Effects of Growth-Related Genes on Body Measurement Traits in Wenshang Barred Chickens

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Body measurement traits (BMTs), which are classical quantitative traits of vital responses to body growth, have been studied in pigs, cattle, and sheep for several decades. In chickens, BMTs mainly cover body slope length, keel length, chest width, chest depth, tibia length, and tibia diameter; however, their genetic markers are yet to be considered. In this study, the Wenshang Barred chicken, a meat-egg-type native breed in China, was used to investigate the association between BMTs and the expression of growth-related genes, including GH, IGF1, IGF2, GHRL, IGF1R, IGFBP2, GHF-1, and TSHB. The results revealed that the single nucleotide polymorphism (SNP) rs3138025 in GH was significantly associated with keel length ($P=0.0455<0.05$), rs313810945 in IGF2 was significantly correlated with chest width ($P=0.0454<0.05$) and chest depth ($P=0.0259<0.05$), and rs317298536 in TSHB significantly affected chest depth ($P=0.0399<0.05$). The SNPs were associated with traits reflecting body size and were potentially involved in bone growth, which was consistent with studies in humans, rodents, and other vertebrate species. In addition, a borderline significant association was found between rs317298536 and body weight ($P=0.0604$). These polymorphic sites may be treated as candidate genetic markers in breeding programs involving Wenshang Barred chickens.

Key words: association, body measurement traits, chicken, growth, SNP

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dwarf chickens, which are characterized by shorter shanks and lower BW than normal-sized chickens (Ouyang et al., 2012). The expression profiles of somatotropic axis genes were related to body size and growth rate in Daweishan mini chickens, Wuding chickens, and a commercial broiler hybrid (Jia et al., 2018).

In this study, Wenshang Barred chickens were used to investigate the association between body measurement traits and BW with eight growth-related genes, including GH, IGF1, IGF2, GHRL, IGF1R, IGFBP2, GHF-1, and TSHB.

Materials and Methods

Animals

A total of 605 Wenshang Barred chickens (498 females and 107 males) from the same cohort and of similar age were used. All chickens were reared in a stair-step caging system, under the same recommended nutritional and environmental conditions. Blood samples were collected from wings at 18 weeks of age. All experimental chickens were maintained, and all studies were conducted according to the guidelines of the experimental animal management of Shandong Agricultural University (SDAUA-2018-018).

Body Measurements

BMTs, including BL, KL, CW, CD, TL, and TD, were measured in the 605 chickens. BL, KL, CW, and CD were measured at 6, 12, 18, and 30 weeks of age, and TL and TD were measured at 0, 6, 12, 18, and 30 weeks of age using a caliper (cm) in Wenshang Barred chickens. BL was measured from the shoulder joint to the ischial tuberosity. KL was measured from the front edge of the keel to its end. CW was defined as the body surface distance between the shoulder joints. CD was defined as the distance from the first thoracic vertebra to the position between the third and fourth toes. TD was defined as the width of the middle part of the tibia. BW of the chickens was measured at 0, 6, 12, 18, and 30 weeks of age using an electronic balance (g).

Genotyping of Growth-related Genes

Genomic DNA was extracted from blood samples of 605 chickens using the TIANamp Blood DNA Kit DP348 (Tiangen, Beijing, China), following the manufacturer’s instructions. The sequence information of the eight chicken growth-related genes (Table 1) was obtained from GenBank (http://www.ncbi.nlm.nih.org). PCR primers were designed using the Primer5.0 program (detailed in Table S1), and the products were sequenced using the Sanger method.

Statistical Analysis and Inference

Association analyses were performed using the following linear model by ASReml (Gilmour et al., 2009).

\[
\text{trait} = \mu + \text{sex} + \text{time} + \text{SNP} + e
\]

In the model, \(\mu\) is the population mean, \(\text{sex}\) is the fixed effect for chicken gender, \(\text{time}\) is the covariate for recorded time points, \(\text{SNP}\) is the SNP effect of growth-related genes, and \(e\) is the residual random. The response variable (\text{trait}) was the BMT of the chickens, namely, BL, KL, CW, CD, TL, TD, and BW.

When a set of statistical inferences was simultaneously considered, multiple comparisons were conducted using the false discovery rate (FDR) in the R project.

Results

Genotypes of Growth-related Genes

For the eight growth-related genes, 12 polymorphic sites, including 10 transitions and two transversions, were genotyped in 605 chickens. Eight polymorphic sites were in the exon, five in the intron, and one in the 3’ UTR (Tables 2 and S2). The SNP success rate in the population was 100% for rs3138025, 99.67% for rs316492824, 99.34% for rs313810945, 96.20% for rs317572724, 100% for rs14011776, 99.83% for rs14011780, 100% for rs312403832, 100% for rs737328551, 100% for rs13687128, 99.83% for rs732246871, 99.67% for rs317298536, and 99.67% for rs313091243.

Effect of Growth-related Genes on BMTs

Local breeds are notorious for missing lineage information, and the Wenshang Barred Chicken is no exception. The

| Gene | Description | Location | Aliases |
|------|-------------|----------|---------|
| GH   | growth hormone (<i>Gallus gallus</i> [chicken]) | Chromosome 27, NC_006114.5 (4034905..4038410, complement) | GH1, cGH |
| IGF1 | insulin like growth factor 1 (<i>Gallus gallus</i> [chicken]) | Chromosome 1, NC_006088.5 (55281097..55329525) | IGF-1, IGF-I |
| IGF2 | insulin like growth factor 2 (<i>Gallus gallus</i> [chicken]) | Chromosome 5, NC_006092.5 (13964428..13981564) | IGF-II |
| GHRL | ghrelin/obestatin prepropeptide (<i>Gallus gallus</i> [chicken]) | Chromosome 12, NC_006099.5 (19849376..19852075, complement) | ghrelin, preproghrelin |
| IGF1R | insulin like growth factor 1 receptor (<i>Gallus gallus</i> [chicken]) | Chromosome 10, NC_006097.5 (17124349..17264663, complement) | IGF-1R |
| IGFBP2 | insulin like growth factor binding protein 2 (<i>Gallus gallus</i> [chicken]) | Chromosome 7, NC_006094.5 (23252168..23328028, complement) | IGFBP-2 |
| GHF-1 | growth hormone factor-1; POU class 1 homeobox 1 (<i>Gallus gallus</i> [chicken]) | Chromosome 1, NC_006088.5 (94255838..94273551) | POU1F1, PIT1, PIT-1, Pit-1 |
| TSHB | thyroid stimulating hormone beta (<i>Gallus gallus</i> [chicken]) | Chromosome 26, NC_006113.5 (3966715..3987181) | TSH-B, TSH-β |
Hardy-Weinberg equilibrium and population stratification strategy can be used to study pedigree in birds. The chickens were sampled from a conserved population during random mating. According to the χ² test, they were in Hardy–Weinberg equilibrium (Table S3). According to principal component analysis, no population stratification existed (Figure S1). The descriptive statistics for the traits evaluated are detailed in Table 3. Using the mixed linear model and FDR multiple comparisons, three polymorphic sites in growth-related genes were found to affect the traits (Table 4). The SNP rs3138025 in GH was significantly associated with KL (P=0.0455 < 0.05), rs313810945 in IGF2 was significantly correlated with CW (P=0.0454 < 0.05) and CD (P=0.0259 < 0.05), and rs317298536 in TSHB was significantly correlated with CD (P=0.0399 < 0.05). In addition, a borderline significant association was found between the variant rs317298536 and BW (P=0.0604), which was validated by a whole genome-wide association study in a larger population with a higher marker density for the fine mapping of candidate sites for chicken growth.

**Discussion**

The somatotropic axis, which consists of certain growth-related genes, regulates metabolism and physiological processes in vertebrates (Buyse and Decuypere, 1999; Renaville et al., 2002), including chickens. Some genes in the axis, namely GH, IGF1, IGF2, and GHF-1, are associated with chicken growth (Nie et al., 2005; Fujita et al., 2019), and SNPs of these genes might influence BW, body size, and

| Table 2. Polymorphism information of the growth-related genes in the Wenshang Barred Chicken population |
| --- |
| Gene | SNP | Position | Variant |
| GH | rs3138025 | exon | G/A |
| IGF1 | rs316492824 | exon | A/G |
| IGF2 | rs313810945 | exon | C/T |
| GHR | rs317572724 | intron | A/G |
| IGFR | rs14011776 | intron | C/A |
| IGF1R | rs14011776 | intron | A/G |
| IGFBP2 | rs312403832 | intron | C/T |
| GHF-1 | rs13687128 | exon | T/C |
| TSHB | rs373328551 | exon | A/T |
| | rs317298536 | exon | G/A |
| | rs313091243 | 3′UTR | A/G |
| | rs732246871 | intron | A/G |

| Table 3. Descriptive statistics for the traits |
| --- |
| Trait | TL (cm) | TD (cm) | BL (cm) | KL (cm) | CD (cm) | CW (cm) | BW (g) |
| Min | 2.35 | 0.28 | 9.00 | 5.00 | 1.14 | 2.68 | 21.11 |
| Max | 12.78 | 1.75 | 26.00 | 15.50 | 13.20 | 9.63 | 2883.00 |
| Mean | 7.44 | 0.91 | 17.04 | 9.74 | 9.44 | 6.07 | 860.71 |
| Standard Error | 0.0499 | 0.0058 | 0.0700 | 0.0340 | 0.0303 | 0.0264 | 11.2911 |

TL, tibia length; TD, tibia diameter; BL, slope length; KL, keel length; CW, chest width; CD, chest depth; BW, body weight.

| Table 4. Effect of significant single nucleotide polymorphisms on chicken body measurement traits |
| --- |
| Trait | SNP | P-value | Genotype | Freq. | Percentage to phenotypic variance |
| KL | rs3138025 | 0.0455* | AA | 0.05 | 0.09% |
| | | | AG | 0.3 | |
| | | | GG | 0.65 | |
| CW | rs313810945 | 0.0454* | CC | 0.9 | 0.01% |
| | | | CT | 0.1 | |
| CD | rs313810945 | 0.0259* | CC | 0.9 | 0.04% |
| | | | CT | 0.1 | |
| CD | rs317298536 | 0.0399* | AA | 0.16 | 0.14% |
| | | | AG | 0.5 | |
| | | | GG | 0.34 | |
| BW | rs317298536 | 0.0604 | AA | 0.16 | 0.03% |
| | | | AG | 0.5 | |
| | | | GG | 0.34 | |

1. When a set of statistical inferences was simultaneously considered, multiple comparisons were conducted using the false discovery rate (FDR) in the R project. * was at the significant level of 0.05, and a borderline significant association was found between rs317298536 and body weight.

2. The percentage of phenotypic variance=2×p×q×a^2/σ², where p and q represent the allele frequencies of different alleles, a represents the estimated SNP effect value, and σ² represents phenotypic variance (Falconer and Mackey, 1996; Huang et al., 2020).
other traits (Ouyang et al., 2012; Jia et al., 2018). In this study, the relationship between body measurement traits of Wenshang Barred chickens and genetic polymorphism of growth-related genes (GH, IGF1, IGF2, GHR, IGF1R, IGFBP2, GHF-1, and TSHB) was explored. The SNP rs3138025 in GH, rs313810945 in IGF2, and rs317298536 in TSHB were found to significantly affect BMTs, especially KL, CW, and CD of Wenshang Barred chickens. These three traits, which reflect body size, are mainly involved in bone growth. Studies in humans, rodents, and other vertebrate species have unequivocally shown that the somatotropic axis is the major controller of skeletal growth and body size (Brown-Borg, 2009; Yakar et al., 2018; Lv et al., 2019a). Growth hormone deficiency leads to decreased bone turnover and low bone mass (Tritos and Klibanski, 2016). Process dysfunctions in the GH-associated axis cause animal dwarfism, and mutations cause aberrant functioning, resulting in defects in the number and diameter of muscle fibers as well as bone development (Lin et al., 2018). In-depth studies have revealed that IGFs produced by the liver and secreted into the circulation (endocrine) and locally by tissues (paracrine/autocrine) affect the effects of GH on bone. IGFs regulate bone length, radial bone growth, and cortical and trabecular bone properties via their effects on osteoblast, osteocyte, and osteoclast functions (Yakar et al., 2018; Halmos and Suba, 2019). Direct effects of TSH on bone traits have been reported, and TSH is a single necessary molecular switch that negatively regulates skeletal remodeling (Siderova et al., 2018). In addition to the action of pituitary TSH on osteoblasts and osteoclasts, the bone marrow microenvironment acts as an endocrine circuit that produces a novel TSH-β subunit variant, which is positively regulated by T3. Therefore, the bone marrow microenvironment accentuates such modulation in the presence of thyroid overactivity and may modulate skeletal physiology (Baliram et al., 2017).

Several chicken QTLs are associated with body size, covering or close to the SNPs detected in this study. The SNP rs3138025 (Chr27: 4,036,381) in GH was mapped to the QTL controlling shank weight (Chr27: 3,878,604–6,356,601) and was located near the QTL for femur bone mineral density (Chr27: 4,201,821–5,063,733). The SNP rs317298536 (Chr26: 3,982,339) in TSHB was located close to QTLs related to blood calcium levels (Chr26: 3,980,313–3,980,317) and shank weight (Chr26: 4,124,861–5,880,121). The SNPs rs313810945 (Chr5: 13,979,291) in IGF2 was mapped to the QTL for shank weight (Chr5: 10,066,856–16,876,788) and carcass weight (Chr5: 10,747,849–58,624,632). The supporting information facilitates the understanding of candidate genetic markers in chicken breeding programs relevant to Wenshang Barred chicken.

The variant rs317298536 in TSHB showed a borderline significant association with BW. However, this is only a suggestive significance associated with BW, almost reaching a significance level of 0.05, probably because the detection power is limited by population structure or marker density. This indicates that the many small-effect loci of this highly polygenic trait may remain borderline or undetected (Lillie et al., 2018). Further, fine-mapping by a larger population or an F2 cross population, as well as genome resequencing, would contribute to the polygenic genetic demonstration of BW.

The percentage of each SNP effect on phenotypic variance was small, ranging from 0.01% to 0.14%. According to previous studies on chickens, a significant SNP could account for a higher percentage of the relevant phenotypic variance (Table S4); however, this percentage was dependent on the trait (Figure S2) (Cruz et al., 2021; Fan et al., 2013). Taken together, the results of this study may provide important insights into chicken breeding programs. However, whether these sites could be used as genetic markers should be validated in further experiments.

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Authors’ Contributions

HT designed the study. CY, JT, and SL collected the samples and performed the experiments. CY and CN analyzed and interpreted the data. CY, DW, JT, HT, QZ, and WW drafted the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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