Myf5 gene polymorphisms and production performance traits in Songliao white geese

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ABSTRACT. To explore the relationship between *Myf5* gene polymorphisms and production performance traits in Songliao white geese, we used the chicken *Myf5* sequence to design primers and amplified part of the exon 1 sequence of the Songliao white goose *Myf5* gene. Results of single-strand conformation polymorphism polymerase chain reaction analysis revealed polymorphisms of the amplified fragment, including three genotypes (AA, AB, and BB). Three varieties were dominated by allele A and were mainly expressed in AA genotypes. We also identified that the *Myf5* gene has one single nucleotide change (A→G) on exon 1 at locus 1344, and another (G→C) at locus 1410. Analysis of variance showed significant differences between genotypes before slaughter in live weight, carcass weight, eviscerated weight, leg muscle weight, weight of the wings, and slaughter rate. There were no significant differences with respect to other growth and carcass traits evaluated.

INTRODUCTION

Myf5 is one of the members of the myogenic determining (*MyoD*) factor gene family. Muscle growth requires myoblast proliferation and differentiation of *MyoD* family regulation. Members of the *MyoD* family encode 4 types of muscle-specific transcription factors, namely *MyoD*, *MyoG*, *Myf5*, and *Myf6* (*MRf4*), which are key regulators of skeletal muscle generation (Naidu et al., 1995). In addition, *MyoD* family genes share the myogenic basic helix-loop-helix (bHLH) transcription factor domain, which can activate muscle-specific genes (Rudnicki et al., 1993; Kim et al., 1999). Although several studies have focused on *MyoD* and *MyoG* genes, the *Myf5* gene, which is difficult to study, has received less attention, particularly in geese. Recently, however, the potential importance of *Myf5* has attracted the attention of researchers.

The Songliao white goose is a new line of goose meat that was developed using advanced molecular breeding methods of existing geese species. Songliao geese are characterized as fast growing, with high velvet meat production, good reproductive performance, high quality meat, and disease resistance (Piao et al., 2010). This study investigated polymorphisms in the goose *Myf5* gene from Songliao white geese, in order to find unique SNP markers and their relationships to carcass traits. The data obtained will help lay the foundation for determining genetic markers for improving goose production traits.

MATERIAL AND METHODS

Materials

Thirty-eight unified, standard-bred Songliao white geese were slaughtered at 90 days of age. Liver tissues were obtained and immediately frozen in liquid nitrogen until used to extract genomic DNA by the phenol-chloroform method.

Reagents

The DNA gel extraction kit was purchased from AXYGEN, the pMD18-T vector kit was purchased from Takara, the *Taq* enzyme and dNTP were purchased from TIANGEN, and competent DH5α cells were obtained from our laboratory's stock population.

Primer design and single-strand conformation polymorphism-polymerase chain reaction (SSCP-PCR) detection

Primers were designed by reference to chicken (GenBank: X73250) sequences. The primer sequences for the exon 1 sequences (upstream primer: 5'-CCAGTTCTCCCCATCC GA-3'; downstream primer: 5'-GGCAGGTGATAGTAGTTCTCC-3') were synthetized by Beijing Luhe Genomics Technology Co., Ltd. The PCR system was run in a 25-µL total volume, including 2.5 µL 10X buffer, 1 µL 2.5 mM dNTP, 0.5 µL of each 10 µM upstream and downstream primer, 1.0 U 100 ng/µL *Taq*, 1 µL DNA template, and double-distilled water. PCR conditions were as follows: denaturation at 95°C for 5 min, denaturation at 94°C for 30 s, 56°C annealing for 30 s, extension at 72°C for 30 s, for 30 cycles, and a final extension at 72°C for 5 min. The amplification products were detected by electrophoresis on 1% agarose

gel. PCR amplification products were denatured at 98°C for 10 min, pre-cooled on ice for 10 min, and subjected to 8% denatured polyacrylamide gel electrophoresis at 180 V for 14-16 h, silver stained, and typed.

Sequencing

Different homozygous genotypes were selected using the DNA gel extraction kit recycling purpose bands, cloned into the pMD18-T vector, transformed into DH5α competent cells, and cultured overnight at 37°C. The positive bacterial cells identified by PCR were sent to Beijing Luhe Genomics Technology Corporation for sequencing.

Statistical analysis

Genotype and gene frequencies were calculated and checked for deviations from Hardy-Weinberg equilibrium. A fixed model was employed to investigate the effects of different genotypes. The SPSS 12.0 statistical software was used for variance analysis of production traits, and single factor and multiple comparisons were used to measure the effects of different marker genotypes on production traits (means ± standard deviation). The linear model used was as follows:

$$Y_{ij} = \mu + marker_j + e_{ij} \qquad\qquad \text{(Equation 1)}$$

where Y_{ij} represents individual phenotypic values, μ is the population mean, $marker_j$ is the marker genotype effect, and e_{ij} is the random error.

Slaughter performance measurement indicators

In this study, ante-mortem live weight, carcass weight, semi-eviscerated weight, eviscerated weight, breast muscle weight, leg muscle weight, abdominal fat weight, wing weight, head weight, and claw poultry slaughter weight were all determined and used to calculate the slaughtering rate. Slaughter performance indicators were measured in accordance with poultry production performance and calculation guidelines published in 1984 by the National Association of Poultry Breeding.

RESULTS

Myf5 gene PCR amplification

The PCR results are shown in Figure 1. The length of the amplification product was 432 bp, and non-specific bands were not observed, indicating that the amplification product could be used for SSCP analysis.

SSCP analysis

After SSCP analysis, there were 3 genotypes detected, defined as AA, AB, and BB (Figure 2).
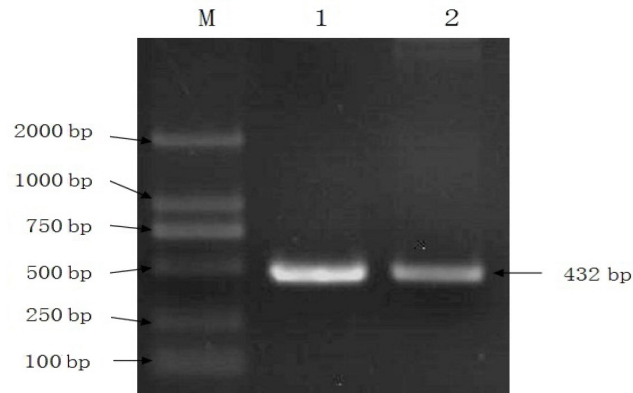
Figure 1. Electrophoresis map of the goose *Myf5* gene PCR amplification. *Lanes 1* and *2* = amplified fragments; *lane M* = D2000.
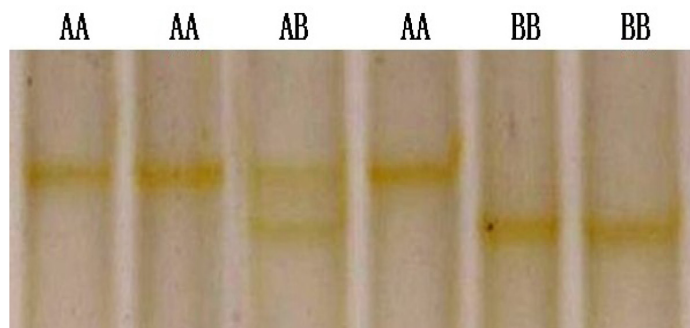

Figure 2. *Myf5* gene SSCP analysis of amplified products.

Sequence analysis results

The genotypes AA and BB were sequenced and matched with the original sequence, revealing one single base change (A → G) at position 1344, and another (G → C) at position 1410 of exon 1 (Figure 3).
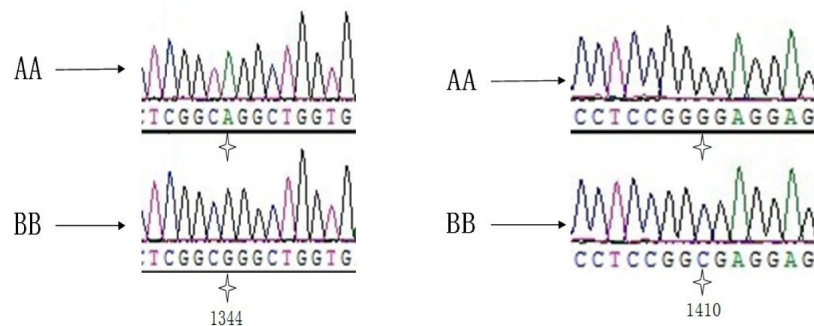

Figure 3. *Myf5* gene sequencing peak comparison charts of different genotypes.

Genotype frequencies

Genotype frequencies were calculated based on the genotyping results, and tested for Hardy-Weinberg equilibrium. Results revealed that the AA genotype was dominant, and that A was the predominant allele (Table 1).

Table 1. Genotype and gene frequency of the Songliao white goose *Myf5* gene.

Genotype frequency			Allele frequency		χ^2	P
AA	BB	AB	A	B		
0.789 (N = 30)	0.132 (N = 5)	0.079 (N = 3)	0.829	0.171	19.787	<0.01

χ^2 distribution of different genotypes for the Hardy-Weinberg equilibrium test values.

Relationships among different genotypes and carcass traits

As shown in Table 2, the AA genotype had a significantly stronger effect on live weight, carcass weight, semi-eviscerated weight, eviscerated weight, and wing weight before slaughter, compared to the BB genotype ($P < 0.05$). Inter-individual differences were not significant with respect to the 3 genotypes. Specifically, genotype AA had positive effects on ante-mortem live weight, carcass weight, semi-eviscerated weight, eviscerated weight, and wing weight. Individuals with the BB genotype had significantly lower leg muscle weights compared to individuals of genotype AA or AB ($P < 0.05$), suggesting a negative impact of genotype BB on leg muscle weight. Individuals of genotype AB had significantly higher semi-eviscerated and eviscerated yield traits compared to individuals of genotype AA ($P < 0.05$ and $P < 0.01$, respectively), suggesting that genotype AB has a positive impact on these traits. No significant differences were detected for breast muscle weight, abdominal fat weight, head weight, claw weight, slaughter rate, breast muscle rate, leg muscle rate, or abdominal fat percentage.

Table 2. *Myf5* gene genotype and carcass traits of Songliao white geese.

Traits	AA type (N = 30)	AB type (N = 3)	BB type (N = 5)
Before slaughter live weight (g)	4506.67 ± 65.08[a]	4273.33 ± 205.78	4076.00 ± 159.40[b]
Carcass weight (g)	3749.37 ± 54.98[a]	3597.67 ± 173.86	3406.00 ± 134.67[b]
Semi-eviscerated weight (g)	3404.20 ± 49.29[a]	3318.33 ± 155.88	3087.40 ± 120.75[b]
Eviscerated weight (g)	2991.70 ± 43.17[a]	2965.00 ± 136.52	2743.40 ± 105.74[b]
Breast muscle weight (g)	496.40 ± 7.94	494.00 ± 25.09	457.80 ± 19.44
Leg muscle weight (g)	501.70 ± 6.93[a]	503.33 ± 21.91[a]	462.00 ± 16.97[b]
Abdominal fat weight (g)	50.93 ± 3.14	46.00 ± 9.93	46.40 ± 7.69
Wings weight (g)	445.33 ± 8.87[a]	421.00 ± 28.04	397.00 ± 21.72[b]
Head weight (g)	159.67 ± 2.90	155.33 ± 9.18	144.40 ± 7.11
Pawl weight (g)	129.46 ± 2.84	117.67 ± 8.98	116.00 ± 6.95
Slaughter rate (%)	83.20 ± 0.24	84.17 ± 0.76	83.52 ± 0.59
Semi-eviscerated rate (%)	75.54 ± 0.30[b]	77.73 ± 0.96[a]	75.73 ± 0.74
Eviscerated rate (%)	66.41 ± 0.33[B]	69.44 ± 1.03[A]	67.25 ± 0.80
Breast muscle rate (%)	16.66 ± 0.23	16.66 ± 0.72	16.65 ± 0.55
Leg muscle rate (%)	16.81 ± 0.18	16.97 ± 0.57	16.80 ± 0.44
Abdominal fat percentage (%)	1.68 ± 0.11	1.55 ± 0.36	1.66 ± 0.28

$P < 0.05$ for significant differences between lowercase letters. $P < 0.01$ for highly significant differences between uppercase letters.

DISCUSSION

*Myf*5 plays a very important role in the growth process of muscles, regulating the number and size of muscle fibers. Therefore, studies of the *Myf*5 gene will be of high significance for livestock poultry production, meat quality, and flavor improvement.

Kiefer and Hauschka (2001) demonstrated dynamic changes in *Myf*5 expression in the chick embryo. *Myf*5 expression first appeared in the stage 3 primitive streak of chicken embryo muscle development, and showed transient lower levels of expression in non-muscle mesoderm. Thereafter, *Myf*5 was concurrently expressed with the occurrence of the sarcomere germ layers in the pre-sub section (pre-segmented mesoderm), and showed dynamic regional expression changes in the I-IV somite (Ernst et al., 1994; Black et al., 1995; Goureau et al., 2001). Up- and downregulation experiments have revealed that *Myf*5 plays a critical role in the myogenic process of vertebrates, especially in the muscle differentiation stage (Tatusova and Madden, 1999). Cooper et al. (1999) suggested that a negative feedback regulation ring might exist between *MyoD* and *Myf*5. Indeed, *Myf*5 has been shown to promote *MyoD* expression, while *MyoD* inhibits *Myf*5 expression (Twardizik et al., 1990).

In a gene level study, Cieslak et al. (2002) showed that the regulatory mechanism of *Myf*5 in mammals was complex. Maak et al. (2006) conducted an in-depth comparative study of *Myf*5, demonstrating that various regulatory factors within the region of the gene in mice, rabbits, pigs, cattle, dogs, and chickens (H1 and 2; M6EH) were all highly conserved. The function of these regulatory factors has been determined in transgenic mice, which are all temporally and spatially expressed independently in different regions during mouse embryo development (Williams et al., 1990; Welsh et al., 1991; Yablonka-Reuveni et al., 1999).

In this study, the PCR-SSCP technique was used to detect Songliao white goose *Myf*5 gene polymorphisms. The analysis revealed 3 genotypes, in which the AA genotype was dominant, and the A allele showed a dominant advantage. The frequency of the A and B alleles were 0.829 and 0.171, respectively. AA, AB, and BB genotype frequencies were 0.789, 0.079, and 0.132, respectively. The χ^2 test showed that the locus is in Hardy-Weinberg disequilibrium (P < 0.01), which may be related to the high homozygosity of the population and the selection of certain traits, indicating that this group can be further adjusted.

Results of this study revealed that different genotypes had significantly different effects on pre-slaughter live weight, carcass weight, semi-eviscerated weight, eviscerated weight, leg muscle weight, wing weight, and dressing percentage traits, whereas most other growth and carcass traits showed no significant differences among genotypes. The AA genotype showed relatively high trait association values, indicating that this genotype could be used as a molecular marker for screening high value slaughter traits that could be used to improve growth of Songliao white geese. Therefore, more in-depth studies of relationships between goose growth traits and *Myf*5 are needed to confirm its suitability as a candidate gene.

ACKNOWLEDGMENTS

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