A Potential Molecular Marker for Selection Against Abdominal Fatness in Chickens

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ABSTRACT The peroxisome proliferators-activated receptor-\(\gamma\) coactivator-1\(\alpha\) (PGC-1\(\alpha\)) was investigated as a candidate gene for growth and fatness traits in chicken because of its prominent role in muscle fiber specialization and adipogenesis. A single nucleotide polymorphism (SNP) from C to T at position 646 of the open reading frame of chicken PGC-1\(\alpha\) gene was identified. The frequencies of alleles and genotypes were significantly different among several chicken breeds. The White Leghorn had the lowest (0.18). The associations of the SNP with the growth and fatness traits were evaluated in 332 F\(2\) birds from an experimental cross of White Plymouth Rock \(\times\) Silkies. No association was found between the SNP and growth-related traits. However, abdominal fat weight at 12 wk of age for birds with genotype CT was 34.26 and 28.71% higher than those with genotypes CC and CT, respectively (\(P < 0.01\)), indicating that the Cys430Ser polymorphism of the PGC-1\(\alpha\) gene could be used as a novel potential molecular marker for selection against abdominal fatness without interfering in regular breeding for growth rate of chickens.

Key words: chicken, abdominal fat, growth, genetic marker, peroxisome proliferators-activated receptor-\(\gamma\) coactivator-1 \(\alpha\) gene

INTRODUCTION

Although intensive selection in broilers has made great improvement in growth rate and feed efficiency, modern strains of broilers exhibit excessive body fat deposition (Mallard and Douaire, 1988; Griffin, 1996). Excessive fat deposition is one of the main problems encountered by the broiler industry today, because it has significant negative effects on feed efficiency and causes great economic loss to processing plants. The economic concern and recognition of consumer aversion to excess fatty tissue deposition have led commercial breeders to incorporate significant selection for reduced body fatness in their breeding programs. Although several strategies of selection for leanness in meat production have been described, it is still laborious and expensive to measure fat deposition (Mallard and Douaire, 1988). Therefore, considerable research effort has been applied to study factors associated with fat deposition and methods to reduce it, and several candidate genes for this complex trait have been identified (Lei et al., 2005; Zhou et al., 2005; Wang et al., 2006).

The peroxisome proliferators-activated receptor-\(\gamma\) coactivator-1\(\alpha\) (PGC-1\(\alpha\)), which was originally identified through its functional interaction with peroxisome proliferators-activated receptor-\(\gamma\), is an important regulator of many metabolic pathways, including adaptive thermogenesis, fatty acid \(\beta\)-oxidation, adipocyte differentiation, hepatic gluconeogenesis, muscle fiber specialization, and glucose uptake (Puigserver et al., 1998; Wu et al., 1999; Vega et al., 2000; Michael et al., 2001; Lin et al., 2002). Polymorphism studies of the PGC-1\(\alpha\) gene in both human and livestock reveal that it is a functional candidate gene in lipid metabolism. A Gly482Ser polymorphism in the human PGC-1\(\alpha\) gene was described as a risk factor for development of type 2 diabetes in Pima Indians (Pratley et al., 1998) and Caucasians (Kunej et al., 2004), associated with obesity indices in middle-aged women (Esterbauer et al., 2002) and with lipid metabolism and insulin secretion (Muller et al., 2003). In pigs, a Cys430Ser polymorphism in PGC-1\(\alpha\) (PPARGC1A) was reported to be related with fat and lean tissue deposition in different pig breeds, suggesting that it may be a candidate gene for QTL influencing fatness and leanness (Kunej et al., 2005). Screening for polymorphisms in the porcine PGC-1\(\alpha\) gene also revealed a significant association between a polymorphism in exon 9 and leaf fat weight in the Meishan-White Composite resource population (Jacobs et al., 2006). In cattle, the PGC-1\(\alpha\) gene was mapped to the region BTA6q17-q19, and a significant association (\(P < 0.05\)) between a single nucleotide polymorphism (SNP) in intron 9 of the PGC-1\(\alpha\) gene and milk fat yield was found, indicating that this gene
could be involved in genetic variation underlying the QTL for milk fat synthesis on BTA6 (Weikard et al., 2005).

In general, the PGC-1α gene has emerged as a major player that integrates signaling pathways in the control of cellular and systemic metabolism (Lin et al., 2005). Because of its key role in energy metabolism, PGC-1α is a potential candidate gene for fatness in poultry. The PGC-1α gene has been cloned from chicken skeletal muscle, and the functional domains of the gene are well conserved among species (Ueda et al., 2005). Previous studies with the PGC-1α gene in other farm animals suggested that mutations in the functional regions of PGC-1α may also affect the transcription level of nuclear hormone receptors, such as peroxisome proliferators-activated receptor-γ, proliferators-activated receptor-α, and liver X receptor α (Puigserver et al., 1998; Vega et al., 2000; Lin et al., 2005), which ultimately influences the mechanism of lipid deposition in chickens. The objectives of the present study were to identify polymorphisms in the coding region of the chicken PGC-1α gene and to evaluate associations between the polymorphisms and economically important traits in an F2 resource population.

**MATERIALS AND METHODS**

**Experimental Populations and Phenotyping**

A total of 856 individuals were used for the analysis of genotypic and allelic frequency, including 2 egg-type populations (White Leghorn and Dwarf Layer), 1 meat-type breed (White Plymouth Rock), and 3 unrelated Chinese indigenous breeds (Blue Shell, Silkes, and Beijing You). In addition, a 3-generation resource population was established by a reciprocal cross between the White Plymouth Rock breed and a genetically distinct breed, the Silkes (Deng et al., 2001). The F1 birds were intercrossed with 5 males mated with 15 females, and 332 F2 birds in 5 batches were used for the current study. All birds could access feed and water ad libitum and were fed a commercial broiler diet (3,100 kcal/kg of ME and 190 g/kg of CP).

Body weight was measured at hatch and in 2-wk intervals up to 12 wk of age. At 12 wk of age, the birds were submitted to at least 10 h of fasting, weighted, slaughtered, and the carcasses were eviscerated by hand immediately. Carcass traits, including breast muscle weight, leg muscle weight, abdominal fat weight (AFW), heart weight, and liver weight, were recorded. And AFW was also expressed as a percentage of BW (%AFW) at 12 wk of age.

**PCR Amplification and Genotyping**

Genome DNA was extracted from venous blood by the phenol-chloroform method of Wang et al. (2006). Primers for amplifying a fragment of 252 bp were designed from chicken PGC-1α gene (GenBank accession no. AB170013), and the sequences were as follows: forward 5′-GACCTGA-GAATTCTGGGAGCA-3′; reverse 5′-ACCTTCCCTC-CAAACCAAC-3′. The PCR amplifications were carried out in 20-μL reactions containing 50 to 100 ng of genomic DNA, 5× PCR buffer, 0.20 mM dNTPs triphosphate, 2.5 mM MgCl2, 0.5 mM each primer, and 0.5 U of Taq DNA polymerase (Dingguo Biotechnology Co., Beijing, China). The reaction conditions were 94°C for 5 min, followed by 35 cycles of 94°C for 20 s, 60°C for 20 s, 72°C for 45 s, and a final extension at 72°C for 7 min.

The PCR products were then subjected to single-stranded conformational polymorphism analysis. The PCR products were mixed 1:3 with loading buffer (98% formamide, 0.09% xylene cyanole, and 0.09% bromophenol blue), denatured at 95°C for 5 min, chilled on ice rapidly, and loaded in 12% polyacrylamide gels (29:1 acrylamide:biacylamide). Gels were electrophoresed at 8 V/cm for 15 h at room temperature in 1× Tris-borate EDTA (10 mM Tris-HCl, 10 mM boric acid, and 1 mM EDTA, pH 8.0). The DNA bands were visualized by silver staining. Based on the single-stranded conformational polymorphism patterns, the PCR products of the 2 different homozygous individuals were sequenced by Dingguo Biotechnology Company. In total, 856 chickens from 6 different breeds and 332 F2 birds from the resource population were genotyped for the polymorphism.

**Statistical Analysis**

The χ² test for genotypic frequency in 6 populations was performed with SAS 8.02 (SAS Institute Inc., Cary, NC). The association between genotypes and traits recorded in 332 F2 individuals was analyzed using the GLM procedure, with genotype, reciprocal cross, and batch as fixed effects. As for the carcass traits, except for %AFW, BW at age for slaughtering was also included as a covariate in the model.

**RESULTS**

**Genotypic and Allelic Frequencies**

Sequencing result of the different homozygotes indicated a SNP from G to A at position 646 of the open reading frame of the chicken PGC-1α gene, which resulted in an Asp to Asn change at codon 216 in exon 5. The genotypic and allelic frequencies of the Asp216Asn polymorphism in 6 chicken breeds are listed in Table 1. The χ² fitness-test results demonstrated that the observed frequencies of the genotypes were in agreement with Hardy-Weinberg equilibrium among all 6 populations (P > 0.05), which indicated that selection had no influence on gene frequency during evolution. The frequency of the G allele (0.67) in the White Plymouth Rock was the highest of all the 6 breeds, whereas the A allele was the most frequent in White Leghorn (0.82).

**Associations of the PGC-1α Gene SNP with Phenotypic Traits**

Effects (least square means) of the PGC-1α genotype on growth and body composition traits in the F2 individuals of the cross between the White Plymouth Rock and Silkes
are shown in Table 2. There were significant \((P < 0.01)\) associations between genotypes and fatness traits (AFW and \%AFW). Abdominal fat weight at 12 wk of age for birds with genotype GG was 34.26 and 28.71% higher than those with genotypes AA and AG, respectively. The \%AFW of those with genotype GG was about 0.8% higher than those with AA and AG. However, no significant associations were found between the SNP and growth-related traits, except that the genotypes were significantly associated with BW at 4 wk of age \((P < 0.05)\).

### DISCUSSION

As abdominal fat is positively correlated with BW, selection for rapid growth and meat yields in broilers over decades has been accompanied by increased abdominal fat and feed intake (Havenstein et al., 2003). It is well known that excessive fat in poultry depresses feed efficiency and lean meat yield, has no commercial value, and is less appreciated by consumers. The traditional selection against abdominal fat involves difficulties in measuring the trait and limited number of samples. The emerging molecular markers that are closely associated with chicken fatness might provide better solutions for the problem.

Several candidate genes, such as insulin-like factor-I (Zhou et al., 2005), insulin-like factor binding protein 2 (Lei et al., 2005), and adipocyte fatty acid-binding protein gene (Wang et al., 2006), have been reported to relate with fatness in chickens. Most SNP in these genes, however, showed significant associations with both growth and body composition traits (including fatness traits). Therefore, selection against AFW in broiler chickens by these SNP might interfere with the routine breeding programs in which growth rate is always the primary trait of breeding significance.

Because of its critical function in activating many nuclear hormone receptors in regulating energy homestasis, thermal regulation, and glucose metabolism in liver, fat tissue, and muscle, the chicken \(PGC-1\alpha\) gene is a potential candidate gene for fat deposition. In the current study, a G to A polymorphism at position 646 of the open reading frame in chicken \(PGC-1\alpha\) gene was identified. And this mutation-causing amino acid changed from Asp to Asn at codon 216. The distribution of allelic frequencies revealed that the G allele was the most frequent \((0.67)\) in the White Plymouth Rock (a meat-type bird). However, in a typical layer, the White Leghorn, the frequency of the G allele was only 0.18. The association study indicated that this SNP was significantly related with AFW and \%AFW \((P < 0.01)\). However, there were no significant influences of this SNP on most growth-related traits. Chickens with genotype GG had nearly 15 g more abdominal fat than

### Table 2. Least square mean of growth and carcass traits for different genotypes of the Asp216Asn polymorphism in the chicken peroxisome proliferators-activated receptor-\(\gamma\) coactivator-1\(\alpha\) (\(PGC-1\alpha\)) gene

<table>
<thead>
<tr>
<th>Traits(^1)</th>
<th>Genotype(^1)</th>
<th>Age (wk)</th>
<th>AA (109)</th>
<th>AG (176)</th>
<th>GG (47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>0</td>
<td>30.90 ± 0.36</td>
<td>30.47 ± 0.30</td>
<td>31.74 ± 0.56</td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>2</td>
<td>133.35 ± 2.24</td>
<td>136.02 ± 1.87</td>
<td>139.63 ± 3.66</td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>4</td>
<td>351.49 ± 5.90(^b)</td>
<td>365.44 ± 5.18(^b)</td>
<td>375.16 ± 9.88(^b)</td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>6</td>
<td>705.81 ± 13.18</td>
<td>711.41 ± 10.71</td>
<td>729.05 ± 20.84</td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>8</td>
<td>1,060.97 ± 21.33</td>
<td>1,079.09 ± 17.02</td>
<td>1,103.84 ± 33.49</td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>10</td>
<td>1,424.95 ± 29.21</td>
<td>1,437.63 ± 22.80</td>
<td>1,486.35 ± 45.18</td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>12</td>
<td>1,703.17 ± 31.01</td>
<td>1,708.38 ± 24.86</td>
<td>1,701.43 ± 48.30</td>
<td></td>
</tr>
<tr>
<td>Breast muscle weight (g)</td>
<td>12</td>
<td>196.06 ± 2.76</td>
<td>198.41 ± 2.24</td>
<td>200.65 ± 4.32</td>
<td></td>
</tr>
<tr>
<td>Leg muscle weight (g)</td>
<td>12</td>
<td>265.95 ± 3.20</td>
<td>265.56 ± 2.60</td>
<td>269.05 ± 5.01</td>
<td></td>
</tr>
<tr>
<td>AFW (g)</td>
<td>12</td>
<td>46.62 ± 2.99(^b)</td>
<td>48.63 ± 2.43(^b)</td>
<td>62.59 ± 4.64(^b)</td>
<td></td>
</tr>
<tr>
<td>%AFW (g/100 g)</td>
<td>12</td>
<td>2.71 ± 0.17(^b)</td>
<td>2.82 ± 0.14(^b)</td>
<td>3.58 ± 0.27(^b)</td>
<td></td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>12</td>
<td>10.11 ± 0.31</td>
<td>9.66 ± 0.25</td>
<td>9.86 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>12</td>
<td>32.95 ± 0.56</td>
<td>33.80 ± 0.45</td>
<td>33.02 ± 0.87</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a,b}\)Means within a row with no common superscript differ significantly \((P < 0.01)\).

\(^{1}\)Numbers in parentheses are the number of individuals measured.

\(^{2}\)AFW = abdominal fat weight; \%AFW = AFW expressed as percentage of BW at 12 wk of age.
those with genotypes AA and AG, whereas all genotypes had similar growth rates. Therefore, given the unique property of the SNP, breeders can reduce abdominal fat significantly by selecting AA birds without affecting the continued improvement of growth rate. Although a high frequency of the unfavorable allele G was found in the White Plymouth Rock, a widely used broiler dam line, the elimination of the allele G might greatly reduce the frequency of the unfavorable allele G was found in the current study might be associated with functional polymorphisms in other positions of PGC-1α, other factors that may influence expression of the gene, or with variations of nearby genes. Further functional genomic investigations are needed to explore the molecular mechanism of the genetic variation in PGC-1α. Once the genetic effects of the molecular marker are verified in other populations, the application of the Asp216Asn SNP identified in the PGC-1α gene to broiler breeding programs may lead to significant improvement in feed efficiency and carcass quality.

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