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Haplotypes in the coding region of myostatin gene in broiler chicken

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ABSTRACT

A study was designed to explore haplotypes of myostatin gene in control broiler chicken. The single stranded-conformation polymorphism technique was used to find out variants of exon 1, 2 and 3 of myostatin gene and all the variants were sequenced. Total 7 haplotypes were observed in the population where h1 haplotype was the most predominant one. Consequently, 6 haplotype combinations were found in the population of which h1h5 haplogroup was the most frequent one while h1h3 haplogroup was the least frequent one. The sex-wise differences of distribution of haplotypes as well as haplogroups were found as nonsignificant. The broiler population did not follow the Hardy-Weinberg equilibrium for this locus. The differences of nucleotides at several locations were found in the haplotypes. Different demographic parameters were also studied with respect to the haplotype distribution in the population.

Key words: Broiler chicken, Coding region, Haplotype, Myostatin gene

Myostatin, a member of transforming growth factor-β super-family is one of the most important factors directly involved in inhibiting muscular growth in chicken. It is expressed in the myotome of developing somites and myogenic lineage throughout development of embryonic stages and in adult animals (Lee and McPherron 2001). Mutation in the gene causes enormous effect on muscular development in vertebrates. Mice with a deleted myostatin gene made the animal approximately twice in size as those of wild type mice on account of muscle fibre hyperplasia and hypertrophy (McPherron *et al.* 1997). Mutated myostatin gene showed double muscling in cattle with drastic increase in skeletal muscle mass (Grobet *et al.* 1998). Thus, it was immense importance to identify the myostatin haplotypes in control broiler chicken.

MATERIALS AND METHODS

Birds: The study was conducted in control broiler chicken maintained at the Institute farm, Rajendranagar, Hyderabad. These birds are unselected line and are mated randomly for regeneration. The birds are kept in the brooder house till the age of 6 weeks and then shifted to the grower house. Finally, they are housed in the cages at the age of 20 weeks. Birds (428) of control broiler line were taken randomly for the present study.

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Sample collection and extraction: Blood samples were collected aseptically from the wing veins of the birds with 20 µl of 0.5M EDTA anticoagulant and transported to the laboratory. Genomic DNA was isolated from blood following the protocol of Sambrook and Russel (2001) with slight modifications.

PCR-SSCP: Myostatin gene consists of 3 exons and all the three exons were considered for this study. Three pairs of primers were designed from available chicken myostatin sequence at NCBI (Accession number AF346599) with DNASTAR software (Lasergene Inc.). The primer pairs used for amplification were–MYTmF: 5'-ATGCAAAAGCTAGCAGTCTATG-3' and MYTE1R: 5'-ACTCCGTAGGCATTGTGATAAT-3' (Exon 1); MYTE2F: 5'-CTGATTTTCTTGTACAAATGGAG-3' and MYTE2R: 5'- CAATCCATCTTCACCCGGTC-3' (Exon 2); MYTE3F: 5'-AACCCATTTTTAGAGGTCAGAG-3' and MYTmR: 5'-TCATGAGCACCCGCAACGAT-3' (Exon 3). The PCR reaction included 50 µg of DNA template,10 ng of each primer, 1.5 mM of $MgCl_2$, 100 µM of each dNTP, 1X assay buffer and 0.25 U of *Taq* DNA polymerase (MBI Fermentas). The PCR programme was initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C (For exon 1 and 2) and 58°C (For exon 3) and extension at 72°C for 30 sec with a final extension at 72°C for 10 min.

SSCP: A 12% native PAGE (50: 1, acrylamide and bis-acrylamide) with 5% glycerol was prepared for SSCP study. The PCR products were electrophoresed at 200 V for 8 h

and stained with silver nitrate to visualize banding patterns of the fragments (Vohra *et al.* 2006).

Sequencing: Sequencing of all the variants of 3 exons was done at Xcelaris lab by automated sequencer (ABI prism) using Sanger's dideoxy chain termination method. The same pairs of primers, which were used for genotyping, were used for sequencing of the variants.

Analysis: The sequence analysis of all the variants was performed by DNASTAR software. Sequences of the variants of 3 exons in each individual were coupled to develop haplotype of myostatin gene for each individual. The sequences of the haplotypes were aligned for elucidating nucleotide differences among them. The frequency of haplotype as well as haplotype combinations were calculated by following gene counting method (Falconer and Mackay 1996). The sex-wise differences of frequency distribution of haplotypes were compared by Student's t test. Chi-square and likelihood ratio test was performed to test the Hardy-Weinberg equilibrium in the populations.

RESULTS AND DISCUSSION

SSCP variants: The size of the exon 1 was 373 bp while the size of the exon 2 and 3 were 374 and 381 bp respectively. Two variants (12 and 13) were observed in exon 1 while in exon 2, two variants, namely, 11 and 12 were observed. In exon 3, three variants, namely, 11, 12 and 13 were observed (Fig. 1). These variants were sequenced and further used for preparation of haplotypes.

Haplotypes: All the variants of three exons were sequenced and aligned for establishing haplotypes and their combinations. The sequences of variants were submitted to the gene bank and the accession numbers were obtained as GU181321, GU181322, GU181323, GU181324, GU181325, GU181326, GU181327 and GU181328. Consequently, 8 haplotypes were observed in the population. These haplotypes showed presence of 7 haplotype combinations in the broiler line. The highest frequency was observed in h1 haplotype (0.50) while the lowest frequency was found in h2, h3 and h7 (0.02). The frequencies of other haplotypes were 0.09 for h4, 0.20 for h5 and 0.15 for h6. The haplotype frequencies in male birds were 0.50, 0.02, 0.02, 0.07, 0.21, 0.15 and 0.03 in h1, h2, h3, h4, h5, h6 and h7 respectively. The Student's t test revealed non-significant differences of distribution of haplotype frequencies between males and females. The highest frequency of haplotype combination was observed in h1h5 group (0.40) while the lowest frequency was found in h1h3 group (0.03). The frequencies in other haplogroups were 0.05, 0.17, 0.30 and 0.04 in h1h2, h1h4, h1h6 and h1h7 respectively. The sex-wise differences of the frequencies of the haplogroups were nonsignificant.

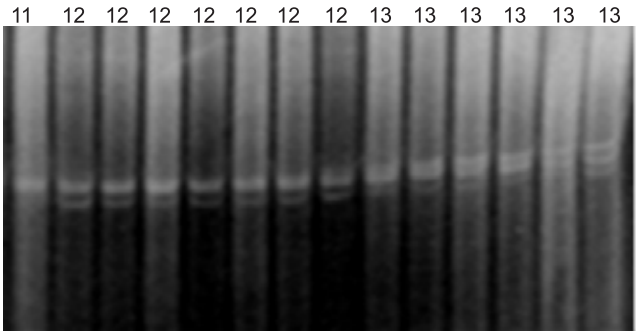

Fig. 1. SSCP variants of exon 3 of myostatin gene in control broiler chicken.

Sequence divergence: The sequences of haplotypes were explored and compared. The changes of nucleotides among haplotypes have been delineated in Table 1. Alignment of haplotypes revealed nucleotide substitution in 11 places of which first 5 positions showed silent mutations and remaining 6 substitutions showed non-synonymous type of mutation. The silent mutations in h1, h2, h3, h4, h5, h6 and h7 haplotypes were 154c/a/a/a/a/a/a; 195c/c/c/c/c/c/g; 234g/a/a/a/a/a/a and 324c/t/t/t/t/t/t respectively. The non-synonymous mutation found in different locations were 478a(Methionine)/a(Methionine)/a(Methionine)/t(Leucine)/t(Leucine)/t(Leucine)/t(Leucine); 908g(Arginine)/a(Lysine)/g(Arginine)/g(Arginine)/a(Lysine)/g(Arginine)/a(Lysine); 998g(Arginine)/a(Lysine)/g(Arginine)/g(Arginine)a(Lysine)/g(Arginine)/a(Lysine); 1006 g(Alanine)/g(Alanine)/c(Proline)/g(Alanine)/g(Alanine)/c(Proline)/g(Alanine); 1069g (Glutamate)/g(Glutamate)/a(Lysine)/g(Glutamate)/g(Glutamate)/a(Lysine)/g(Glutamate) and 1120g(Glycine)/g(Glycine)/t(Cysteine)/g(Glycine)/g(Glycine)/t(Cysteine)/g(Glycine) in h1, h2, h3, h4, h5, h6 and h7 haplotypes respectively. The percentage of transitional mutation was 40 while transversional mutations were 60 in the haplotypes.

Demographic studies: The chi-square test revealed that the

Table 1. Nucleotide variabilities at different locations of the haplotypes

Haplotype	…	154	…	234	…	324	…	477	478	…	908	…	998	…	1006	…	1069	…	1120	…
h1	…	C	…	G	…	C	…	T	A	…	G	…	G	…	G	…	G	…	G	…
h2	…	A	…	A	…	T	…	T	A	…	A	…	A	…	G	…	G	…	G	…
h3	…	A	…	A	…	T	…	T	A	…	G	…	G	…	C	…	A	…	T	…
h4	…	A	…	A	…	T	…	C	T	…	G	…	G	…	G	…	G	…	G	…
h5	…	A	…	A	…	T	…	C	T	…	A	…	A	…	G	…	G	…	G	…
h6	…	A	…	A	…	T	…	C	T	…	G	…	G	…	C	…	A	…	T	…
h7	…	A	…	A	…	T	…	C	T	…	A	…	A	…	G	…	G	…	G	…

control broiler population did not follow Hardy-Weinberg equilibrium. The likelihood ratio test also concluded similar pattern of allelic distribution in both the population. In this population, the number of polymorphic alleles observed was 8 while the effective number of polymorphic alleles was 3.15. The Shanon's information index in this population was 1.46. The observed homozygosity in the population was nil while heterozygosity was 1.0. The expected homozygosity was calculated as 0.31 while the expected heterozygosity in the population was 0.69. The Nei's expected heterozygosity was also predicted as 0.69. The Wright's fixation index revealed heterozygote excess for all the haplotypes and the magnitudes were estimated as 1.00 for h1, 0.02 for h2, 0.01 for h3, 0.09 for h4, 0.24 for h5, 0.17 for h6 and 0.02 for h7 haplotypes. Thus, our control broiler population was more heterozygotic which could be due to possible random mating followed constantly in the every generation in the population. In fact, the results indicate that the heterozygotes were providing better fitness in the population. The Ewens-Watterson test for neutrality revealed significant contribution of natural selection in the population. The mean test statistics for neutrality was 0.39±0.02, which falls in the range of 0.20 to 0.73.

In conclusion, it may be stated that the control broiler population was highly polymorphic for myostatin gene revealing the existence of seven haplotypes with non-significant differences of haplotype distribution between two sexes.

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