



Myf5 and MyoG gene SNPs associated with Bian chicken growth trait

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ABSTRACT. The growth trait is important in poultry production. We analyzed the association between single nucleotide polymorphisms (SNPs) in the *Myf5* and *MyoG* gene and Bian chicken growth traits. SNPs in candidate genes of the Bian chickens were detected by the polymerase chain reaction-single strand conformation polymorphism method. Two mutation loci and six genotypes were identified in each candidate gene. In terms of growth traits, least square analysis showed that the FF genotype of the *MyoG* was the advantage genotype and the IJ genotype of the *Myf5* was the disadvantage genotype for growth

trait in Bian chicken. Correlation analysis suggested that the different combination genotypes between *Myf5* and *MyoG* genes had a significant effect on growth traits in Bian chickens. The result suggested that *MyoG* and *Myf5* genes can be used in marker-assisted selection for improving the growth trait in Bian chicken.

Key words: Bian chickens; *Myf5*; *MyoG*; Growth trait; SNP; Conjoint analysis

INTRODUCTION

Body weight, a high heritability trait, is an important economic factor for broiler chickens and reflects the production level and economic benefits of a farm (Wang and Chen, 2004). Therefore, research into the genetic mechanism of growth traits is valuable. Using a candidate gene, quantitative trait loci that are related to growth traits are identified and isolated to provide theoretical basis for marker-assisted breeding (Bai et al., 2006).

The myogenic determination factor (MRF) family has four members: *Myf5*, *MyoD*, *MyoG* and *Myf6*. The factors contain a basic helix-loop-helix domain that can dimerize with the E-protein to form protein dimers (Murre et al., 1989; Sun and Baltimore, 1991; Anthony-Cahill et al., 1992). They not only contribute to the differentiation and development of skeletal muscle precursor cells during the growth of embryo, but also related to skeletal muscle repair and hypertrophy (Lin and Konieczny, 1992; Perry and Rudnick, 2000). In chickens, the *Myf5* gene is located on chromosome 1 and comprises three exons and two introns. It is 1215 bp long. The *MyoG* gene is located on chromosome 26 and also comprises three exons and two introns. It is 3402 bp long. Gene knockout studies have clearly established the roles of the MRFs in myogenesis during mouse embryo development. *Myf5* is expressed first during embryonic muscle development and initiates the conversion of muscle satellite cells to muscle stem cell with myoblast characteristics (Rescan, 2001). The *MyoG* gene determines myoblast differentiation to myotubes (Neville et al., 1992; Naka et al., 2013). There appears to be a hierarchical and partially redundant relationship among the MRFs (Megeny and Rudnicki, 1995; Rudnicki and Jaenisch, 1995; Wang et al., 1996). Therefore, studying *Myf5* and *MyoG* genes are of great significance for the improvement of meat production in livestock and poultry.

In this study, the Bian chicken breed, which is a national poultry genetic resource and is characterized by adaptability to poor quality feeds and environment, was chosen as the research object. To identify growth related molecular markers and provide a theoretical basis for breeding and conservation of Bian chickens at the molecular level, single nucleotide polymorphisms (SNPs) in the exons of the *Myf5* and *MyoG* genes were detected by polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP).

MATERIAL AND METHODS

Chicken population

Blood samples were collected from 360 female Bian chickens that had been randomly selected from the same feeding batch at Animal Husbandry and Veterinary Institute of Shanxi.

The body weight of each female Bian chicken was measured at 0, 6, 8, 10, 12, 14, 16, 18, and 20 weeks. Genomic DNA was extracted by the phenol-chloroform extraction method and dissolved in TE buffer (TE is derived from Tris and ethylene diamine tetraacetic acid). Before conducting the PCR, the concentration and purity of the DNA samples were measured by spectrophotometer.

Primer design and PCR amplification

Based on the chicken *Myf5* and *MyoG* gene sequence (GenBank accession Nos. NW_003763474.1 and NC_006113.3), the primers were designed for amplifying all of the exon regions using the Primer Premier 5.0 software and were synthesized by the Sangon Biotech (Shanghai) Co., Ltd.

The PCR was performed in a 20- μ L mixture that contained 1 μ L chicken genomic DNA (50 ng/ μ L), 2 μ L 10X buffer, 1.6 μ L deoxyribonucleotide triphosphates (dNTPs, 10 mM), 1 μ L forward primer (10 μ M), 1 μ L reverse primer (10 μ M), 0.2 μ L *Taq* DNA polymerase, and 13.2 μ L sterilized water. The amplification program was as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, the annealing temperature for 30 s (see Table 1), extension at 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR products were verified by 1% agarose gel electrophoresis.

Table 1. Primer sequences for polymerase chain reaction (PCR) amplification of the chicken *Myf5* and *MyoG* genes.

Primer	Sequence (5'→3')	Annealing temperature (°C)	Fragment size (bp)	Location
MY5	F: AGATGGAGGTGATGGACAGC R: GGACGTGTTCTCTTCCTCA	57.0	174	Exon 3
MYG	F: CTCCTCCCTCTCTCAGAT R: CTTTGCGCCAGCTCAGTT	56.0	152	Exon 3

F = forward; R = reverse.

SSCP and sequencing

A mixture of the PCR production (2 μ L) and a denaturing buffer (7 μ L) was heated at 98°C for 10 min and then cooled on ice for 10 min. The denatured PCR products were detected by 10% non-denaturing polyacrylamide gel electrophoresis at 220 V for 5 min and then 10 V/cm for 10-12 h. After electrophoresis, the gels were developed by silver staining. Each genotype sample was sent for sequencing and alignment was conducted.

Statistical analysis

The general linear model was implemented using statistical software SPSS17.0 for multiple comparisons. The following linear model was used for least-squares analysis between growth traits and different genotypes in each gene

$$Y_i = \mu + G + e_i \quad (\text{Equation 1})$$

where μ was the overall mean, G was the effect of the *Myf5* or *MyoG* gene, and e_i was the random error.

The following linear model was used for candidate genes conjoint analysis

$$Y_{ijk} = \mu + G_i + G_j + G_{ij} + e_{ijk} \quad (\text{Equation 2})$$

where μ was the overall mean, G_i was the effect of the *Myf5* gene, G_j was the effect of the *MyoG* gene, and e_{ijk} was the random error. Unless otherwise stated, data are reported as means \pm standard error.

RESULTS

Detection and sequencing results of PCR-SSCP

The products amplified by primers MYG of the *MyoG* gene and MY5 of the *Myf5* gene showed polymorphisms. For primers MYG and MYF5, six genotypes were observed (Figure 1). Comparisons between the sequencing results and the original sequences of the candidate genes revealed nucleotide mutations. The mutations were T2927C and G2957A in exon 3 of *MyoG* (Figure 2), and C238T and G264A in exon 1 of *Myf5* (Figure 3). Analysis using DNAMAN software showed that all mutation loci in the candidate genes were synonymous mutations.

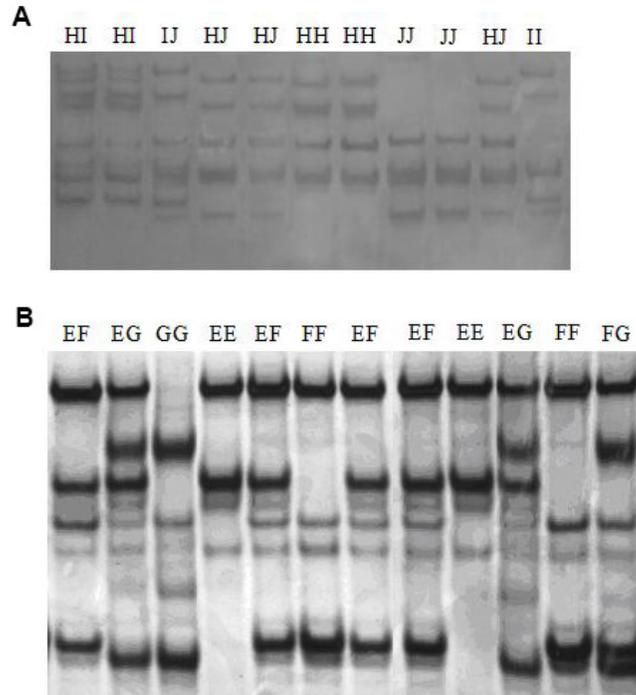


Figure 1. Polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) electrophoresis showing the different genotypes of the *MyoG* (A) and *Myf5* (B) genes in Bian chickens.

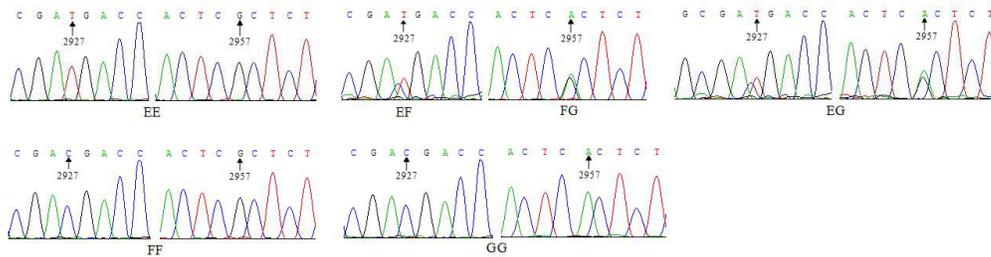


Figure 2. Sequence of different genotypes of primer MYG in Bian chickens.

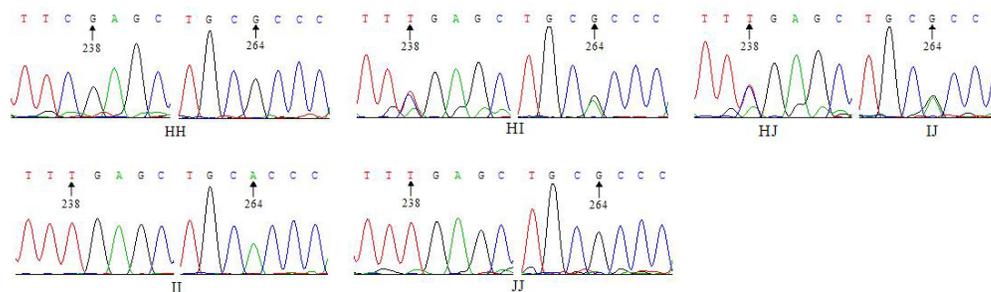


Figure 3. Sequence of different genotypes of primer MY5 in Bian chickens.

Association between different genotypes of the MYG locus in the *MyoG* gene with growth traits

The correlation analysis suggested that the MYG locus had a significant effect on growth traits from 0-20 weeks in Bian chickens (Table 2). Association analysis revealed that the FF genotype produced a significantly higher body weight at 0 week old compared with the EF genotype ($P < 0.05$). Body weight in the FF genotype was also significantly higher than in the EE genotype at 6-10 weeks old ($P < 0.05$). Furthermore, no significant association between the different genotypes and body weight at the other time-points were detected ($P > 0.05$).

Table 2. Associations between the genotypes and growth traits in the MYG loci of the *MyoG* gene in Bian chickens.

Body weight (g)	Genotypes					
	EE (119)	FF (32)	GG (3)	EF (119)	EG (53)	FG (34)
0 weeks	36.57 ± 0.30 ^{ab}	37.63 ± 0.58 ^a	37.33 ± 1.89 ^{ab}	36.38 ± 0.30 ^b	36.98 ± 0.45 ^{ab}	36.71 ± 0.56 ^{ab}
6 weeks	439.81 ± 4.13 ^b	460.56 ± 7.98 ^a	428.33 ± 26.05 ^{ab}	442.69 ± 4.13 ^{ab}	444.45 ± 6.20 ^{ab}	443.29 ± 7.74 ^{ab}
8 weeks	617.82 ± 6.05 ^b	643.75 ± 11.66 ^a	601.67 ± 38.07 ^{ab}	622.73 ± 6.05 ^{ab}	625.45 ± 9.06 ^{ab}	623.09 ± 11.3 ^{ab}
10 weeks	785.50 ± 7.29 ^b	818.41 ± 14.06 ^a	762.67 ± 45.90 ^{ab}	796.07 ± 7.29 ^{ab}	795.93 ± 10.92 ^{ab}	798.85 ± 13.64 ^{ab}
12 weeks	977.43 ± 9.17	1009.75 ± 17.68	956.00 ± 57.76	992.03 ± 9.17	981.93 ± 13.74	984.85 ± 17.16
14 weeks	1122.10 ± 10.96	1161.00 ± 21.12	1090.67 ± 69.00	1145.49 ± 10.96	1120.68 ± 16.42	1125.88 ± 20.49
16 weeks	1171.74 ± 13.60	1201.78 ± 26.22	1140.67 ± 85.64	1205.61 ± 13.60	1167.36 ± 20.38	1189.35 ± 25.44
18 weeks	1301.70 ± 14.97	1353.06 ± 28.88	1282.67 ± 94.30	1334.61 ± 14.97	1294.19 ± 22.44	1306.35 ± 28.01
20 weeks	1427.97 ± 17.25	1490.19 ± 33.27	1422.33 ± 108.66	1466.33 ± 17.25	1431.74 ± 25.85	1441.65 ± 32.28

In the same line, different small letters correspond to a statistically significant difference where $P < 0.05$.

Association between different genotypes of the MY5 locus in the *Myf5* gene with growth traits

Correlation analysis suggested that the MY5 locus had a significant effect on growth traits at 0-20 weeks in Bian chickens (Table 3). Association analysis revealed that body weight in the II genotype was significantly higher than in the HJ genotype ($P < 0.01$) and also significantly higher than HH, JJ and HI genotypes ($P < 0.05$) at 0 week old. Body weight in the IJ genotype was significantly lower than in the HH genotype ($P < 0.01$) and also significantly lower than in the other genotypes ($P < 0.05$) at 6 weeks old. Body weight in the IJ genotype was significantly lower than in the other genotypes except for the JJ genotype at 8 and 10 weeks old ($P < 0.05$). Body weight in the IJ genotype was significantly lower than in the HH and HI genotypes and also lower than in the other genotypes at 12 weeks old ($P < 0.05$). Body weight in the IJ genotype was lower than in the HH genotype at 14 weeks old ($P < 0.05$). The HH genotype was extremely significantly higher than IJ genotype ($P < 0.01$) and significantly higher than in the JJ genotypes at 16 to 20 weeks old ($P < 0.05$). Furthermore, no significant associations between the different genotypes and body weight at the other time-points were detected ($P > 0.05$).

Table 3. Associations between the genotypes and growth traits in the MY5 loci of the *Myf5* gene in Bian chickens.

Body weight (g)	Genotypes					
	HH (84)	II (7)	JJ (57)	HI (51)	HJ (136)	IJ (25)
0 weeks	36.54 ± 0.36 ^B	39.85 ± 1.23 ^{Aa}	36.36 ± 0.43 ^B	36.17 ± 0.46 ^B	36.76 ± 0.28 ^{ABb}	37.56 ± 0.65 ^{AB}
6 weeks	449.68 ± 4.89 ^A	462.57 ± 16.94 ^{ABa}	438.44 ± 5.94 ^{ABa}	447.06 ± 6.28 ^{ABa}	443.77 ± 3.85 ^{ABa}	420.56 ± 8.97 ^{Bb}
8 weeks	627.53 ± 7.15 ^a	654.00 ± 24.77 ^a	613.00 ± 8.68 ^{ab}	630.61 ± 9.18 ^a	625.56 ± 5.62 ^a	595.80 ± 13.11 ^b
10 weeks	802.48 ± 8.61 ^a	837.00 ± 29.85 ^a	784.36 ± 10.46 ^{ab}	803.02 ± 11.06 ^a	795.25 ± 6.77 ^a	757.68 ± 15.79 ^b
12 weeks	995.51 ± 10.80 ^A	1038.57 ± 37.43 ^{ABa}	981.36 ± 13.12 ^{ABa}	996.94 ± 13.87 ^A	985.86 ± 8.49 ^{ABa}	932.84 ± 19.81 ^{Bb}
14 weeks	1147.12 ± 13.01 ^a	1172.28 ± 45.08 ^{ab}	1122.17 ± 15.80 ^{ab}	1136.98 ± 16.70 ^{ab}	1134.50 ± 10.22 ^{ab}	1085.44 ± 23.85 ^b
16 weeks	1216.08 ± 16.00 ^{Aa}	1199.71 ± 55.44 ^{AB}	1156.45 ± 19.43 ^{ABb}	1205.00 ± 20.5 ^{AB}	1186.14 ± 12.57 ^{AB}	1114.16 ± 29.33 ^{Bb}
18 weeks	1347.38 ± 17.69 ^{Aa}	1346.85 ± 61.28 ^{AB}	1291.86 ± 21.47 ^{ABb}	1334.61 ± 14.97 ^{AB}	1294.19 ± 22.44 ^{AB}	1306.35 ± 28.01 ^{Bb}
20 weeks	1490.09 ± 20.37 ^{Aa}	1442.47 ± 16.02 ^{AB}	1427.72 ± 24.73 ^{ABb}	1453.90 ± 26.15 ^{AB}	1442.47 ± 16.01 ^{AB}	1365.04 ± 37.35 ^{Bb}

In the same line, different capital letters correspond to a statistically significant difference where $P < 0.01$; different small letters correspond to a statistically significant difference where $P < 0.05$.

Association between the different combination genotypes of the *MyoG* and *Myf5* genes with growth traits

Correlation analysis suggested that the different combination genotypes in the *Myf5* and *MyoG* genes had a significant effect on growth traits, especially in the later time- periods in Bian chickens (Table 4). Association analysis revealed that the combination genotypes had a significantly effect on body weight at 12 weeks ($P < 0.05$) and a significantly effect at 16, 18 and 20 weeks ($P < 0.01$). There was not significant effect on weight at the other time-points.

Table 4. Associations between the different genotype combinations and growth traits of the candidate genes in Bian chickens.

Body weight	P value	Body weight	P value	Body weight	P value
0 weeks	0.063	10 weeks	0.072	16 weeks	0.003
6 weeks	0.111	12 weeks	0.029	18 weeks	0.007
8 weeks	0.111	14 weeks	0.088	20 weeks	0.009

DISCUSSION

The MRFs play key and different roles in the myogenic pathway. MyoD and Myf5 have overlapping functions in the differentiation and proliferation of muscle precursor cells such that deletion of one or the other does not significantly affect muscle development (Braun et al., 1992; Rudnicki et al., 1992), whereas deletion of both genes completely eliminates the skeletal muscle lineage (Rudnicki et al., 1993). *MyoG* and *Myf6* function downstream of the myogenic pathway. The *MyoG* factor controls myoblast differentiation to myotubes. In its absence, myoblasts are properly specified and positioned but there is a severe deficiency of muscle fibers (Hasty et al., 1993; Nabeshima et al., 1993). The *Myf6* controls myotubes and the further integration of muscle fibers, but it is not essential for muscle development (Braun and Arnold, 1995; Patapoutian et al., 1995). The MRFs also have the ability to trigger skeletal muscle differentiation in non-muscle cells *in vivo* (Delfini and Duprez, 2004) and *in vitro* (Weintraub et al., 1991).

Using the PCR-SSCP technique, three mutations have been found in the *MyoG* gene 5' regulatory region in seven chicken breeds. The result suggested that these variations might be associated with muscle fibers, thereby affecting the chicken growth (Wang et al., 2008). One study has shown that two polymorphic loci of the chicken *MyoG* gene 5' regulatory region have a significant effect on muscle fiber density, muscle weight, leg muscle weight, and the rate of development of leg muscles (Wang et al., 2007). A study conducted by Ma (2010) showed that the different genotypes combinations of *Myf5* gene in Gushi-Anka chickens had a significant effect on body weights at 8, 10 and 12 weeks.

In this study, we found six genotypes in each of the candidate gene including *MyoG* and *Myf5* gene. Association analysis revealed that the FF genotype of the *MyoG* gene was the advantage genotype and the IJ genotype of *Myf5* gene was disadvantage genotype for growth trait in Bian chickens. As we know, a large number of SNPs exist in a genome. Although a single SNP can only provide limited information owing to its dimorphism, there is usually more than one SNP in a candidate gene. These SNPs can be used for association analysis between one or more candidate genes and traits (Cao et al., 2012). Correlation analysis suggested that the different combination genotypes in the *Myf5* and *MyoG* genes had significant effects on growth traits from 16 to 20 weeks in Bian chickens. An interesting result of the body weight conferred by the advantage genotype combination in each gene did not necessarily occur at the same time-point (not listed in this article). However, we found that body weight conferred by the disadvantage genotype combination was the lowest in both candidate genes. The body weight in the EF/IJ combination was significantly lower than in the other associated genotypes from 6 to 20 weeks. Therefore, it is a possible to increase the average weight by reducing the number of EF/IJ combinations.

We plan to increase the number of experimental groups in order to verify the reliability of the EF/IJ combination used for the Bian chicken breeding. Meanwhile, we will continue to research the genotypes to discover the most that is advantageous combination for improving growth traits.

CONCLUSIONS

In this study, six SNPs were detected by PCR-SSCP in the exons of the candidate genes

in Bian chickens. Correlation analysis showed that reducing the number of EF/IJ combination could be used to improve the growth trait in Bian chickens. The findings in this study not only provide a basis for marker-assisted selection of Bian chickens, but also represent a reference for further studies in other chicken breeds.

Conflicts of interest

The authors declare no conflict of interest.

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