Identification of AvaI Polymorphisms in the Third Intron of GH Gene and Their Associations with Abdominal Fat in Chickens

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ABSTRACT Growth hormone (GH) plays a diverse role in animals together with other hormones of somatotropic axis. In the current research, chicken GH (cGH) as a candidate gene affecting carcass traits was investigated in the chickens from 2 local chicken breeds [Mountainous Black-Bone (Wugu) and Caoke chicken] in the Sichuan province, 1 pure line of a quality chicken (Sanhuang chicken) from the Guangdong province, and commercial crossbreds. The RFLP method was used to identify polymorphisms of the cGH gene. Three restriction enzyme polymorphic sites were detected in the cGH gene. Sequence alignment from GenBank revealed 2 mutations in the third intron of the cGH gene, which were identified by the AvaI enzyme. Two novel AvaI polymorphic sites were genotyped in 240 chickens from the above-mentioned chicken populations. One EcoRV polymorphic site, the previously reported polymorphism, was also detected in these populations. Significant differences in allelic and genotypic frequencies among all the chicken populations were observed. In AvaI polymorphic sites, allele A2 and B1 had higher frequencies than allele A1 and B2, respectively. In EcoRV polymorphic sites, the frequency of allele N2 was higher than that of allele N1. Associations of polymorphisms of the cGH gene with carcass traits were analyzed by using a GLM procedure. Significant associations were found between AvaI genotypes or combined genotypes and abdominal fat weight and abdominal fat percentage (P ≤ 0.05). The allele A2 and B1 had a beneficial effect on increasing the live BW, breast muscle weight, and breast muscle percentage while decreasing the abdominal fat weight, abdominal fat percentage, and s.c. fat thickness. No significant associations were observed between EcoRV genotypes and carcass traits. In conclusion, the cGH gene may be a potential marker affecting the abdominal fat trait of chickens.

Key words: chicken, growth hormone gene, polymorphism, carcass trait, abdominal fat

INTRODUCTION

The molecular markers, either linked to QTL influencing economically important traits or directly having an effect, are unaffected by environmental conditions. Therefore, they could enhance the speed and effectiveness of progress in animal breeding. Once an association between a DNA polymorphism and an important trait is found, the DNA marker could be used in the molecular MAS.

The approach of using a candidate gene, selected because of known relationships between physiology and production traits, is a useful method to investigate associations of gene polymorphisms directly, with certain traits of interest in farm animals (Rothschild and Soller, 1997). Growth hormone (GH), a polypeptide hormone, is very important in animals for its broad range of activities. It plays important roles in promoting-growth, protein and muscle accretion, and fat catabolism together with other hormones of the somatotropic axis (Hou and Cheng, 1984; Etherton and Bauman, 1998). Studies in animals have shown that treatment with GH in vivo and in vitro, increases average daily weight gain, feed conversion efficiency, and milk production and reduces fat deposition (Hoj et al., 1993; Vasilatos-Younken, 1995; Klindt et al., 1996). Therefore, GH may be a potential candidate gene for MAS schemes.

In farm animals, many polymorphisms have been identified in the GH gene of pigs (Kirkpatrick and Huff, 1991; Franco et al., 2005), bovine (Lucy et al., 1993; Grochowska et al., 2001), and goat (Malveiro et al., 2001). Compared with other animals, the intron regions of the chicken GH (cGH) gene is highly polymorphic, and the studies using RFLP showed that these polymorphisms are associated with abdominal fat, egg production, resistance to Marek’s disease or avian leucosis, and meat yield traits (Fotouhi et al., 1993; Kuhnlein et al., 1997; Yan et al., 2003). The
objectives of the current study were to detect polymorphisms in the third intron of the cGH gene using the RFLP method and to analyze the associations of these polymorphisms with carcass traits in the Chinese local populations of chickens.

MATERIALS AND METHODS

Resource Populations

The chicken populations were the Mountainous Black-Bone chicken (n = 120), Caoke chicken (CK; n = 60), Sanhuang chicken (SH; n = 60), and a commercial crossbred (CC) chicken (n = 30). The Mountainous Black-Bone and CK chickens are indigenous breeds in the Sichuan province, having spotty feathers and black or yellow skin. These chickens have favorable meat quality but grow slowly. The SH chicken is an indigenous breed in the Guangdong province. It was named by its yellow plumage, skin, and shank and has a high quality of meat. The CC was designed by crossing SH cocks with CK hens. All birds were hatched on the same day, housed on the deep-litter bedding, and moved to the growing pens at the age of 7 wk. Birds had access to feed (commercial cornsoybean diets meeting NRC requirements) and water ad libitum. Before slaughter, blood was collected, and the genomic DNA was isolated by phenolic extraction and was used to genotype the GH gene.

Phenotyping for Carcass Traits

At the age of 90 d, BW was measured on live birds after 12 h with no access to feed. After slaughter at the same day of age, the carcass traits were measured, including carcass weight (CW), eviscerated weight, semieviscerated weight, breast muscle weight (BMW), leg muscle weight, abdominal fat weight (AW), and s.c. fat thickness (SFT). The CW was measured on the chilled carcass after removal of the feathers. Semieviscerated weight was measured on the carcass after removal of the trachea, esophagus, gastrointestinal tract, spleen, pancreas, and gonad. The eviscerated weight was measured on the semieviscerated weight after removal of the head, claws, heart, liver, gizzard, glandular stomach, and abdominal fat. The ratios of these traits to CW were calculated as eviscerated percentage, semieviscerated percentage, breast muscle percentage (BMP), leg muscle percentage, and abdominal fat percentage (AP). Subcutaneous fat thickness was measured at the caudal spondyle including the skin and fat width with a vernier caliper after processing.

Amplification and Population Genotyping

Primer pairs 5'-GTC CGT GCT TTT CTA TTA TC-3' (forward) and 5'-GCC AGG CTT CCA TCA GTA T-3' (reverse) were used (Yan et al., 2003) to amplify the fragment (664 bp) of the third intron of the cGH gene. The PCR was performed in a final volume of 15 μL containing 0.3 μM each primer, 0.5 U of Taq polymerase with its buffer, 0.2 mM each deoxynucleotide triphosphate, 1.5 mM MgCl₂, and 50 ng of DNA. Genomic DNA was denatured for 4 min at 94°C, and the PCR was run at 94°C for 30 s for 35 cycles, 62°C for 45 s for annealing, 72°C for 50 s for extension, and 72°C for 7 min as final elongation.

The polymorphic sites could be detected by the alignment of DNA sequences deposited in the GenBank (AF289468, D10484, and AY461843), and the AvaI restriction enzyme sites in the sequence were predicted by DNASTAR (DNASTAR Inc., Madison, WI). The amplified fragment was digested with restriction enzymes AvaI and EcoRV, respectively. In a total volume of 15 μL of reaction buffer containing 7 μL of PCR product and 8 μL of enzyme after maintaining at 37°C overnight, restriction patterns with the AvaI enzyme were visualized by electrophoresis of the digestion product through 8% acrylamide gel. The digests with the EcoRV enzyme were electrophoresed through 2% agarose gel, and gels were both visualized on Gel Doc EQ170-8060 (Bio-Rad Laboratories Inc., Hercules, CA) and photographed.

Statistical Analysis

Data were analyzed with the GLM procedures of SAS (SAS Institute Inc., Cary, NC). The genetic effects were analyzed by a GLM procedure in the SAS package, and the following model was used: Y = μ + B + S + G + (S × G) + e, where Y = the traits measured; μ = the population mean; e = the random error; B = the fixed effect of breed; S = the fixed effect of sex; G = the fixed effect associated with the genotype; and S × G = the interaction between the breed and sex. The S × G interaction was excluded from the model if its effect was P > 0.05 for a given trait. The values were presented as least square means ± SEM. The significant differences of least square means were tested with Duncan’s multiple range tests (P ≤ 0.05).

The data of some carcass traits were not normally distributed. The BW, CW, leg muscle weight, AW, and SFT were analyzed as the linear model, with parameters estimated on the square root scale. The eviscerated percentage, semieviscerated percentage, BP, and AP traits were shifted and rescaled to give approximate normality and equality of variance.

RESULTS

Genotypic and Allelic Frequencies of cGH Gene With the AvaI Enzyme

Chickens (240) from 4 breeds or strains were examined for the cGH gene polymorphisms in the current study. Two new polymorphic sites were identified in the 664-bp fragment with the AvaI enzyme. The polymorphisms were at 240 bp (A locus) and 347 bp (B locus). In locus A, allele A1, with the polymorphic restriction site, was cut into 240- and 424-bp fragments, and allele A2 was characterized by a 664-bp fragment. In locus B, allele B1, with the polymorphic restriction site, was cut into 317-
The amplified product was digested with EcoRV restriction enzyme, and 1 polymorphic site was found. The fragment sizes of 431 and 233 bp were designated as the N1 allele, whereas the allele N2 showed only 1 fragment of 664 bp.

The genotypic and allelic frequencies of EcoRV polymorphism in 4 chicken populations are shown in Table 1. The frequency of N1N1 was very low, even 0 in the CK and CC populations. The frequency of N2N2 was the highest, and N2 was the dominant allele among all populations.

**Genotypic and Allelic Frequencies of cGH Gene With the EcoRV Enzyme**

The results of the GLM analysis of associations between the GH RFLP polymorphisms and carcass traits in the local chicken populations are summarized in Table 2. In locus A, AW and AP were significantly associated with GH genotypes (P = 0.0219 and P = 0.013). The AW of A1A1 chickens was notably higher than that of A2A2 (P < 0.05). There were no differences among other genotypes (P > 0.05). The A1A1 chickens had higher AP than A1A2 and A2A2 chickens by 0.12 and 0.45%, respectively (P < 0.05), and A1A2 chickens had 1.64% AP, which was higher than that of the A2A2 chickens (P < 0.05). No significant differences were detected for other carcass traits. The allele A2 had a favorably positive effect on the BW, CW, BMW, and BMP, and it also beneficially decreased the AW, AP, and SFT of chickens. In locus B, differences among AvaI genotypes were also significant for trait AW (P = 0.0283) and AP (P = 0.0080). The B1B1 chickens had significantly lower AW than B2B2 chickens (P < 0.05), but there was no difference when compared with B2B2 (P > 0.05). The AW of the B2B2 chickens was not different from that of the B1B2 chickens (P > 0.05). The AP of B1B1 chickens was 2.53%, which was lower than that of B2B2 by 0.48% (P < 0.01) and lower than that of B1B2 by 0.33% (P < 0.05). There was no significant difference between B1B2 and B2B2 chickens (P > 0.05). No differences were observed for other carcass traits. The B1 allele had a favorably positive effect on BW, BMW, and BMP and also beneficially decreased the AW, AP, and SFT of chickens.

**Association Between AvaI Combined Genotypes and Carcass Traits**

Based on the 2 polymorphic sites, 5 combined genotypes were constructed as M1 (A1A1-B1B1), M2 (A2A2-B1B1), M3 (A1A1-B2B2), M4 (A1A2-B1B1 and A1A1-B1B2), and M5 (A1A2-B1B2). The frequency of the M4 combined genotype was very low (only 4 chickens), so it was deleted from further analysis. There were significant associations of combined genotypes with AW and AP (P = 0.0487 and P = 0.0334). The AW and AP of the M2 chickens were lower than that of M3 by 7.64 g (P = 0.0108) and 0.47% (P = 0.0104), respectively (Table 2). No associations were observed between combined genotypes and other carcass traits.

**Association Between EcoRV Genotypes and Growth Traits**

The associations of EcoRV genotypes with carcass traits in chickens were analyzed. No significant associations between genotypes and carcass traits were observed, although the results indicated that allele N1 had an additive effect on all the carcass traits.

**DISCUSSION**

Because essential GH is for growth and metabolism of the chicken, the cGH gene, which directly controls the synthesis of chicken GH, has received a lot of attention for a long time. The cGH gene contains 4 exons and 5 introns, and the intron sequences of cGH gene are longer.
Table 2. Effect of chicken growth hormone genotypes identified with AvaI enzyme on the carcass traits (least square mean and SE)

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<td>AW (g)</td>
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<td>48.81±2.61</td>
<td>45.76±2.12</td>
<td>41.86±1.83</td>
<td>41.73±1.78</td>
<td>45.39±2.23</td>
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<td>46.89±5.45</td>
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<td>25.94±0.11</td>
<td>49.26±2.68</td>
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<td>%AP</td>
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<td>2.98±0.15</td>
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AW = abdominal fat weight; %AP = AW to carcass weight.

a Means within a row with no common superscripts differ significantly (P ≤ 0.05).

The results indicated the possibility that these sequences are involved in the regulation of cGH production. However, it is unclear whether the introns indeed regulate the cGH expression and how the introns exert their roles during the transcription or translation.

Abdominal fat has been recognized as by-product in the processing, and numerous studies have been carried out to detect the genes whose expression or mutations are related to AF. To investigate the possible function of the mutations, the associations of cGH genotypes with carcass traits were analyzed. The AvaI genotypes were significantly associated with AW and AP. The mutations in the third intron in the current study did not change amino acids in the protein sequence but possibly affected the efficiency of transcription or translation and interfered with the quantity of GH secretion. The distribution of energy and metabolism is controlled by hormone factors, so expression profiles of these genes could affect the metabolism of animals.

The EcoRV polymorphic site was detected by Yan et al. (2003). Studies of this polymorphism in the F2 offspring derived from the populations of crossing Mingxing chickens with Silkie found a significant association of the genotypes with AP. However, the present results did not show significant associations between this polymorphism and carcass traits. The effects of this mutation have varied in different studies, which could be caused by the different populations studied, different statistical models used, and numbers of animal genotyped. Therefore, further study is still needed to identify the effects of EcoRV genotypes.

In the current study, the new AvaI polymorphisms were found in the third intron. Associations between cGH genotypes and abdominal fat were identified. The associations with polymorphisms do not necessarily mean that selection based upon these polymorphisms will have a direct effect on AW and AP. Resource chickens in the current study had lower abdominal fat, s.c. fat, and more i.m. fat in comparison with fast-growing chickens. Therefore, the results from the association analysis implied that 3 polymorphic sites could be used as genetic markers notably affecting the abdominal fat deposition. Considering that allele A2 and B1 had a beneficial effect on decreasing abdominal fat, it would be possible to make selection schemes favoring the 2 alleles for decreasing the abdominal fat in chickens; this hypothesis must be tested in selection experiments. To make the selection schemes applicable, it would be necessary to further analyze the effects of...
cGH polymorphisms by using populations from different genetic backgrounds and increasing the size of samples.

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REFERENCES


