

*Full Length Research Paper*

# Polymorphisms in the myostatin gene and their association with growth and carcass traits in duck

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**The objective of this study was to assess the association of polymorphisms in myostatin (MSTN) genes with growth and carcass traits in lines of Peking ducks. The polymorphism of the ducks MSTN gene promoter region was researched by polymerase chain reaction-single-stranded conformation polymorphism (PCR-SSCP) and DNA sequencing methods. Three mutations: G-753A, G-658T and G-235C were detected in the duck MSTN gene. Altogether, 300 Peking ducks of three lines (Z<sub>2</sub> line, Z<sub>4</sub> line and Z<sub>2</sub>×Z<sub>4</sub> cross line) were genotyped and allele frequencies were determined. The effects of MSTN polymorphisms on growth and carcass traits were analyzed. The G-753A was significantly associated with breast meat percentages (BMP) (P = 0.0225). The G-235C indicated significant association with abdominal fat percentages (AFP) (P = 0.0297). No significant association, however, was detected between any of the marker genotype and other traits measured in this study. Results from this study suggest that MSTN gene-specific single-nucleotide polymorphism (SNP) may be a useful marker for growth and carcass traits in future marker assisted selection programs in ducks.**

**Key words:** Duck, myostatin gene, SNPs, PCR-SSCP, growth and carcass traits, association analysis.

## INTRODUCTION

Myostatin (MSTN), also known as growth differentiation factor 8 (GDF-8), is a member of the transforming growth factor- $\beta$  superfamily, which is an essential factor for the growth and development of muscle mass (McPherron and Lee 1997; Mendias et al., 2008). The MSTN gene mainly expresses itself in skeletal muscle and cardiac muscle (Hennebry et al., 2009) and the protein functions as a negative regulator in the process of muscle development in animal breeding (Bellinge et al., 2005), and the polymorphisms of this gene are directly related to the double muscling phenotype (Wiener et al., 2002; Esmailizadeh et al., 2008; Grisolia et al., 2009; Phocas, 2009) which makes it a consolidate candidate gene for the enhancement of productivity in terrestrial livestock and fowl. This shows the meaningful study of the association between the polymorphisms of MSTN and the growth and carcass traits of animals.

The MSTN gene consists of three exons and two intronic regions in many species such as pig (AY208121),

buffalo (AH013313), zebra fish (AY323521), gilthead sea bream (AF258447) and chicken (AF346599). All elements of the MSTN gene seem to necessarily have negative effect on muscularity of animals. Loss of function mutations in the MSTN gene coding regions are associated with increased skeletal muscle mass (double muscling) in mice (Mendias et al., 2008), dogs (Mosher et al., 2007), sheep (Clop et al., 2006; Kijas et al., 2007), cattle (Marchitelli et al., 2003) and humans (Schuelke et al., 2004). Besides, the SNPs in promoter, intron and the 3'untranslated region of the gene have also been identified to have impact on carcass traits (Kijas et al., 2007; Zhou et al., 2008). Despite the large effects of MSTN mutations on different livestock and fowl breeds, no mutations with major effects have been described in ducks, especially in the promoter region of MSTN gene. By searching "tag" SNPs in the promoter of MSTN in different Peking duck lines (Z<sub>2</sub> line, Z<sub>4</sub> line, Z<sub>2</sub>×Z<sub>4</sub> cross line) in this work, these SNPs could be used to reveal the association between the polymorphisms and the production traits of individuals, which is an increasingly common approach to genetic association studies. The results of this study could add new important evidences that MSTN could be an important candidate gene to be used for the

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**Table 1.** Primers of the duck MSTN gene designed for PCR-SSCP analysis.

| Locus | Primer sequence (5' to 3') <sup>a</sup>                   | Location <sup>b</sup> | Length (bp) | Annealing temperature (°C) |
|-------|---|-----------------------|-------------|----------------------------|
| Frag1 | F: ATCTCGTGCAGAAAGCATATTGA<br>R: TGTAATAATGAGTTTGCCTGGTCA | -889~-637             | 253         | 55                         |
| Frag2 | F: GAGATATTTTGAAGTGAATTR:<br>R: GTGCATATTTGTCTGTTTGTCC    | -769~-561             | 204         | 53                         |
| Frag3 | F: CTTTAGAGAGAGCTCTGCCTTGA<br>R: CCAACAATGAATCTAGCTGTCAG  | -331~-88              | 244         | 58.2                       |

<sup>a</sup> F is the forward primer and R is the reverse primer; <sup>b</sup> the complete promoter of Pecking duck myostatin gene sequence (GenBank accession no: AY329600) was 1159 bp and the location represents the relative position from the start codon of the MSTN gene.

selection of growth and carcass traits in the duck industry.

## MATERIALS AND METHODS

### Animals, growth and carcass traits

A total of 300 individuals from three lines of Peking ducks: Z<sub>2</sub> (50♀ and 50♂), Z<sub>4</sub> (50♀ and 50♂) and Z<sub>2</sub>×Z<sub>4</sub> Cross (50♀ and 50♂) were derived from the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. All individuals were raised in the same farm and given self-help feeding. The average age was six weeks. Genomic DNA was extracted from whole blood by the standard phenol/chloroform/isoamyl alcohol extraction protocol (Mullenbach et al., 1989), then dissolved in TE buffer (10 mmol/L Tris-HCl and 1 mmol/L EDTA, pH 8.0), and kept at -20°C. Growth and carcass traits measured in the population were six-week weight (WW), eviscerated weight (EW), breast meat percentages (BMP), leg meat percentages (LMP), abdominal fat percentages (AFP) and skin fat percentage (SFP).

### Primer design and PCR amplification

Three pairs of primers were designed according to the duck (*Anas platyrhynchos*) MSTN gene (GeneBank accession no: AY329600) to cover the complete region of the MSTN gene promoter. Primers' information is shown in Table 1. PCR amplification was performed in a reaction volume of 20 µl including 50 ng of genomic DNA, 25 pmol of each primer, 0.25 mM dNTPs, 1.5 mM MgCl<sub>2</sub> and 1.0 U Taq DNA polymerase. The condition for PCR was as follows: 5 min at 95°C, 35 cycles of 35 s at 94°C, 30 s at different temperature (corresponding to different primer pairs, Table 1), 45 s at 72°C; and a final 8 min extension at 72°C. The PCR products were separated on 1.5% agarose gel (Promega) including 0.5 µg/ml of ethidium bromide, and photographed under UV light.

### Genotype determination of PCR-SSCP and DNA sequencing analysis

SSCP method was used to scan mutations within the amplified regions. Aliquots of 5 µl PCR products were mixed with 5 µl denaturing solution (95% formamide deionized, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled in ice immediately for 5 min. Denatured DNA was subjected to 10% PAGE (polyacrylamide gel electrophoresis) in 1×TAE buffer and constant voltage (140 V) for 10 to 12 h at a constant temperature of 4°C, and then gels were stained with 0.1%

silver nitrate. After the polymorphism was detected, the PCR products representing different electrophoresis patterns in different breeds were subcloned to T-vector (Promega) and sequenced in both directions in ABI PRISM 377 DNA sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) by Shanghai Invitrogen Biotechnology Ltd. Co., (Shanghai, P. R. China).

### Statistical analysis

Differences in genotypic and allelic frequencies of duck MSTN gene among three lines were analyzed by Popgene32 (Yeh et al., 1997). Data of the breeds were analysed by ANOVA (SAS software GLM procedure) using the following model:  $Y_{ijklm} = \mu + \text{Line}_i + \text{Age}_j + \text{Sex}_k + \text{Marker}_l + e_{ijklm}$ , where,  $Y_{ijklm}$  is the observation of the traits,  $\mu$  is the least square mean,  $\text{Line}_i$  is the effect of line,  $\text{Age}_j$  is the effect of age,  $\text{Sex}_k$  is the effect of sex,  $\text{Marker}_l$  is the effect of marker genotype and  $e_{ijklm}$  is the residual effect.

## RESULTS AND DISCUSSION

### Identification of SNPs polymorphism and genotyping

Through PCR-SSCP and DNA sequencing method, three SNPs at -753, -658 and -235 (relative to ATG start codon) of duck MSTN gene, respectively, could be detected. These new allelic variants corresponded to the GA,GT and GC mutations at positions -753, -658 and -235 of duck MSTN gene promoter, respectively. The PCR-SSCP analysis for the three SNPs showed that in the three lines populations, both alleles of G was not predominant. The value of the GG genotype frequency was minimum in all the populations. The genotype frequencies of the MSTN gene SNPs in different populations are shown in Table 2.

### Gene-specific SNP marker association analysis with economic traits

Myostatin gene acts as a negative regulation of skeletal muscle growth and keeps the skeletal musculature within appropriate proportions. Comparison of the homology of myostatin demonstrates that myostatin has been highly

**Table 2.** Genotype frequencies of MSTN gene SNPs in three lines of Peking duck.

| SNP    | Genotype | Line                 |                      |                                      |
|--------|----------|----------------------|----------------------|--------------------------------------|
|        |          | Z <sub>2</sub> (100) | Z <sub>4</sub> (100) | Z <sub>2</sub> ×Z <sub>4</sub> (100) |
| G-753A | AA       | 0.43                 | 0.59                 | 0.38                                 |
|        | AG       | 0.57                 | 0.41                 | 0.57                                 |
|        | GG       | 0                    | 0                    | 0.05                                 |
| G-658T | GG       | 0.22                 | 0.24                 | 0.16                                 |
|        | GT       | 0.45                 | 0.48                 | 0.41                                 |
|        | TT       | 0.33                 | 0.28                 | 0.43                                 |
| G-235C | CC       | 0.48                 | 0.45                 | 0.66                                 |
|        | GC       | 0.34                 | 0.50                 | 0.13                                 |
|        | GG       | 0.18                 | 0.05                 | 0.21                                 |

**Table 3.** Association of the MSTN genotypes and growth and carcass traits in different populations.

| SNP    | Genotype | N   | Traits(LSMEAN ± SE) <sup>a</sup> |              |                          |            |                         |            |
|--------|----------|-----|----------------------------------|--------------|--------------------------|------------|-------------------------|------------|
|        |          |     | WW (g)                           | EW (g)       | BMP (%)                  | LMP (%)    | AFP (%)                 | SFP (%)    |
| G-753A | AA       | 140 | 2652.64±14.22                    | 1618.2±12.59 | 12.49 <sup>a</sup> ±0.05 | 17.82±0.15 | 2.26±0.03               | 30.82±0.48 |
|        | AG       | 155 | 2611.12±16.22                    | 1590.1±13.47 | 10.97 <sup>b</sup> ±0.03 | 17.83±0.14 | 2.40±0.04               | 31.76±0.46 |
|        | GG       | 5   | 2568.76±21.79                    | 1540.1±17.41 | 10.46 <sup>b</sup> ±0.13 | 17.86±0.23 | 2.42±0.10               | 31.86±0.96 |
|        | <i>P</i> |     | 0.5613                           | 0.5821       | 0.0225                   | 0.7662     | 0.1232                  | 0.2687     |
| G-658T | GG       | 62  | 2807.27±21.66                    | 1620.3±13.44 | 10.24±0.10               | 2.46±0.04  | 2.46±0.05               | 32.79±0.56 |
|        | GT       | 134 | 2736.15±28.64                    | 1587.3±15.36 | 10.05±0.11               | 2.55±0.02  | 2.62±0.04               | 32.46±0.45 |
|        | TT       | 104 | 2672.16±25.61                    | 1538.3±11.38 | 9.65±0.08                | 2.62±0.07  | 2.55±0.04               | 33.64±0.51 |
|        | <i>P</i> |     | 0.1178                           | 0.0485       | 0.5704                   | 0.4850     | 0.7754                  | 0.6410     |
| G-235C | CC       | 159 | 3171.34±27.17                    | 1880.7±12.57 | 11.51±0.16               | 16.77±0.17 | 2.43 <sup>a</sup> ±0.03 | 29.57±0.46 |
|        | CG       | 97  | 3091.52±25.52                    | 1820.7±12.54 | 11.41±0.12               | 16.67±0.18 | 2.48 <sup>a</sup> ±0.06 | 30.16±0.66 |
|        | GG       | 44  | 3010.08±19.67                    | 1781.9±13.42 | 11.28±0.11               | 16.58±0.19 | 2.56 <sup>b</sup> ±0.02 | 30.25±0.73 |
|        | <i>P</i> |     | 0.4978                           | 0.9171       | 0.1400                   | 0.9813     | 0.0297                  | 0.1468     |

<sup>a</sup>Least squares means value ± standard errors; WW, 6-week weight; EW, eviscerated weight; BMP, breast meat percentages; LMP, leg meat percentages; AFP, abdominal fat percentages; SFP, skin fat percentage. Different letters (<sup>a</sup> and <sup>b</sup>) denote extremely significant differences between groups ( $P < 0.05$ ).

conserved during animal evolution. The predicted amino acid sequence has an overall similarity with a comparable region of Turkey (99%), domestic goose (98%), chicken (99%), human (92%) and pig (92%). Investigations of myostatin developmental expression in skeletal muscles (Shibata et al., 2003) and its function in myogenesis and adipogenesis showed that myostatin expression is related to animal growth (Lin et al., 2002; Joulia et al., 2003; Rebbapragada et al., 2003; Wagner et al., 2005). Mutations in MSTN promoter could lead to changes of the gene expression and thereby influence growth and development. Some research results showed that the lack of MSTN gene strongly affects muscle phenotype in humans and other animal species (Joulia-Ekaza and

Cabello, 2007). Piedmontese crossbreds with an inactive MSTN allele have higher birth weights and yearling weights (Casas et al., 1999). In mice, the MSTN gene knockout causes a significant increase in muscle mass through muscle cell hypertrophy and hyperplasia (Grobet et al., 2003; Whitemore et al., 2003; Tang et al., 2007; Welle et al., 2007). There is no similar research about the association analysis of the MSTN gene with growth and carcass traits in duck. In this study, the MSTN gene is considered to be one of the potential candidate genes influencing growth and carcass quality traits in duck; the gene-specific SNP markers association analysis were evaluated and shown in Table 3. The -G753A SNP marker was significantly associated with BMP.

Individuals with the AA genotype indicated significant association with higher BMP than AG and GG genotype ( $P = 0.0225$ ). The pattern of myostatin expression was closely parallel to the trend of breast muscle growth, suggesting that myostatin might play an important role in breast muscle development. It was postulated that myostatin may be a major determinant of muscle mass in breast muscle, as also shown in other species. Significant association between the G-235C locus and AFP was found. Ducks of genotype CC had significant lower AFP than those of genotype CG and CC ( $P = 0.0297$ ). No significant association, however, was detected between the marker genotypes and growth and carcass traits measured in this study (Table 3). Results from this study show that the mutations in the promoter region of the duck MSTN gene were important reasons for the variance of growth and carcass traits, opening up possibilities for duck breeding and improvement in gene-assisted selection. Therefore, the MSTN gene could act as a candidate gene for the duck breeding and industry.

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