Journal of Animal and Veterinary Advances 10 (6): 704-708, 2011
ISSN: 1680-5593

Association of *MyF5, MyF6* and *MyOG* Gene Polymorphisms with Carcass Traits in Chinese Meat Type Quality Chicken Populations

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Abstract: The *MyoD* 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.

Key words: 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 Tapscott, 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 Murre, 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; Yun 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; Jin *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 muscularity 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 (Soumillion *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 molecular-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_006088.2, NC_006088.2 and AF487518) by OLIGO 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 Mg^{2+}, dNTP, Taq DNA Polymerse; Beijing Tianwei Biology Technique Corporation), 3.3 μL ddH_2O 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 bromophinol 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%

Table 1: Details of primers used for detecting SNPs

Primers	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing temperatures (°C)	Product length (bp)
Myf5-1	CTGCCAGTTCTCCCCATCCGA	TCCGCCGGTCCATGGT	61	245
Myf5-2	CCGGTGCCAGGCTACGCGA	CATAGCGCCCTGGGTAGGTCC	65	208
Myf5-3	GCCCCTGCTCGCCATTTGTCC	ATGACGGGGCTCTACGGGGTG	61	253
Myf6-1	TCCTCACCCCTCTCCGCATCT	AGCACGACGCACGCGAAAC	59	193
Myf6-2	AACCTGCAAGAGAAAGTCGGC	TCTCGATGTAGCTGATGGCC	59	179
Myf6-3	CGGCTCCTATTTCTTCTAC	CGACTTTCTCTTGCAGGTTTT	58	244
MyoG-1	GGTGGGTGTGGGGAATGTGCT	CCGGCTTTGTCTCTAATCCT	57	203
MyoG-2	AAACCCATCCCATTGTGC	CATCATCTGGTCCCTTCAGTT	60	236
MyoG-3	AACCACCTCACCCCATAACTG	AACCTGAGCCCACCCTAAG	58	220

$AgNO_3$ 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:

$$Y = \mu+L_i+S_k+G_j+e_{ijkf}$$

Where:

Y = The traits measured on chickens
μ = 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 G×S and G×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 SSPC 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 deteted 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 EW ($p<0.05$) but not with other slaughter traits ($p>0.05$). After multiple regression analysis it was found that the LW, CW, SEW and EW 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 2: The distribution of genotypic and allelic frequencies in 6 strains

		Genotype frequency			Allele frequency		Hady-Weinberg Equilibrium
SNP	Strains	AA	AB	BB	A	B	(p-values)
Myf5 87T>C	S01	0.333 (20)	0.500 (30)	0.167 (10)	0.583	0.417	0.876
	S02	0.333 (20)	0.533 (32)	0.1330 (8)	0.600	0.400	0.543
	S03	0.200 (12)	0.433 (26)	0.367 (22)	0.417	0.583	0.552
	S05	0.200 (12)	0.467 (28)	0.333 (20)	0.433	0.567	0.785
	S06	0.367 (22)	0.533 (32)	0.1000 (6)	0.543	0.457	0.595
	D99	0.0000 (0)	0.700 (42)	0.300 (18)	0.350	0.650	0.003
MyoG 154T>C	S01	0.200 (12)	0.567 (34)	0.233 (14)	0.483	0.517	0.461
	S02	0.1000 (6)	0.500 (30)	0.400 (24)	0.350	0.650	0.588
	S03	0.1000 (6)	0.633 (38)	0.267 (16)	0.417	0.583	0.047
	S05	0.200 (12)	0.500 (30)	0.300 (18)	0.450	0.550	0.955
	S06	0.1330 (8)	0.600 (36)	0.267 (16)	0.433	0.567	0.225
	D99	0.1330 (8)	0.533 (32)	0.333 (20)	0.400	0.600	0.543

The test of Hardy-Weinberg Equilibrium. $p<0.05$ suggested the significant deviation form Hardy-Weinberg Equilibrium

Table 3: Least square means of the carcass traits, by genotype, of chicken *Myf5* and *MyoG* gene

	Myf5-SNP (87T>C)			MyoG-SNP (154T>C)		
Traits	AA	AB	BB	AA	AB	BB
LW (g)	1704.22±39.73^{A}	1697.17±27.78^{A}	1715.03±41.23^{B}	1760.33±47.66^{A}	1672.46±25.86^{B}	1730.47±36.97^{B}
CW (g)	1524.27±37.27^{A}	1520.68±26.06^{A}	1541.63±38.67^{B}	1586.61±44.65^{A}	1496.35±24.23^{B}	1550.80±34.63^{B}
SEW (g)	1421.09±38.66^{A}	1404.68±27.03^{A}	1433.96±40.12^{B}	1436.57±46.66	1395.49±25.32	1444.27±36.20
EW (g)	1181.00±29.68^{A}	1178.71±20.75^{A}	1187.93±30.79^{B}	1224.30±35.63^{A}	1160.41±19.33^{B}	1197.89±27.64^{B}
BMW(g)	90.89±2.720	93.97±1.900	93.06±2.820	97.90±3.260^{A}	90.74±1.770^{B}	94.32±2.530^{B}
LMW (g)	127.97±4.090	126.66±2.870	126.52±4.250	132.88±4.900	123.51±2.660	130.37±3.810
AW (g)	32.48±2.580	33.93±1.800	34.94±2.720	32.13±3.100	33.20±1.690	35.99±2.450
BMP (%)	7.66±0.140	7.94±0.090	7.84±0.140	7.96±0.160	7.79±0.080	7.86±0.130
LMP (%)	10.82±0.150	10.68±0.100	10.63±0.150	10.85±0.180	10.60±0.090	10.84±0.140
AP (%)	1.79±0.140	1.97±0.100	1.96±0.150	1.83±0.180	1.92±0.090	1.97±0.140

LW = Live Weight (g); CW = Carcass Weight (g); SEW = Semi-Eviscerated Weight (g); EW = Eviscerated Weight (g) ; BMW = Breast Muscle Weight (g); LMW = Leg Muscle Weight; AFW = Abdominal Fat Weight (g); SFT = Subcutaneous Fat Thickness (mm); Percentage sign (%) indicates these traits relative to CW. Different uppercase letters mean significant difference at the $p<0.05$ levels for chickens with different genotypes of a given SNP

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. Danuta *et al*. (2002) analyzed Hinf I 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 Vykoukalova *et al*. (2003) in intron 1 of the chicken Myf6 and showed these polymorphic sites to have significant effect on muscle growth (Vykoukalova *et al*., 2003).

The Ava I polymorphisms of the *Myf6* 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 rate 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 *Myf6* 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 *Myf6* 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.

ACKNOWLEDGEMENTS

The researchers thank Xiao-Song Jiang, Hua-Rui Du and Xiao-Cheng Li for sample collecting and thank Prof. W. Bruce Currie for revising the manuscript. This study was financially supported by the Program for New Century Excellent Talents in University (NCET-10-0889) and the Science Fund for Yong Scholars in Sichuan Province (ZQ 026-017).

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