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Myostatin Gene Polymorphism and Its Association with Production Traits in Western Azerbaijan Native Chickens

Research Article

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ABSTRACT

In the present study, the polymorphism of myostatin gene (MSTN) in native chickens of Western Azerbaijan Rearing and Breeding Institute was investigated. The blood samples were collected from eighty two randomly selected hens. Genomic DNA was extracted from blood samples and a fragment of myostatin including 599 bp in promoter and exon 1 was amplified using PCR method. Breeding values for body weight and carcass traits were predicted by univariate animal mixed model analysis, using WOMBAT software. The effects of different SSCP genotypes on breeding and phenotypic values of the studied traits were evaluated by general linear model analysis. Three different single strand conformational polymorphism (SSCP) genotypes as AA, AB and AC were identified, with frequencies of 0.244, 0.549 and 0.207, respectively. Shannon and Nei gene diversity indices and number of effective alleles in the studied population were 0.88, 0.53 and 2.2, respectively, which indicated a high diversity of the studied population. Moreover, the studied population was not in Hardy-Weinberg equilibrium. The effect of the SSCP genotypes on breeding and phenotypic values was significant only in the case of breeding value for body weight at 12 weeks of age, whereas, the AC genotype individuals, significantly ($P<0.05$) had the lowest breeding value for body weight at 12 weeks of age. Based on the results obtained, it could be concluded that the studied fragment of myostatin gene is polymorphic in native chickens of Azerbaijan and could be used for marker assisted selection.

KEY WORDS breeding value, myostatin gene, native chicken, PCR-SSCP.

INTRODUCTION

Myostatin (MSTN) is a member of the transforming growth factor-beta (TGF-beta) super family (Karim *et al*. 2000; Mc Croskery *et al*. 2003; Ye *et al*. 2007). The sequence of MSTN gene is known in human and several mammalian species, including cattle, sheep, goat, swine, monkey and horse. MSTN was first discovered in mice (Mc Pherron *et al*. 1997; Marchitelli *et al*. 2003) and was then identified in cattle as the gene responsible for double muscling (Mc Pherron and Lee, 1997; Kambadur *et al*. 1997; Grobet *et al*. 1998). MSTN is mainly expressed in muscular tissues, and several studies have reported that MSTN negatively regulates skeletal muscle growth (Thomas *et al*. 2000; Mc Croskery *et al*. 2003). Mutations in MSTN regulatory regions have been shown to be associated with abdominal fat weight, abdominal fat percentage, birth weight, and breast muscle percentage and weight in chickens (Karim *et al*. 2000). In chicken, MSTN gene is composed of three exons and two introns. The first, second and third exons are 373 bp, 374 bp and 1567 bp, respectively (Baron *et al*. 2002). Ye *et al*. (2007) studied the effects of MSTN polymorphism on mortality, growth, feed conversion efficiency, ultrasound breast depth, breast percentage, eviscerated carcass weight,

leg defects, blood oxygen level, and hen antibody titer to the infectious bursal disease virus in three elite commercial broiler chicken lines.

They identified 13 single nucleotide polymorphisms (SNPs) in the amplified regions of exons 1, 2 and 3 and both introns of MSTN gene, where 11 SNPs (MST2100, MST2109, MST2244, MST2283, MST2346, MST2373, MST2416, MST4842, MST7434, MST7435 and MST7436) were in the exons and two SNPs (MST4405 and MST4954) were in the introns. Five identified MSTN SNPs were significantly or consistently associated with body weight at day 7 and/or day 40 in at least one broiler line (Ye *et al*. 2007). Baron *et al*. (2002) identified seven SNPs and one deletion in exon 2 of the MSTN gene in broiler and layer chicken lines. Zhiliang *et al*. (2004) identified three SNPs in the 5' regulatory region and two SNPs in the 3' regulatory region in the chicken, and these differed in allele frequencies between breeds. They found that in a F2 generation from a cross of broiler and silky chickens, homozygous genotypes AA and BB at a locus in the 5' regulatory region have a higher abdominal fat weight and abdominal fat percentage than AB genotype (Zhiliang *et al*. 2004).

The objective of the present study was investigation of allelic variation and heterozygosity indices in a region of MSTN gene and association of the identified genotypes with production traits in Iranian western Azerbaijan native chickens.

MATERIALS AND METHODS

A total number of 82 hens were randomly selected from the population of Native Chicken Breeding Centre of Western Azerbaijan. Blood samples were collected from brachial vein and stored in EDTA contained tubes. All blood samples were kept at -20 ˚C until DNA extraction. At 10 weeks of age, the chickens were slaughtered and carcass, breast muscle, leg muscle and abdominal fat weights were recorded.

DNA extraction and PCR

Genomic DNA was extracted from whole blood samples according to the procedure described by Aljanabi and Martines (1997). A fragment of 599 bp, in promoter region and exon 1 of myostatin was amplified using 300 ng extracted genomic DNA in a final volume of 25 µl containing 10X PCR buffer, 2 m*M* $MgCl_2$, 25 µ*M* dNTP mixture, 1 U *Taq* polymerase, 25 µ*M* of each primer according to Ye *et al*. (2007) as follows:

Forward: 5´-AACCAATCGTCGGTTTTGAC-3´
Reverse: 5´-CGAAAGCAGCAGGGTTGTTA-3´

Reactions were run on a Mastercycler Gradient 5331 thermal cycler (Eppendorf and Germany) under the following thermal conditions: Initial denaturation at 94 ˚C for 5 min, followed by 35 cycles consisting of denaturation at 94 ˚C for 45 s, annealing at 57 ˚C for 45 s, extension at 72 ˚C for 50 s and a final extension at 72 ˚C for 2 min.

SSCP analysis

The SSCP technique was used to allow sequence variants to be detected from migration shifts in PCR amplified fragments of the gene (Orita *et al*. 1989a; Orita *et al*. 1989b). Five µL of each PCR product was mixed with 10 µL of denaturing loading buffer (0.05% xylene cyanole, 0.05% bromophenol blue, 5.5 m*M* EDTA, with pH 8.0, in formamide), denatured at 95 ˚C for 5 min, and snap-chilled in ice for at least 5 min. Then the samples were loaded onto 8% poly acrylamide gel and were run using 0.5ẊTBE (Barroso *et al*. 1998).

The bands were visualized by silver-staining according to the procedure described by Bassam *et al*. (1991). Allelic and genotype frequencies, heterozygosity, G and Chi tests, Shannon and Nei indices of diversity, and number of effective alleles were estimated using POPGENE software (Yeh *et al*. 1997).

Prediction of breeding values for the studied traits

Breeding values for the studied traits were predicted using all available data for body weight records, including 23828 records for body weights at day one and at eight weeks of age (BW1 and BW8, respectively), and 23319 records for the body weight at 12 weeks of age (BW12). The data used for prediction of breeding values for carcass traits were the records measured on the premeditated birds, which included 82 records of abdominal fat percentage (AFP), breast muscle percentage (BMP), leg muscle percentage (LMP), dressing percentage (DP), age at sexual maturity (ASM) and weight at sexual maturity (WSM). The breeding values were predicted by restricted maximum likelihood analysis of univariate animal mixed models, using WOMBAT software (Meyer, 2009). The model used was:

$$y = Xb + Za + e$$

Where:
y: is the vector of observed records.
b: is the vector of fixed effects, including sex (male and female) and generation-hatch number (1-20) for body weight traits and hatch number (1-4) for carcass traits.
a: is the vector of additive genetic random effects.
X and Z: are incidence matrices and e is the vector of random residual effects.

The association of different genotypes with phenotypic and breeding values of the studied traits was evaluated by linear model analysis, using SAS software (SAS, 2004). The model used for this analysis was:

$$y_{ij} = \mu + P_i + e_{ij}$$

Where:

y_{ij}: is observed phenotypic value or predicted breeding value for j^{th} individual with i^{th} SSCP pattern.

μ: is the overall mean.

P_i: is the effect of the i^{th} SSCP pattern.

e_{ij}: is residual effects.

RESULTS AND DISCUSSION

Allelic variation and genotype frequencies

PCR-SSCP analysis of the amplified fragment of MSTN gene in the studied population showed three different SSCP genotypes as AA, AB and AC (Figure 1).

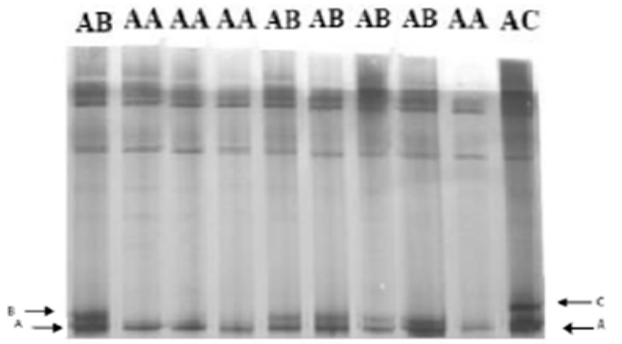

Figure 1 The PCR-SSCP patterns observed for MSTN gene and identified genotypes

Three alleles of A, B and C were observed, with allelic frequencies of 0.622, 0.274 and 0.104, respectively. Three different genotypes as AA, AB and AC with frequencies of 0.244, 0.549 and 0.207 respectively, were observed. However, regarding the observed homozygosity and heterozygosity, the number of effective alleles was 2.2.

Nei's gene diversity was 0.53 and Shannon's index of diversity was 0.88. This shows a high diversity of MSTN in the studied population.

Chi-square and G square tests based on observed and expected frequencies of different genotypic variants of MSTN gene showed a significant deviation from Hardy-Weinberg equilibrium.

Based on the obtained results, it was concluded that the MSTN gene had a high degree of polymorphism and could be considered as a candidate gene for the marker assisted selection in poultry.

Association of MSTN polymorphism and the studied traits

The effects of the SSCP patterns on phenotypic and breeding values of the studied traits are presented in Tables 1 and 2, respectively. The SSCP patterns did not have any significant effect on phenotypic values of different traits, meaning that the average phenotypic values of different SSCP patterns were similar (Table 1). The breeding value of the body weight at 12 weeks of age was significantly affected by SSCP pattern (P=0.0086). The average breeding values of AA and AB individuals for body weight at 12 weeks of age were significantly higher than the AC pattern (Table 2). The estimated breeding values for other traits were not significantly affected by SSCP patterns (Table 2). MSTN has some features in common to other members of the transforming growth factor β (TGF-β) super family of genes (Mc Pherron and Lee, 1997). A high level of polymorphism in MSTN gene sequences has been detected in different chicken lines (Gu *et al*. 2002; Zhang *et al*. 2011). Growth rate is very important in poultry industry because of its high economic value.

For that reason, it has been improved dramatically in the past decades through different methods, such as mass selection. The growth is a complex process that involves an increase in body mass and differentiation and maturation of many tissues, especially for skeletal muscles. Therefore, a number of complications (such as reduced reproductive performance, increased carcass fat, skeletal abnormalities and ascites) have been arisen with intensive mass selection for the high growth rate (Zhiliang *et al*. 2004).

In the present study, the chicken MSTN gene was chosen as a candidate gene to evaluate single strand conformational polymorphism (SSCP) with the aim to investigate the genetic association of MSTN SSCP with growth traits. The results showed that the SSCP was not associated with skeletal muscle growth and fat deposition in the chickens. However, a significant relationship was found between the SSCP patterns and breeding values at 12 months of age. These findings suggest that MSTN could be used as a genetic marker in poultry breeding programs for selecting chicken.

Ye *et al*. (2007) reported the associations of MSTN gene polymorphisms with performance and mortality traits in broiler chickens.

They found that MSTN SNPs had significant associations with growth, mortality and blood oxygen, and hen antibody titer to infectious bursal disease virus vaccine in three commercial broiler lines.

Baron *et al*. (2002) identified seven SNPs and one deletion in exon 2 of the MSTN gene in broiler and / or layer chicken lines.

Table 1 The effect of MSTN genotype on phenotypic values of the studied traits

Traits \ genotypes	AA (N=20)	AB (N=45)	AC (N=17)	SEM	P-value
BW1	42.49±4.10	63.41±3.51	42.07±2.35	0.725	0.6445
BW8	737.0±78.3	737.5±126.9	758.2±74.3	0.776	0.7767
BW12	1758.7±116.2	1795.7±150.6	1735.3±54.7	29.13	0.2779
AFP	3.12±2.05	3.86±2.28	4.11±2.25	0.458	0.3480
BMP	24.51±1.55	24.43±1.84	24.48±1.33	0.340	0.9825
LMP	13.91±1.18	13.96±22.1	13.75±0.79	0.230	0.7996
DP	66.81±8.28	66.72±6.09	66.39±8.03	1.57	0.9825
ASM	181.3±17.5	174.4±13.4	178.3±21.9	3.71	0.3082
WSM	2321.1±172.7	2372.0±157.1	2344.6±120.6	35.63	0.5031

BW1, BW8 and BW12: body weights at 1 day, 8 and 12 weeks of age, respectively; AFP: abdominal fat percentage; BMP: breast muscle percentage; LMP: leg muscles percentage; DP: dressing percentage; ASM: age at sexual maturity and WSM: weight at sexual maturity.
SEM: standard error of the means.
AA, AB and AC: observed genotypes.

Table 2 The effect of MSTN genotype on estimated breeding values for the studied traits

Traits \ genotypes	AA (N=20)	AB (N=45)	AC (N=17)	SEM	P-value
BW1	-0.0756±2.570	-0.5296±1.797	-0.001±1.509	0.4089	0.5308
BW8	-7.53±24.53	-3.78±33.12	-18.12±23.90	6.17	0.2394
BW12	2.30^{a}±58.08	-5.32^{a}±58.88	-53.42^{b}±49.24	12.36	0.0086
AFP	-0.00001±0.00002	0.00000±0.00003	0.00001±0.00002	0.00001	0.2661
BMP	-0.00000±0.00007	0.00003±0.00009	-0.00001±0.00008	0.00002	0.9942
LMP	-0.00001±0.00008	0.00000±0.00009	-0.00003±0.00006	0.00002	0.3964
DP	0.3096±6.2150	0.0976±5.5430	0.7145±6.1450	1.2190	0.9330
ASM	0.4011±1.3930	-0.1172±1.2200	-0.0002±1.7360	0.3130	0.4129
WSM	0.931±4.380	0.300±4.390	-0.774±3.308	0.965	0.5120

BW1, BW8 and BW12: body weights at 1 day, 8 and 12 weeks of age, respectively; AFP: abdominal fat percentage; BMP: breast muscle percentage; LMP: leg muscles percentage; DP: dressing percentage; ASM: age at sexual maturity and WSM: weight at sexual maturity.
SEM: standard error of the means.
AA, AB and AC: observed genotypes.

Gu *et al.* (2002) identified three SNPs in the 5′-regulatory region and two SNPs in the 3'-regulatory region in the chicken, and these differed in allele frequencies between breeds. They found homozygous genotypes AA and BB at a locus in the 5′-regulatory region to be associated with higher abdominal fat weight and abdominal fat percentage than AB in the F2 chickens from a cross of broiler and Silky chickens.

In the present investigation, the SSCP patterns of the studied fragment of myostatin did not have appreciable effects on most of the studied traits. A bigger sample size could increase the power of the test and the probability to find a significant effect in other studies to be conducted in the future.

CONCLUSION

In conclusion, the results showed that the MSTN gene had a high degree of polymorphism. The effect of the SSCP genotypes on breeding and phenotypic values was significant only in relation to breeding value for body weight at 12 weeks of age, where the AC individuals had the lowest breeding value for body weight at 12 weeks of age ($P<0.05$). The obtained results indicate that the fragment of myostatin gene evaluated in the present study is polymorphic in native chickens of Azerbaijan and can be noticed for the marker assisted selection, especially to improve the body weight at 12 weeks of age.

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