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Original Article

Association Analysis of Indel Variant of IGFBP2 Gene with Slaughter Performances of Jiaji Muscovy Ducks

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ABSTRACT

The *IGFBP2* gene plays an important role in regulating animal energy metabolism. It is one of the key candidate genes for poultry growth and meat-related traits. Therefore, the present study screened the indel loci of the *IGFBP2* gene to investigate the effects of these indels on the growth and slaughter performance of Jiaji Muscovy ducks. Using our previous genome resequencing data, a 14 bp indel was found on the first intron of the *IGFBP2* gene. Specific primers were designed and synthesized, and genotyping was carried out in 102 Jiaji Muscovy ducks using PCR and agarose gel electrophoresis. Association analyses between growth and slaughter traits, and genotyping results were conducted for the Jiaji Muscovy ducks. Meanwhile, the expressions of *IGFBP2* mRNA in the breast muscle of individuals with different genotypes were detected using RT-qPCR. The results showed that the pre-slaughter body weight, carcass weight, half-clean rump weight, full-clean rump weight, leg muscle weight, pectoral muscle weight, abdominal fat, and skin fat content of Jiaji Muscovy ducks with indel mutation were significantly reduced, and the expression level of *IGFBP2* mRNA was increased in the breast muscle of the mutant individuals. This indel locus can regulate the expression level of the *IGFBP2* gene in muscles, thus affecting the growth and slaughter performance of Jiaji Muscovy ducks.

INTRODUCTION

Jiaji Muscovy ducks, also known simply as Muscovy ducks, were introduced into Southeast Asian countries by Qiong Chinese more than 300 years ago. They are very popular because of their fast growth, tasty meat, and higher intramuscular fat (Kokoszyński *et al*., 2020; Guo *et al*., 2021; Kierończyk *et al*., 2021). However, with the improvement of people's living standards, the demand for high-quality duck is increasing. For a long time, the market for Jiaji Muscovy ducks has been in short supply. The feed consumption of Jiaji Muscovy ducks is relatively high, and their feeding cycle is relatively long. This leads to an increase in breeding costs due to time costs. The body fat content, body weight, and meat production performance of duck meat are important economic indicators. Therefore, exploring the genes that control these traits and understanding their influencing mechanisms can help in genetic improvement efforts. Such efforts will also promote the development of the duck breeding industry.

IGF is a polypeptide that regulates the growth of organisms by regulating the secretion of GH (Ohlsson *et al*., 2009). The vast majority of *IGFs* bind to one of the members of the IGFBP protein family, which includes six members that are more or less associated with *IGF1* and its receptors, which are involved in cell proliferation by regulating *IGF*

signaling (Allard & Duan, 2018). Moreover, *IGF* also plays an important role in cardiovascular disease, tumor progression, fetal growth, and neuronutrition (Song *et al*., 2021).

IGFBP2 is a secreted protein and a member of the IGFBP family. It constitutes a system with insulin-like growth factor 1/2 (IGF1/2) and IGF receptors (IGF1R and IGF2R). *IGFBP2* can inhibit the biological effects of IGF *in vivo* via endocrine or paracrine mechanisms (Hosnedlova *et al*., 2020). It affects collagen (SHC) and IRS-1 by influencing the binding of *IGF1* to the IGF1-R receptor. Meanwhile, IRS-2 is attached to the IGF-1R linker, which activates the corresponding pathway for cell proliferation and growth (Hosnedlova *et al*., 2020). Moreover, in the studies of other species such as chickens, pigeons, and pigs, the *IGFBP2* gene has been found to be associated with *in vivo* fat deposition and growth traits (Li *et al*., 2006; Prasongsook *et al*., 2015; Wu *et al*., 2025). The gene has a significant impact on the slaughter performance in multiple species. Therefore, we selected *IGFBP2* as a candidate gene for study on its effects on various growth indexes of Jiaji Muscovy ducks.

The candidate gene method is an important research method to study the relationship between genes and poultry economic performance. This method is not uncommon in studying the association of *IGFBP2* gene with biological traits, but it is rarely used in the field of meat ducks. Therefore, the purpose of this study was to investigate whether the *IGFBP2* indel mutation site was associated with the slaughter performance of Jiaji Muscovy ducks through molecular marker technology.

MATERIALS AND METHODS

Sample and Data Collection

The Jiaji Muscovy ducks were raised under constant temperature and good ventilation in Hainan Chuanwei Muscovy Duck Breeding Co., Ltd. Growth traits such as body weight, trunk length, half diving length, keel length, breast depth, breast width, shank length, shank circumference, and pelvis width were measured in 102 randomly selected female Jiaji Muscovy ducks at 90 days of age (Gu *et al*., 2020) Anticoagulated blood samples were collected from the wing vein and stored in a -80°C refrigerator. Jiaji Muscovy ducks slaughter performance was measured according to the Technical Specification for the Determination of Production Performance of Meat Ducks (GB/T 29389-2024), including preslaughter body weight, carcass weight, half eviscerated weight, eviscerated weight, leg muscle weight, breast muscle weight, abdominal fat weight and skin fat weight. The breast muscle samples were collected and immediately frozen in liquid nitrogen for gene expression analysis. All experiments in this study involving animals were approved by the Faculty Animal Policy and Welfare Committee of HAUST (Henan University of Science and Technology). Moreover, the care and use of experimental animals completely conformed with local animal welfare laws, guidelines, and policies.

Genomic DNA Extraction

Genomic DNA was extracted from blood samples using a Tiangen Genomic DNA Kit (DP204, Beijing, China). The concentration and quality of genomic DNA were detected using a Thermo NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA) and 0.8% agarose gel electrophoresis. Each DNA sample was diluted to 50 ng/μL and stored in a -80°C refrigerator.

Detection of indel variants in the IGFBP2 gene

Indel variants were scanned and filtered from 10 Jiaji Muscovy ducks genome resequencing data (unpublished) using GATK4.3 software The location of indel variants in the *IGFBP2* gene was determined using Blast analysis. The core promoter element of the *IGFBP2* gene was predicted using the NNPP program (https://www.fruitfly.org/seq_tools/promoter.html) and TFBSs (transcription factor binding sites) were predicted using the AliBaba2.1 program (http://gene-regulation.com/pub/programs/alibaba2/).

Genotyping of the indel variant

According to the *IGFBP2* gene sequence published on NCBI (https://www.ncbi.nlm.nih.gov/gene), the primer P1 (Table 1) was designed to amplify the DNA fragment including the indel variant. Each 20 μL PCR amplification volume contained 1 μL of DNA, 1.4 μL of primer, 10 μL 2 × Taq Master mix (TSINGKE, Beijing, China), and 7.6 μL of double-distilled water. The PCR parameters were as follows: 95 °C for 5 min, 35 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. The PCR products were separated by 2% agarose gel electrophoresis with the aim of genotyping indel polymorphisms in 102 Jiaji Muscovy ducks. Sequencing of the PCR products of different homozygous genotypes was subsequently performed to verify the mutation.

Table 1 – Primer information.

Primer name	Primer sequence (5'-3')	Tm (°C)	Product length (bp)	Purpose
P1	TCTCCATCAAGTCCCACC CTGCATTGCTGCTGCTAC	56	219	genotyping
P2	CCATCACAACCACGAGGAC GGGGATGTGGAGGGAATAG	58	154	qPCR
GAPDH	GGTTGTCTCCTGCGACTTCA TCCTTGGATGCCATGTGGAC	60	165	Internal reference

RNA Extraction and Quantitative RT-PCR

The total RNA from the tissue samples was extracted using the Trizol reagent (TaKaRa Biotech Co. Ltd., Dalian, China) in accordance with the manufacturer's recommendations. The same amount of RNA of each sample was reverse transcribed using a PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Dalian, China). Quantitative real-time polymerase chain reaction (qPCR) was done on a BioRad CFX-96 thermal cycler (BioRad, California, USA) using the TB Green® Premix DimerEraser™ kit (TaKaRa, Dalian, China). All qPCR assays were performed in triplicate, and the GAPDH gene was used as an internal control. The designed primers are listed in Table 1. The qPCR conditions were as follows: 95°C for 3 min, 95°C for 30 s, 60°C for 30 s, and 72°C for 10 s for a total of 36 cycles. The results were analyzed using the 2–ΔΔCT method (Yuan *et al*., 2023).

Statistical Analyses

Results of body size and slaughter performance are presented as means ± SD.

General linear model (GLM) procedures were used to determine the important economic traits with different indel genotypes according to the formula Yij = μ + Gj + eij, where Yij is the phenotypic value of body size and slaughter performance, μ is the overall population mean, Gj is the fixed effect of genotype, and eij is the random error (Zhang *et al*., 2023). All data were checked for normal distribution by SPSS software (version 25.0), and then one-way ANOVA with Bonferroni correction for multiple comparisons was applied to analyze the differences in *IGFBP2* mRNA expression in different genotype groups. Differences were considered statistically significant at $p<0.05$.

RESULTS

Identification of indel loci in the *IGFBP2* gene

The results of genome resequencing showed a potential 14 bp indel variant (TCTTTCCATGTGTA) in intron 1 of the *IGFBP2* gene of Jiaji Muscovy ducks. The PCR products were separated by 2% agarose gel electrophoresis, which revealed three genotypes, the 233-bp homozygous AA genotype, the heterozygous AB genotype (233 bp and 219 bp) and the 219-bp homozygous BB genotype (Figure 1A). The PCR-amplified DNA sequences of homozygous individuals were confirmed by Sanger sequencing and genotyped by DNASTAR 7.0 (Figure 1B).

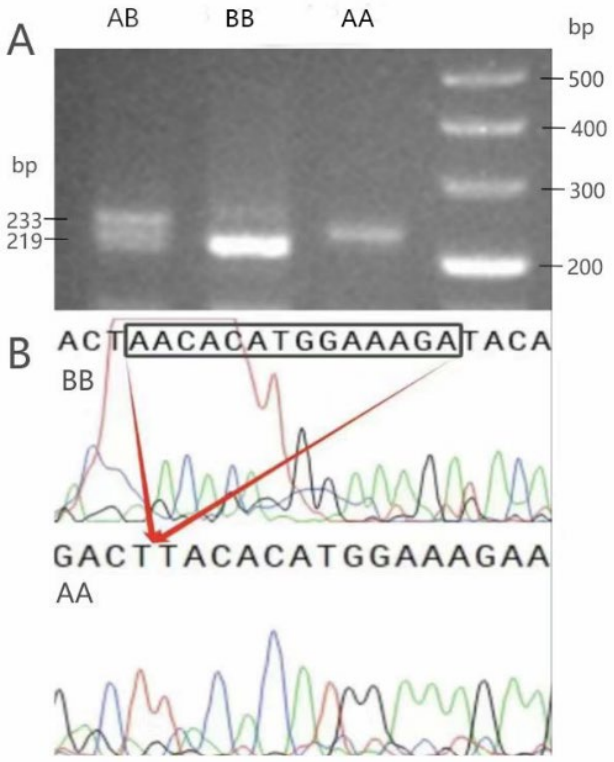

Figure 1 – Results of Electrophoresis and Sanger Sequencing to identify the genetic polymorphism of *IGFBP2*. Figure 1A. Results of the PCR amplification of the *IGFBP2* gene. Figure 1B. Sanger sequencing peak of the *IGFBP2* gene.

Population genetics parameters of the 14-bp Indel locus

Population genetics parameters of the 14 bp indel locus are shown in Table 2. The PIC values indicated that the 14-bp indel locus had moderate polymorphism (PIC = 0.4993). The genotypic frequency of the 14-bp indel locus satisfied the Hardy-Weinberg equilibrium (X^2 value=0.45, p=0.80>0.05).

Table 2 – Population genetics parameters of the indel locus.

Locus	numbers	Frequencies of each genotype			Allele frequencies		PIC[1]	X^2 value	*p*-value
		AA	AB	BB	A	B			
Indel	102	26	49	30	0.4810	0,5190	0.4993	0.45	0.80

Note: PIC, polymorphism information content.

Association Analysis Between Slaughtering performance, growth traits and Indel locus

The 14-bp indel locus of the *IGFBP2* gene has a significant effect on muscle development in Jiaji Muscovy ducks. PBW, CW, HEW, EW and SFW were significantly lower in AA genotype individuals than in BB genotype individuals ($p<0.01$), and LMW, BMW and AFW were significantly lower in AA genotype individuals than in BB genotype individuals ($p<0.05$) (Table 3).

Table 3 – Association of indel variant in the *IGFBP2* gene with key economic traits.

Economic traits	AA[1]	AB[2]	BB[3]	F	*p*
PBW(g)[4]	2058±140^{a}	1985±132^{b}	1910±149^{c}	7.55	0.001
CW(g)[5]	1831±139^{a}	1761±135^{a}	1689±149^{b}	6.86	0.002
HEW(g)[6]	1696±152^{a}	1617±150^{b}	1559±144^{b}	5.63	0.005
EW(g)[7]	1559±136^{a}	1482±129^{b}	1430±131^{b}	6.44	0.002
LMW(g)[8]	102.48±11.82^{a}	99.15±11.37^{ab}	94.62±11.06^{b}	3.19	0.046
BMW(g)[9]	109.05±30.70	101.92±25.50	96.16±26.18	1.51	0.227
AF(g)[10]	36.35±13.90^{a}	37.94±13.57^{ab}	30.03±10.55^{b}	3.35	0.039
SFW(g)[11]	361±53^{a}	341±60^{a}	300±59^{b}	7.84	0.001
PC (%)[12]	88.90±1.32	88.67±1.43	88.36±1.62	0.93	0.397
PHE (%)[13]	82.29±2.96	81.38±3.92	81.60±3.37	0.56	0.574
PE (%)[14]	75.65±2.42	74.62±2.96	74.86±2.76	1.21	0.301
PL (%)[15]	13.17±1.28	13.41±1.37	13.26±1.41	0.28	0.757
PB (%)[16]	13.92±3.45	13.74±3.15	13.31±2.83	0.27	0.764
PAF (%)[17]	2.29±0.92	2.48±0.84	2.04±0.61	2.63	0.077
PLM (%)[18]	27.09±3.57	27.15±3.20	26.57±2.87	0.30	0.738
PSF (%)[19]	25.53±3.50^{a}	25.53±4.05^{a}	23.07±4.21^{b}	3.81	0.025
TL (cm)[20]	23.30±1.61	22.93±1.73	23.21±1.95	0.46	0.633
HD (cm)[21]	45.23±2.35	44.70±2.50	45.40±1.83	1.20	0.306
KL (cm)[22]	14.27±1.39	13.85±1.12	13.42±1.26	3.08	0.056
BD (cm)[23]	8.30±0.82	8.21±0.90	8.30±0.95	0.12	0.888
BW (cm)[24]	9.88±0.52	9.74±1.19	9.79±1.48	0.12	0.886
SL (cm)[25]	6.74±0.54	6.76±0.61	6.50±0.59	1.81	0.169
SC (cm)[26]	4.43±0.47	4.37±0.42	4.41±0.51	0.16	0.852
PW (cm)[27]	6.34±0.54	6.11±0.49	6.15±0.56	1.66	0.196

[1]AA--Indicates Wild genotype. [2]AB--Indicates Heterozygote genotype. [3]BB--Indicates Homozygotic indel genotype. [4]PBW--Preslaughter Body Weight. [5]CW--Carcass Weight. [6]HEW--Half Eviscerated Weight. [7]EW--Eviscerated Weight. [8]LMW--Leg Muscle Weight. [9]BMW--Breast Muscle Weight. [10]AF--Abdominal Fat. [11]SFW--Skin Fat Weight. [12]PC--Percentage of Carcass. [13]PHE--Percentage of Half Eviscerated Yield. [14]PE--Percentage of Eviscerated Yield.[15]PL--Percentage of Leg Muscle. [16]PB--Percentage of Breast Muscle. [17]PAF--Percentage of Abdominal Fat. [18]PLM--Percentage of Lean Meat. [19]PSF--Percentage of Skin Fat. [20]TL--Trunk Length. [21]HD--Half Diving Depth. [22]KL--Keel Length. [23]BD--Breast Depth. [24]BW--Breast Width. [25]SL--Shank Length. [26]SC--Shank Circumference. [27]PW--Pelvis Width

The 14-bp indel locus of the *IGFBP2* gene had a significant effect on the PSF of Jiaji Muscovy ducks, indicating that this locus is also involved in the regulation of fat deposition. The PSF of individuals with the BB genotype (23.07%) was significantly lower than that of individuals with the AA genotype (25.53%) ($p<0.05$).

The 14 bp indel locus had no significant effect on the body size traits of Jiaji Muscovy ducks. It can be observed that KL and PW were higher in individuals with the AA genotype than the BB genotype, but not to a statistically significant level ($p>0.05$).

Functional analysis of the 14-bp indel locus on expression of *IGFBP2* mRNA

To further analyze the potential function of the 14-bp indel locus in Jiaji Muscovy ducks, transcription factor binding sites (TFBS) around this locus were predicted using the AliBaba2.1 program, and the result showed that an important TFBS of estrogen receptor (ER) disappeared (Figure 2). To further analyze the effect of the indel locus on the expression of *IGFBP2* mRNA, the relative expression of *IGFBP2* mRNA in the breast muscles of individuals with different genotypes was determined using RT-qPCR. The results showed that the expression level of the *IGFBP2* mRNA was significantly lower in individuals with the AA genotype than in individuals with the BB genotype (Figure 3).

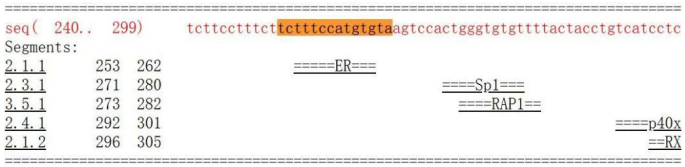

Figure 2 – The result of prediction of transcription factor binding sites (TFBS) around this 14-bp indel locus.

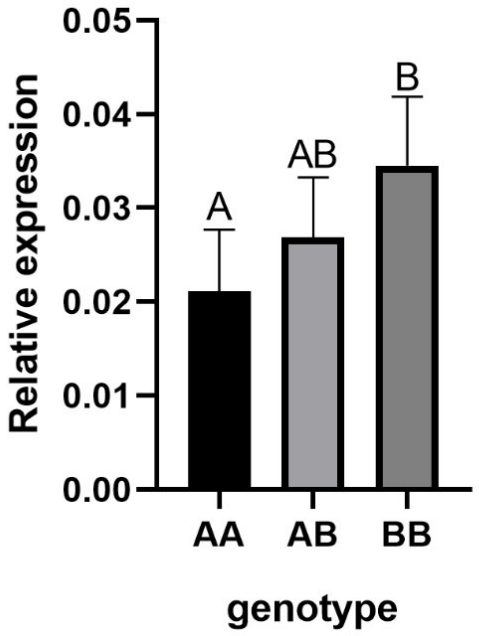

Figure 3 – Expression level of *IGFBP2* gene mRNA in breast muscle tissue of individuals with different genotypes.

DISCUSSION

The growth and development of animals are not only related to the intake of nutrients, but also to the regulation of gene expression. The *IGFBP2* gene plays an important role in the regulation of energy metabolism in animals and is a potential candidate gene for economic traits in poultry. An indel locus was found on *IGFBP2* in Hubbard F15 chickens, which resulted in a significant reduction in pre-slaughter body weight, breast muscle weight, and hamstring muscle weight at 14 days of age (Hosnedlova *et al*., 2020). Previous studies have shown that single nucleotide polymorphism (SNPs) in the introns of the *IGFBP2* gene reduce body weight, bone weight, and fat deposition in chickens (Li *et al*., 2006). In the present study, we found that a 14 bp indel on the first intron of the *IGFBP2* gene significantly reduced pre-slaughter body weight and pectoral muscle weight, hamstring muscle weight, and fat deposition in Jiaji Muscovy ducks, which was consistent with the findings in chickens, again suggesting that *IGFBP2* is a key candidate gene in the regulation of growth and fat deposition in poultry.

The expression level of *IGFBP2* mRNA plays an important role in regulating animal growth and fat deposition. *IGFBP2* transgenic mice had higher levels of *IGFBP2* mRNA in muscle tissues, as well as decreased carcass weight and IMF, which were similar to the phenotypes of the mutant individuals in this study (Ohde *et al*., 2020). *IGFBP2* protein regulates *IGF1* activity and insulin levels to block adipogenesis and inhibit 3T3-L1 cell differentiation (Boughanem *et al*., 2021; Rauzier *et al*., 2024). In addition, *IGFBP2* can inhibit fat deposition by regulating the expression of genes related to fat synthesis (Zhai *et al*., 2024). Overfeeding of geese resulted in a significant decrease in *IGFBP2* mRNA in the liver, along with a significant increase in fat deposition(Li *et al*., 2023). These studies suggest that the expression level of *IGFBP2* mRNA can regulate muscle mass and fat deposition.

The expression of a large number of genes is regulated by transcription factors. One transcription factor binding site (TFBS) of ER was identified on the 14 bp indel sequence of Jiaji Muscovy ducks by transcription factor binding site prediction. ER can influence growth and fat deposition in animals by regulating energy metabolism (Ropero *et al*., 2008). Activated ERs affect gene expression by binding to target DNA (Yoh *et al*., 2023). Most importantly, after estrogen binds to estrogen receptors (ERs) in the cytoplasm or nucleus, the resulting receptor-hormone complexes are phosphorylated. These phosphorylated receptor-hormone complexes either directly bind to specific sequences on DNA within the nucleus or enter the nucleus first and then bind to such sequences, thereby influencing gene transcription (Fuentes & Silveyra, 2019). For example, ER inhibits the expression of the mitochondrial polymerase subunit by binding to the promoter region, thereby reducing fat deposition in precursor adipocytes (Zhou *et al*., 2020). Our experimental conclusion is similar to this result: after the 14-bp fragment (ER binding site) disappeared, the amount of *IGFBP2* mRNA increased. This demonstrates that ER inhibits the expression of the *IGFBP2* gene by binding to the intron.

CONCLUSION

The 14 bp indel in the first intron of the *IGFBP2* gene may have elevated *IGFBP2* mRNA levels in muscle tissues, resulting in significant reductions in pre-slaughter body weights, pectoral muscle weights, leg muscle weights, and sebum percentage in Jiaji Muscovy ducks.

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AUTHOR CONTRIBUTIONS

Conceptualization, Hu J and Zhang X; methodology, Hu J and Ji X; software, Qi Y; validation, Hu J, Ji X and Zhang X; formal analysis, Xu T; investigation, Gu L; resources, Zhang X; data curation, Ji X ; writing—original draft preparation, Hu J; writing—review and editing, Junting Hu and Zhang X; visualization, Xu T; supervision, Gu L; project administration, Qi Y; funding acquisition, Zhang X and Qi Y. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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