Association of Myf5, Myf6 and Myog Gene Polymorphisms with Carcass Traits in Chinese Meat Type Quality Chicken Populations

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Abstract: The MysD gene family has been proposed to profoundly modulate muscle development and carcass performance in farm animals. In this study, researchers examined Single Nucleotide Polymorphisms (SNPs) in the exons of the Myf5, Myf6 and Myog genes using Polymerase Chain Reaction (PCR)-Single Strand Conformation Polymorphisms (SSCP) and DNA sequencing methods in 360 individuals from 6 commercial pure lines of Sichuan Daheng meat type quality chickens. About 2 SNPs (87T>C and 96C>T) in exon 1 of Myf5, 1 SNP (154T>C) in exon 1 of Myog and no variation in Myf6 were detected. The 96C>T SNP in Myf5 was a rare variant and was not analyzed further. The association analysis of genotypes with carcass traits revealed that the genotypes of SNP (87T>C) in Myf5 were significantly associated with Live Weight (LW), Carcass Weight (CW), Semi-Eviscerated Weight (SEW) and Eviscerated Weight (EW) (p<0.05). The SNP genotypes (154T>C) in Myog were significantly associated with Live Weight (LW), Carcass Weight (CW), Eviscerated Weight (EW) and Breast Muscle Weight (BMW) (p<0.05). The results suggested that Myf5 and Myog genes are potential major genes or are in close linkage disequilibrium with the QTL affecting carcass traits in this population of chickens. The 2 SNPs may potentially have use as markers for Marker-Assisted Selection (MAS) in chicken breeding.

Keywords: Chicken, carcass traits, Myf5/Myf6/Myog genes, meat quality, polymorphism, genotypes

INTRODUCTION

Chinese indigenous chickens are known for their delicious and nutritious meat. However, slower growth and lower production performance have constrained the industrialization of indigenous chicken production (Ding et al., 2000). Attempts to increase the growth rate of indigenous chickens while maintaining excellent meat quality have been a key objective for breeders. Because many important economic traits are controlled by several minor genes (Deeb and Lamont, 2002), selection for these important traits using traditional breeding methods is difficult which greatly limits genetic improvement. Marker Assisted Selection (MAS) has been proposed as an aid to circumvent difficulties in this field (Heifetz et al., 2005). Molecular markers, linked with Quantitative Trait Loci (QTL) can be identified by the candidate gene approach and by whole-genome scanning (Wang et al., 2006).

The candidate gene approach is a cost-effective method to find QTLs responsible for variation in traits of interest (Linville et al., 2001). The combining of traditional breeding and modern molecular genetics for poultry breeding is expected to be the future of poultry breeding and will effectively improve breeding power (Li et al., 2005). Development of skeletal muscles in the vertebrate embryo are controlled by the Myogenic Regulatory Factors (MRFs) also known as Myogenic determination gene (MyoD), including MyoD1, Myogenin (Myog), Myf5 and MRF4 (Te Pas et al., 2000; Berkes and Tassone, 2005). The corresponding proteins belong to the family of basic helix-loop-helix transcription factors that control determination of the myogenic cell lineage and differentiation of myoblasts in all muscle-forming regions of the embryo (Massari and Murie, 2000).

In vitro, each MRF efficiently binds to consensus CANNTG sites (E boxes) which are present in the promoters and enhancers of muscle-specific genes (Lassar et al., 1989; Blackwell and Weintraub, 1990). Myf5, together with MyoD1 are mainly expressed in the myoblast proliferation of skeletal muscle cells and are subject to distinct cell cycle regulation and the Myf5 is the 1st member of this family to be expressed in the embryo (Braun et al., 1989; Yen and Wold, 1996). The Myf6 gene is the down-stream gene of MyoD family and is transiently expressed in the mouse myotome at mouse embryonic day 9.0 (E9.0) until E11.5 and reappears at E16.0 during differentiation of muscle fibers. Thus, the complex

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temporal expression pattern of Myf6 suggests potential roles in both muscle determination and terminal differentiation (Maak et al., 2006; Lin et al., 2007). Myogenin is required for the myoblast differentiation established by the initial expression of the Myf5 or MyoD1 genes which are thus responsible for the determination and specialization of myoblasts so, myogenin could be considered to be a differentiation factor (Bergstrom and Tapscott, 2001). These genes, therefore could have major effects on musculature and body growth. In recent years it has been found that some Single Nucleotide Polymorphisms (SNPs) in Myf5, Myf6, and MyoG genes are associated with growth and carcass traits in pigs (Soumillon et al., 1997; Te Pas et al., 1999; Cieslak et al., 2002).

At present, many candidate genes of carcass traits have been intensively studied in chickens. There are few reports however, about associations of polymorphisms of Myf5, Myf6 and MyoG genes with production traits in chickens. In the present study, 6 high-quality broiler strains served as research materials and were screened for SNP loci in the CDS regions of Myf5, Myf6, and MyoG genes using Polymerase Chain Reaction-Single Strand Conformational Polymorphism methodology (PCR-SSCP). Associations of the SNPs with production traits were then investigated to potentially provide a theoretical basis for the molecularly-aided breeding of superior chickens.

MATERIALS AND METHODS

Chicken populations: In this study, 360 Daheng meat type quality chickens from 6 pure lines (S01, S02, S03, S04, S05, S06 and D99) were collected from the Sichuan Daheng Poultry Breeding Company and the Sichuan Animal Science Academy. From each line, 60 chickens (male: female = 1:1) were randomly sampled for collecting blood and slaughter. All birds were hatched on the same day, housed on deep-litter bedding and moved to growing house at 7 weeks of age. Birds had ad libitum access to feed (commercial corn-soybean diets meeting NRC requirements) and water.

Phenotypic measurements: Before slaughter at 91 days of age the birds were fasted for 12 h and blood was collected from a wing vein and stored at -20°C for isolation of genomic DNA by phenol-chloroform extraction. Body Weight (BW) and 7 carcass traits were then measured. These included: Carcass Weight (CW), Semi-Eviscerated Weight (SEW), Eviscerated Weight (EW), Breast Muscle Weight (BMW), Leg Muscle Weight (LMW) and Abdominal fat Weight (AW). The CW was measured on the chilled carcass after removal of the feathers. Semi-eviscerated 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 semi-eviscerated 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, semi-eviscerated percentage, Breast Muscle Percentage (BMP), leg muscle percentage and Abdominal fat Percentage (AP).

Amplification and population genotyping: Primer pairs (Table 1) were designed from reference sequences of Gallus gallus Myf5, Myf6 and MyoG genes in GenBank (Accession Nos: NC_0060882.1, NC_0060882.2 and AF487518) by OLG 6.0 software (Molecular Biology Insights, Inc., Cascade, Co.) and PRIMER 5.0.

The PCR amplification was performed in a total reaction volume of 10 μL which containing 0.9 μL template DNA (100 ng μL⁻¹), 5 μL 2× Taq PCR Master mix (including Mg²⁺, dNTP, Taq DNA Polymerase; Beijing Tianwei Biology Technique Corporation), 3.3 μL dH₂O and 0.4 μL of each primer (10 pmol μL⁻¹). The PCR amplifications were carried out as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 45 sec, 61°C (or other apt temperature shown in Table 1) for 35 sec, 72°C for 1 min and ended with a final elongation at 72°C for 10 min. The PCR products were mixed with Single Strand Conformation Polymorphism (SSCP) buffer (95% formamide deionized, 0.05% of bromophenol blue, 0.25% xylene cyanole and 10% glycerol). Before being loaded into the gel the samples were denatured for 10 min at 99.9°C then quickly chilled on ice for 5 min. Denatured PCR products were electrophoresed for 16 h at 8 V cm⁻¹ on 12% polyacrylamide gels. The DNA was stained with 0.1%
AgNO₃ for 15 min and developed for 15-20 min sodium tetraborate containing formaldehyde then stopped with 10% acetic acid. Individual SSCP banding patterns were determined under visible light. After detecting different homozygous genotypes, representative PCR products were purified and sequenced by a commercial sequencing company (Shanghai Yingjun Biology Technique Corporation, Shanghai, China).

**Statistical analysis:** Data and genetic effects were analyzed with General Linear Model (GLM) procedures of SAS V8.02 package (SAS Inst. Inc., Cary, NC, USA) using the following model:

\[
Y = \mu + L_i + S_k + G_j + e_{ijk}
\]

Where:
- \(Y\) = The traits measured on chickens
- \(\mu\) = The population mean
- \(L_i\) = The fixed effect of line
- \(S_k\) = The fixed effect of sex
- \(G_j\) = The fixed effect associated with the genotype
- \(e_{ijk}\) = The random error

The interaction \(S \times G\) and \(G \times A\) were not significant for any trait and therefore was not included in this model. Significant differences (\(p<0.05\)) were found among different genotypes in the light of least square means using Duncan’s multiple-range test.

**RESULTS AND DISCUSSION**

**Genetic polymorphisms of Myf5, Myf6 and MyoG genes in chicken populations:** A PCR-based SSCP method was successfully developed for nucleotide substitutions in Myf5, Myf6 and MyoG genes. About 2 nucleotide substitutions (c. 87T>C and c. 96 C>T) in Myf5 gene and 1 nucleotide substitutions (c. 154T>C) were finally detected by directly sequencing the polymorphic fragment based on SSCP banding pattern.

**Frequencies of Myf5 and Myf6 genotypes and alleles:** The genotypic and allelic frequencies of the identified SNPs in the Myf5 and Myf6 gene were analyzed in the 6 strains (Table 2). As the variant (c. 96C>T) of Myf5 gene was rare (only detected in 2 individuals) in the samples it was excluded from further analysis. In the Myf5 gene the frequency of allele A exceeded that of allele B in strains S01, S02 and S06 the reverse was the case in strains S03, S05 and D99. The AA genotype was at low frequency in S03, S05 and D99, even zero in D99 and the frequency of BB homozygous genotype was the lowest in S01, S02 and S06 while the AB genotype was most prevalent in all strains. In the MyoG gene, allele B was consistently the dominant allele (average 0.578). The AB genotype was the most frequent and the AA homozygous genotype least frequent in the 6 strains. Based on the Chi-squared test, for the Myf5 gene, all strains were in Hardy-Weinberg equilibrium except for D99 (\(p<0.01\)). For the MyoG gene, only strain S03 was not in Hardy-Weinberg equilibrium (\(p<0.05\)).

**Analysis of slaughter traits in different genotypes of Myf5 and MyoG:** The associations of Myf5 and MyoG genotypes with slaughter traits in chicken were analyzed and the least square means of the 6 genotypes are shown in Table 3. For the Myf5 gene, 3 genotypes were significantly associated with LW, CW, SEW and BW (\(p<0.05\)) but not with other slaughter traits (\(p>0.05\)). After multiple regression analysis it was found that the LW, CW, SEW and BW in individuals of the BB genotype were significantly higher than those in individuals of AA and AB genotypes. For the MyoG gene, 3 genotypes had significant influence on LW, CW, EW and BMW (\(p<0.05\)) but not on other slaughter traits (\(p>0.05\)); LW, CW, EW and BMW in AA individuals were significantly higher than those in individuals with AB and BB genotypes. Muscle development is genetically controlled by multiple genes and its final expression is the result of

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
<th>Hardy-Weinberg Equilibrium</th>
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<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
<td>BB</td>
</tr>
<tr>
<td>Myf5 87T&gt;C</td>
<td>0.333 (20)</td>
<td>0.500 (30)</td>
<td>0.167 (10)</td>
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<td></td>
<td>0.333 (20)</td>
<td>0.533 (32)</td>
<td>0.133 (8)</td>
</tr>
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<td></td>
<td>0.200 (12)</td>
<td>0.433 (26)</td>
<td>0.367 (22)</td>
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<td>0.200 (12)</td>
<td>0.467 (28)</td>
<td>0.333 (20)</td>
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<td></td>
<td>0.367 (22)</td>
<td>0.533 (32)</td>
<td>0.1000 (6)</td>
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<td>0.0000 (0)</td>
<td>0.700 (12)</td>
<td>0.300 (18)</td>
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<td></td>
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<td>0.500 (20)</td>
<td>0.233 (14)</td>
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<tr>
<td></td>
<td>0.1000 (6)</td>
<td>0.500 (30)</td>
<td>0.400 (24)</td>
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<tr>
<td></td>
<td>0.1000 (6)</td>
<td>0.633 (38)</td>
<td>0.267 (16)</td>
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<tr>
<td></td>
<td>0.200 (12)</td>
<td>0.500 (20)</td>
<td>0.300 (18)</td>
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<td></td>
<td>0.1330 (8)</td>
<td>0.660 (26)</td>
<td>0.267 (16)</td>
</tr>
<tr>
<td>D99</td>
<td>0.1330 (8)</td>
<td>0.533 (22)</td>
<td>0.333 (20)</td>
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The test of Hardy-Weinberg Equilibrium, \(p<0.05\) suggested the significant deviation from Hardy-Weinberg Equilibrium.
among genetic, nutritional and environmental factors (Scanes et al., 1984). More complete interaction understanding of the genetic basis of muscle development in chickens will provide an opportunity for its genetic improvement. The MyoD gene family is critical for the determination and terminal differentiation of skeletal muscle (Fomin et al., 2004). Recently, members of this family were identified as positional candidate genes for muscle growth in farm animals. Dunata et al. (2002) analyzed Hif1 polymorphic loci in the Myf5 gene in 1216 pigs from 2 strains and found that this gene was significantly correlated with lean-meat percentage but was not associated with birth weight, weight at slaughter, growth rate, meat weight or subcutaneous fat thickness. About 3 SNPs were identified by Vyvkoukalova et al. (2003) in intron 1 of the chicken Myf5 and showed these polymorphic sites to have significant effect on muscle growth (Vyvkoukalova et al., 2003).

The Ava I polymorphisms of the Myf5 gene were detected by (Zhu and Li, 2005) from 12 pig breeds and the B allele can increase carcass lean meat percentage, loin eye area and slaughter weight while reducing subcutaneous fat, thereby enhancing the quality of the carcass (Zhu and Li, 2005). Sun et al. (2008) reported a SNP in exon 1 of the chicken Myo6 gene and this mutation was correlated with live weight, carcass weight, breast muscle weight and density of muscle fibers (Sun et al., 2008).

In the present study, 3 members of the chicken MyoD gene family were investigated. There were 3 SNPs detected in the Myf5 and MyoG genes, consistent with the findings of Wang et al. (2007), no polymorphism was detected in the Myf5 gene. None of these 3 SNPs result in amino acid change. Although, none of them leads to amino acid change but the efficiency of transcription or translation of these genes might be affected and the extent of such changes might differ (Zhao et al., 2009). For the purpose of investigating the possible function of the mutations the association of genotypes of Myf5 and MyoG genes with carcass traits was analyzed. Significant association between single SNPs of chicken Myf5 and MyoG genes and carcass traits were found in these chickens.

**CONCLUSION**

Results from the current study showed that these SNPs of the Myf5 and MyoG genes were associated with growth and carcass traits of Sichuan-Daheng chickens, suggesting that the MyoD gene family may be potential major genes or in close linkage disequilibrium with QTLs for muscle growth of the chickens. Further study of the MyoD gene family should lay a good foundation for molecular-aided selection and production of these birds.

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