Rep. 2016;6:36433.
3. Wang X, Yang Q, Luo J, Wang Y, Feng Q, Zhang Z, Lei C, Chen H, Lan X. Novel 16-bp insertion/deletion variant of ZNF132 gene and its influence on growth traits in goats. JAPS: Journal of Animal Plant Sciences. 2016;26(6):1813–88.
4. Williams NM, Glaser B, Norton N, Williams H, Pierce T, Moskvina V, Monks S, Del Favero J, Goossens D, Rujescu D, et al. Strong evidence that GNB1L is associated with schizophrenia. Human molecular genetics. 2008;17(4):555–66.
5. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology. 2001;156(2–3):234–58.

6. Li Y, Zhao Q, Wang T, Liu J, Li J, Li T, Zeng Z, Feng G, He L, Shi Y. Association study between GNB1L and three major mental disorders in Chinese Han populations. Psychiatry research. 2011;187(3):457–9.
7. Ishiguro H, Koga M, Horiuchi Y, Noguchi E, Morikawa M, Suzuki Y, Arai M, Niizato K, Iritani S, Itokawa M, et al. Supportive evidence for reduced expression of GNB1L in schizophrenia. Schizophr Bull. 2010;36(4):756–65.
8. Noorai RE, Freese NH, Wright LM, Chapman SC, Clark LA. Genome-wide association mapping and identification of candidate genes for the rumpless and ear-tufted traits of the Araucana chicken. PloS one. 2012;7(7):1–6.
9. Bottje W, Kong B-W, Song J, Lee J, Hargis B, Lassiter K, Wing T, Hardiman J. Gene expression in breast muscle associated with feed efficiency in a single male broiler line using a chicken 44K microarray. II. Differentially expressed focus genes. Poult Sci. 2012;91(10):2576–87.
10. Mills RE, Luttig CT, Larkins CE, Beauchamp A, Tsui C, Pittard WS, Devine SE. An initial map of insertion and deletion (INDEL) variation in the human genome. Genome Res. 2006;16(9):1182–90.
11. Ren T, Zhou Y, Zhou Y, Tian W, Gu Z, Zhao S, Chen Y, Han R, Liu X, Kang X. Identification and association of novel lncRNA pouMU1 gene mutations with chicken performance traits. J Genet. 2017;96(6):1–10.
12. Li J, Zhu X, Ma L, Xu H, Cao X, Luo R, Chen H, Sun X, Cai Y, Lan X. Detection of a new 20-bp insertion/deletion (Indel) within sheep PRND gene using mathematical expectation (ME) method. Prion. 2017;11(2):143–50.
13. Xu Y, Shi T, Zhou Y, Liu M, Klaus S, Lan X, Lei C, Chen H. A novel PAX7 10-bp Indel variant modulates promoter activity, gene expression and contributes to different phenotypes of Chinese cattle. Scientific reports. 2018;8(1):1–10.
14. Xu W, He H, Zheng L, Xu JW, Lei CZ, Zhang GM, Dang RH, Niu H, Qi XL, Chen H, et al. Detection of 19-bp deletion within PLAG1 gene and its effect on growth traits in cattle. Gene. 2018;675:0378111918306978-.
15. Hui Y, Zhang Y, Wang K, Pan C, Chen H, Qu L, Song X, Lan X. Goat DNMT3B: An Indel mutation detection, association analysis with litter size and mRNA expression in gonads. *Theriogenology* 2020.
16. Wang K, Kang Z, Jiang E, Yan H, Zhu H, Liu J, Qu L, Lan X, Pan C. Genetic effects of DSCAML1 identified in genome-wide association study revealing strong associations with litter size and semen quality in goat (Capra hircus). Theriogenology. 2020;146:20–5.
17. Zang L, Wang Y, Sun B, Zhang X, Yang C, Kang L, Zhao Z, Jiang Y. Identification of a 13 bp Indel polymorphism in the 3'-UTR of DGAT2 gene associated with backfat thickness and lean percentage in pigs. Gene. 2016;576(2):729–33.
18. Ren T, Li W, Liu D, Liang K, Wang X, Li H, Jiang R, Tian Y, Kang X, Li Z. Two insertion/deletion variants in the promoter region of the QPCTL gene are significantly associated with body weight and carcass traits in chickens. Animal genetics. 2019;50(3):279–82.

19. Li W, Liu D, Tang S, Li D, Han R, Tian Y, Li H, Li G, Li W, Liu X. A multiallelic Indel in the promoter region of the Cyclin-dependent kinase inhibitor 3 gene is significantly associated with body weight and carcass traits in chickens. Poultry science. 2019;98(2):556–65.
20. Han R, Wang X, Wang X, Guo Y, Li D, Li G, Wang Y, Kang X, Li Z. Chicken ZNF764L gene: mRNA expression profile, alternative splicing analysis and association analysis between first exon Indel mutation and economic traits. Gene. 2019;695:92–8.
21. Fu R, Ren T, Li W, Liang J, Mo G, Luo W, He D, Liang S, Zhang X. A Novel 65-bp Indel in the GOLGB1 Gene Is Associated with Chicken Growth and Carcass Traits. Animals. 2020;10(3):475.
22. Liang K, Wang X, Tian X, Geng R, Li W, Jing Z, Han R, Tian Y, Liu X, Kang X, et al. Molecular characterization and an 80-bp Indel polymorphism within the prolactin receptor (PRLR) gene and its associations with chicken growth and carcass traits. 3 Biotech. 2019;9(8):296.
23. Wiener P, Wilkinson S. Deciphering the genetic basis of animal domestication. Proc Biol Sci. 2011;278(1722):3161–70.
24. Li Z, Ren T, Li W, Zhou Y, Han R, Li H, Jiang R, Yan F, Sun G, Liu X, et al. Association between the methylation statuses at CpG sites in the promoter region of SLCO1B3, RNA expression and color change in blue eggshells in Lushi chickens. Frontiers in genetics. 2019;10:161.
25. Niknafs S, Nejati-Javaremi A, Mehrabani-Yeganeh H, Fatemi SA. Estimation of genetic parameters for body weight and egg production traits in Mazandaran native chicken. Tropical Animal Health Production. 2012;44(7):1437–43.
26. Schreurs F. Post-mortem changes in chicken muscle. World's Poult Sci J. 2000;56(4):319–46.
27. De Wit NJ, Bosch-Vermeulen H, de Groot PJ, Hooiveld GJ, Bromhaar MMG, Jansen J, Müller M, van der Meer R. The role of the small intestine in the development of dietary fat-induced obesity and insulin resistance in C57BL/6J mice. BMC Med Genom. 2008;1(1):14.
28. Malodobra-Mazur M, Bednarska-Chabowska D, Olewinski R, Chmielecki Z, Adamiec R, Dobosz T. Single nucleotide polymorphisms in 5'-UTR of the SLC2A4 gene regulate solute carrier family 2 member 4 gene expression in visceral adipose tissue. Gene. 2016;576(1):499–504.
29. Southam L, Rodriguez-Lopez J, Wilkins JM, Pombo-Suarez M, Snelling S, Gomez-Reino JJ, Chapman K, Gonzalez A, Loughlin J. An SNP in the 5'-UTR of GDF5 is associated with osteoarthritis susceptibility in Europeans and with in vivo differences in allelic expression in articular cartilage. Human molecular genetics. 2007;16(18):2226–32.
30. Lei MM, Nie QH, Peng X, Zhang DX, Zhang XQ. Single nucleotide polymorphisms of the chicken insulin-like factor binding protein 2 gene associated with chicken growth and carcass traits. Poult Sci. 2005;84(8):1191–8.
31. Nei M, Roychoudhury AK. Sampling variances of heterozygosity and genetic distance. Genetics. 1974;76(2):379–90.
32. Yeh FC, Boyle T, Rongcai Y, Ye Z, Xian J, Yeh F, Yeh F, Boyle T, Ye Z, Xiyan J. POPGENE version 1.31. A Microsoft window based freeware for population genetic analysis. 1999.

Tables

Table 1. Genotypic and allelic frequencies and related genetic parameters of the chicken *GNB1L* gene.

Breeds/n	Genotypic and allelic frequencies					He	Ne	PIC	*P*-value
	DD	*ID*	*II*	*D*	*I*				
F2/360	0.20	0.35	0.45	0.375	0.625	0.47	1.89	0.36	0.00
ND/95	0.08	0.61	0.31	0.385	0.615	0.48	1.91	0.36	0.01
RW/55	0.29	0.35	0.36	0.465	0.535	0.50	1.99	0.37	0.02
ISA/64	0.16	0.53	0.31	0.425	0.575	0.49	1.95	0.37	0.48
GX/71	0.13	0.46	0.41	0.36	0.64	0.46	1.85	0.35	0.93
WC/65	0.2	0.49	0.31	0.445	0.555	0.49	1.97	0.37	0.98
QY/70	0.15	0.45	0.4	0.375	0.625	0.47	1.88	0.36	0.76
LS/39	0.30	0.61	0.08	0.605	0.395	0.47	1.90	0.36	0.06

Note: F2: F2 resource population (F2, n = 360), ND: Ningdu chickens, RW: Recessive White Rock chickens, ISA: ISA Brown laying hen, GX: Guangxi chickens, WC: Wenchang chickens, QY: Qingyuan Partridge chickens, LS: Lushi chickens. He: gene heterozygosity; Ne: effective allele numbers; PIC: polymorphism information content; *P*-value: *P*-value of Hardy–Weinberg equilibrium.

Table 2. Mean ± SE associations of the different genotypes with growth traits in the Xinghua × Recessive White Rock F2 populations.

Traits	Mean±SE			*P*-value
	DD	ID	II	
BW0 (g)	30.2±0.3	29.9±0.2	29.7±0.2	0.359
BW7 (g)	61.9±1.1^{a}	60.6±0.8^{a}	58.1±0.7^{b}	0.004
BW14 (g)	130.0±2.1^{a}	128.2±1.6^{a}	119.7±1.4^{b}	0.000
BW21 (g)	221.2±3.9^{a}	219.5±2.9^{a}	203.2±2.6^{b}	0.000
BW28 (g)	326.3±6.2^{a}	319.6±4.6^{a}	300±4.1^{b}	0.000
BW35 (g)	459.1±9.0^{a}	449.8±6.8^{a}	423.2±6.1^{b}	0.001
BW42 (g)	599.0±12.5^{a}	594.5±9.2^{a}	552.3±8.2^{b}	0.000
BW49 (g)	739.4±14.4^{a}	735.3±10.7^{a}	682.5±9.6^{b}	0.000
BW56 (g)	885.0±17.0	889.9±12.6^{a}	837.9±11.2^{b}	0.004
BW63 (g)	1051.1±23.0	1032.6±19.2	993.2±16.5	0.088
BW70 (g)	1117.6±26.1	1161.8±17.9	1120.7±15.9	0.180
BW77 (g)	1327.9±29.6	1359.6±20.2	1321.2±18.1	0.353
BW84 (g)	1475±38.2	1514.8±27.7	1487.4±22.7	0.640
SL42 (mm)	61.4±0.6	61.1±0.4	60.1±0.4	0.063
SL49 (mm)	69.4±0.7	67.4±0.7	67.1±0.5	0.033
SL56 (mm)	72.9±0.6	72.8±0.4	71.9±0.4	0.205
SL63 (mm)	79.9±1.4	78.2±1.1	79.3±0.9	0.608
SL70 (mm)	82.5±0.8	83.2±0.5	81.7±0.5	0.111
SL77 (mm)	89.4±1.3	88.4±1.0	88.5±0.8	0.827
SL84 (mm)	88.7±0.9	89.6±0.7	88.9±0.6	0.651
SD42 (mm)	7.9±0.1	8.0±0.1	7.8±0.1	0.174
SD49 (mm)	8.6±0.1	8.6±0.1	8.4±0.1	0.472
SD56 (mm)	8.8±0.1	8.9±0.1	8.7±0.1	0.258
SD63 (mm)	9.3±0.2	9.3±0.2	9.3±0.1	0.892
SD70 (mm)	9.4±0.1	9.5±0.1	9.4±0.1	0.670
SD77 (mm)	9.7±0.2	10.0±0.2	10.0±0.1	0.404
SD84 (mm)	10.0±0.2	10.1±0.1	10.0±0.1	0.725
0-4 DG (g/week)	10.6±0.2^{a}	10.3±0.2^{a}	9.6±0.1^{b}	0.000
4-8 DG (g/week)	20.1±0.5	20.3±0.3	19.2±0.3	0.043

Note: SE = standard error of the mean; BW0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84 = body weight at ages of 0, 7, 14, 21, 28, 35, 42, 49, 56, 63,70, 77 and 84 days; SL42, 49, 56, 63, 70, 77 and 84 = shank length at the age of 42, 49, 56, 63, 70, 77 and 84 days; SD42, 49, 56, 63, 70, 77 and 84 = shank diameter at the age of 42, 49, 56, 63,70, 77 and 84 days; 0 to 4 and 4 to 8 DG = daily gain of 0 to 4 and 4 to 8 weeks. Means with different superscripts indicate highly significant differences (different lowercase letters indicate $P < 0.01$; and the same letters indicate $P > 0.01$).

Table 3. Mean ± SE associations of the different genotypes with carcass traits in the Xinghua × Recessive White F2 populations.

Traits	Mean±SE			*P*-value
	DD	*ID*	*II*	
LWS (kg)	1.5±0.0	1.5±0.0	1.5±0.0	0.522
BWH (mm)	67.3±0.68^{ab}	67.9±0.5^{a}	65.8±0.5^{b}	0.006
BP (mm)	96.8±1.023^{ab}	96.7±0.8^{a}	93.9±0.7^{b}	0.009
BSL (cm)	22.9±0.2	23.1±0.1	22.9±0.1	0.606
BAW (°)	60.9±0.6	60.8±0.4	60.3±0.4	0.599
CW (g)	1353.2±27.1	1379.6±20.2	1347.3±18.0	0.477
SFT (mm)	4.1±0.1	4.2±0.1	4.1±0.1	0.837
FCW (mm)	11.2±0.4	11.8±0.3	12.5±0.3	0.038
SEW (g)	1241.3±24.3	1264.0±18.2	1228.7±16.2	0.347
EW (g)	1073.8±21.6	1097.0±16.1	1066.4±14.4	0.358
BMW (g)	95.9±2.05^{a}	95.5±1.5^{a}	88.5±1.4^{b}	0.001
LMW (g)	115.0±2.6	120.1±1.9	117.3±1.7	0.268
WB (g)	66.2±1.3	67.0±1.0	64.7±0.9	0.193
AFW (g)	29.7±2.2	27.6±1.6	27.4±1.4	0.651
SIL (cm)	144.9±1.952^{a}	140.3±1.5^{ab}	136.7±1.31^{b}	0.002

Note: SE = standard error of the mean; LWS = Live weight before slaughter; BWH = Breast width; BP = Breast deep; BSL = Body slanting length; BAW = Breast angle width; CW = Carcass weight; SFT = Subcutaneous fat thickness; FCW = Fat cingula width; SEW = Semi-Eviscerated weight; EW = Eviscerated weight; BMW = Breast meat weight; LMW = Leg meat weight; WW = Wing weight; AFW = Abdominal fat weight; SIL = Small intestine length. Means with different superscripts indicate highly significant differences (different lowercase letters indicate $P < 0.01$; and the same letters indicate $P > 0.01$).

Figures

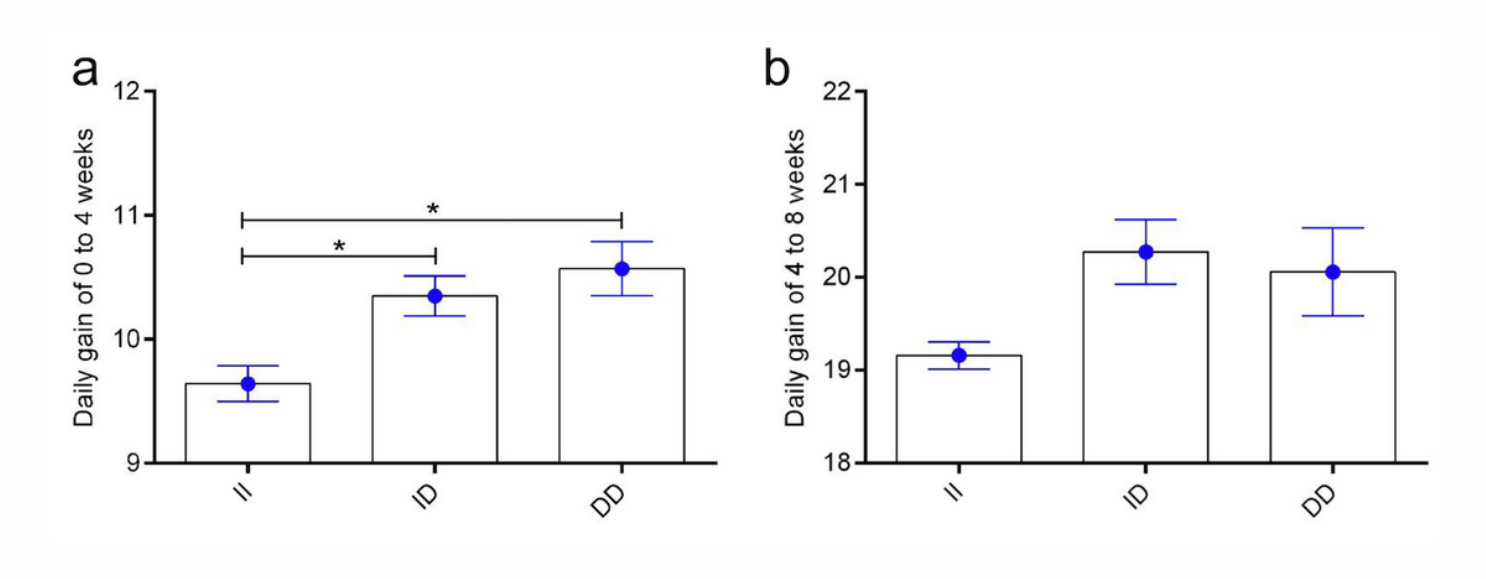

Figure 1

The daily gain of three different genotypes at different growth stages. * indicate P < 0.01.

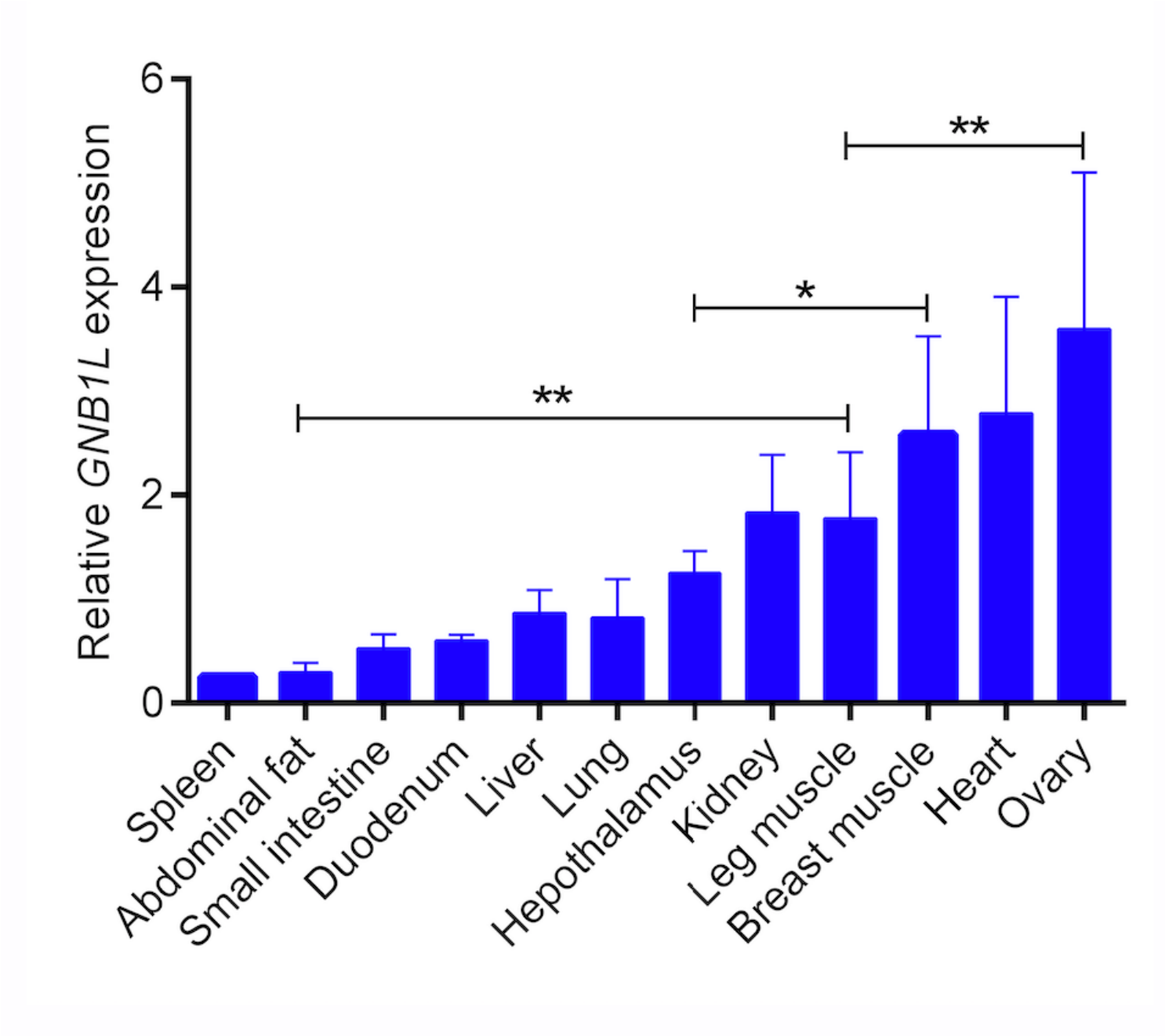

Figure 2

Relative expression patterns of GNB1L in different tissues. The data represent Mean ± SD (n = 3).

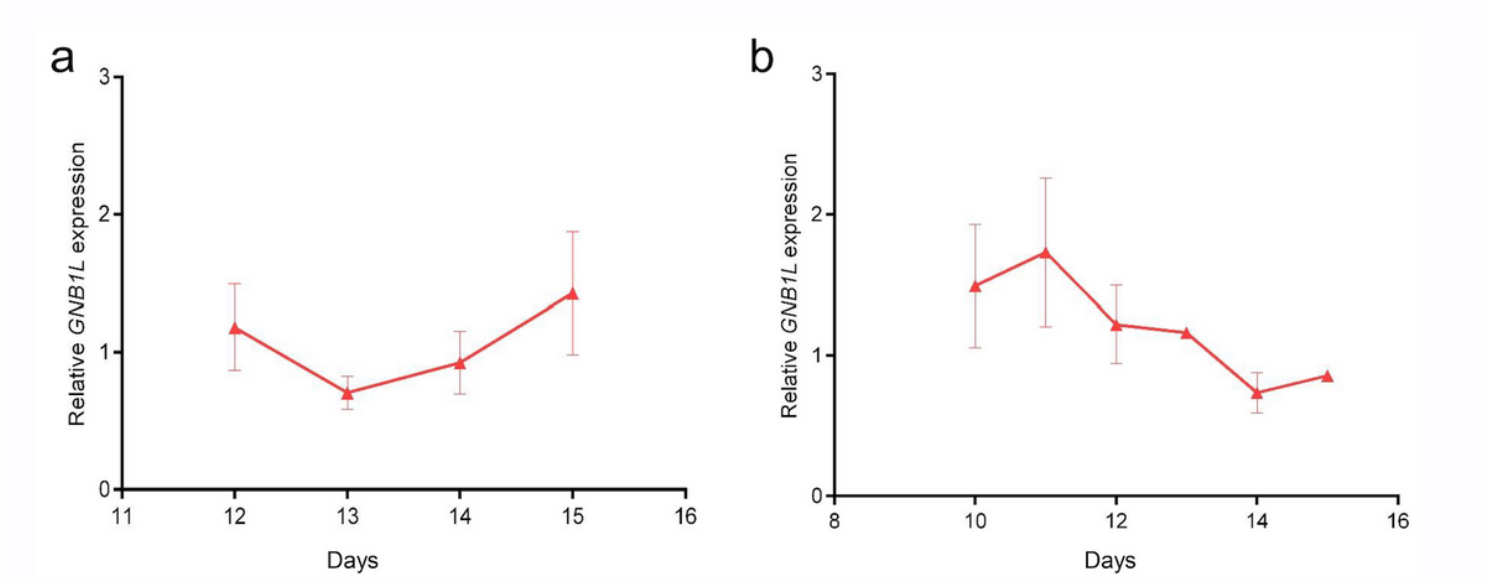

Figure 3

a. Expression of the GNB1L gene in breast muscle at different embryonic stages; b. Expression of the GNB1L gene in leg muscle at different embryonic stages. The data represent Mean ± SD (n = 3).

Supplementary Files

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- Additionalfile1.docx