Nucleotide variability in partial promoter of IGF-1 gene and its association with body weight in fast growing chicken

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ABSTRACT

Present study was carried out to unravel the variability in the nucleotide sequence of the promoter region of the IGF-1 gene and to delineate the association of polymorphism with body weight, in fast growing chicken. It was observed that the IGF-1 was monomorphic and being monomorphic, no association was observed with body weights in fast growing chicken

Keywords: Insulin Like Growth Factor, Polymorphism, Body Weight, Promoter

Growth of a whole animal, or its organs, or tissues, is clearly under a coordinated and integrated control system, of which there is limited understanding (Conlon and Raff, 1999). Quest for unraveling the genetic mechanism, controlling the growth of the muscle has resulted into development of several factors, regulating the muscles growth. One of the most important muscle growths limiting factors is Insulin like Growth Factor-1 (IGF-1) (Arnold *et al*., 2001). Chicken insulin-like growth factor 1 (IGF-1) is a polypeptide hormone structurally related to insulin with multifunctional metabolic activities that exerts its function by binding to specific type I transmembrane receptor (Zhou *et al*., 1995). IGF-I stimulates glucose uptake, amino acid uptake and protein synthesis, and inhibits protein degradation by satellite cell derived myotubes (Duclos *et al*., 1993; McMurtry, 1998). The IGF-1 gene plays important roles in growth of multiple tissues, including muscle cells (Zapf and

Froesch, 1999). The IGF-1 stimulates the proliferation, differentiation and metabolism of myogenic cells (Florini *et al*., 1996).

The chicken IGF-1 gene encompasses 50 kb and maps on chromosome 1 (Klein *et al*., 1996). It comprises of 4 exons and three introns (Kajimoto and Rotwein, 1991,). IGF-1 gene is transcribed and processed into 1.9- and 2.6-kb mRNAs. This structural organization is also found in rat and human IGF-I genes. IGF-1 gene has been reported to be associated with several economic traits of the poultry but most of the work has been concentrated on variation in coding and intron regions. Negligible work has been done on the transcriptional regulation of the IGF-1 gene. This work was carried out, to study the variability in the nucleotide sequence of the promoter region of the IGF-1 gene and to delineate the association of SNP/s in the promoter region of IGF-1 with body weight in fast growing chicken.

MATERIALS AND METHODS

Experimental birds

A total of 206 birds, of fast growing chicken line control broiler (CB), raised at Project Directorate on Poultry (PDP) farm, Hyderabad were included in the present experiment. CB has been bred randomly without practicing selection and is extensively used for estimation of phenotypic and genetic effects in the selected line due to application of selection for a specific trait. All the birds were reared on deep litter system in the same shed under intensive management of farming, providing same managemental regime with *ad-lib* feeding and watering. All the birds were hatched at the same time and housed all along in the same shed. Cooling facilities were provided during summer season through water sprinkling on the roof and proper lighting were arranged in the shed so that birds get congenial environment for performing in optimum potential.

Collection of blood samples

0.5-1 ml of blood was collected from the wing vein of each bird in a sterile polypropylene 2ml centrifuge tubes containing 2.7% EDTA (60-70µl/1 ml of blood) as an anti-coagulant under sterile conditions. After collection of blood, the tubes were tightly capped and shaken gently to facilitate thorough mixing of blood with the anti-coagulant. The vials were then kept immediately in icebox containing ice and gel cool packs and were transported to the laboratory immediately. After reaching the laboratory, samples were kept in deep freeze at –20°C till the isolation of DNA.

Extraction of Genomic DNA

Genomic DNA was isolated from the blood samples by Phenol: chloroform extraction method as described by Sambrook and Russell (2001) with slight modification.

Quality of genomic DNA

Horizontal submarine 0.8% agarose (w/v) gel electrophoresis was performed to check the quality of genomic DNA. On completion of electrophoresis, the gel was visualized under UV transilluminator and documented by gel documentation system. DNA samples showing intact band and devoid of smearing were used for further analysis.

The purity of genomic DNA was checked by using UV-Spectrophotometry. Genomic DNA samples lying in the ranges of OD ratio (260:280) between 1.7 to 1.9 were considered good and were used for further study (PCR amplification).

PCR amplification of IGF-1 promoter

A forward (5'-3') TCAAGAGAAGCCCTTCAAGC) and reverse (5'-3') GTG CACATTTCATTCATGCAG primers, for amplification of IGF-1 gene promoter was designed using DNAstar laser gene software. A PCR reaction of 25ml was set up using 10 x PCR assay buffer, dNTPs mix (2.5 mM), primers (forward and reverse) 40ng each, $MgCl_2$ (25 mM), Taq DNA polymerase 0.3 units (Fermentas USA) and genomic DNA (80-100 ng) of individual bird. IGF-1 gene was amplified using initial denaturation at 95^0C for 5 min, followed by 35 cycles of denaturation at 95^0C for 30 sec, annealing at 58.8 ^{0}C for 45 sec and extension at 72 ^{0}C for 1 min. The final extension used in this amplification was at 72 ^{0}C for 10 min.

Nucleotide variability study

The SSCP was carried out on 12% native PAGE (50:1, acrylamide and bis-acrylamide) with 5% glycerol. A volume of 3 µl PCR product mixed with 15 µl formamide dye [95% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue, 0.5M ethylene diamine tetra-acetic acid (EDTA)] was denatured at 95^0C for 5 minutes followed by snap cooling on ice for 15 min. Then, the product was loaded in the gel and electrophoresis was performed at 4^0C for 12 hrs at 200 V. After electrophoresis was over, the gel was stained with silver nitrate to visualize banding patterns of the fragments (Vohra *et al*., 2006; Bhattacharya *et al*, 2013).

PCR products amplified in this study using HotStar HiFidelity DNA Polymerase (MBI Farmentas) were sequenced using fragment-specific primers from both ends by the automated dye–terminator cycle sequencing method in ABI PRIZM 377 DNA sequencer (Perkin-Elmer).

RESULTS AND DISCUSSION

Polymorphism of IGF-1 partial promoter

A PCR product of 348bp of IGF-1 promoter was amplified in fast growing chicken. PCR-SSCP results showed only one SSCP pattern of IGF-1 promoter in fast growing chicken indicating that partial promoter of IGF-1 was monomorphic in fast growing chicken (Fig1). This observation was is in contrast to one study which reported polymorphism in promoter as well as 5' UTR of IGF-1 gene (Hossein and Mohsen,

2011). Bhattachrya *et al.* (2013) reported 16 haplotypes in the coding regions of the IGF-1 in chicken There was no report of study of polymorphism in matching region of IGF-1 in chicken that may be why no polymorphism was reported IGF-

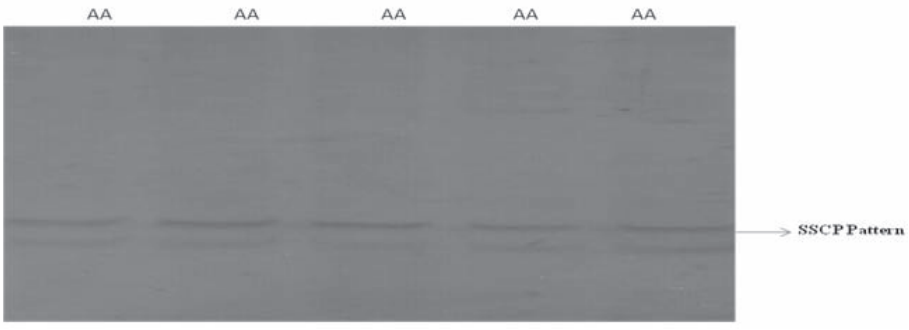

Fig. 1: SSCP Patterns of IGF1PF1 in CB

1 promoter region in present study.

Nucleotide variability of IGF-1 promoter

PCR products amplified in this study were sequenced using fragment-specific primers from both ends by the automated dye–terminator cycle sequencing method. Sequenced nucleotides were compared with the original sequence from which primer was designed, present study revealed transition mutation at 65 A>G. It was observed that this mutation was common to all the individuals of the population that may be reason of whole population being monomorphic for IGF-1 promoter fragment (Fig2). This is in contrast to earlier findings which reported polymorphisms in several

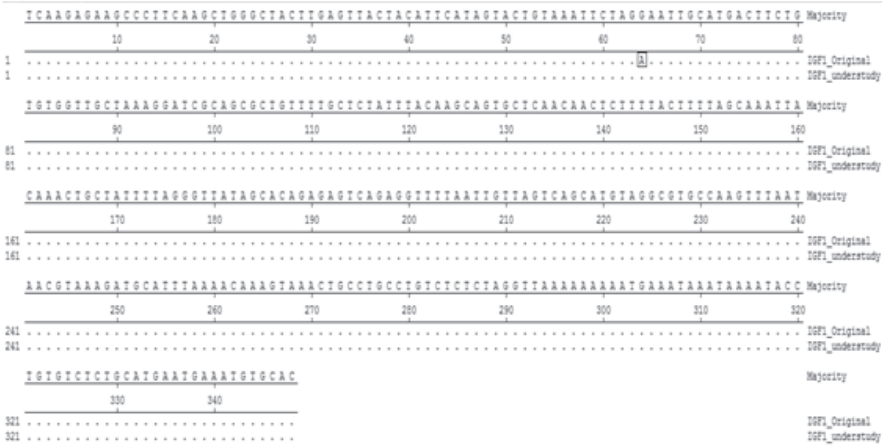

Fig. 2: Nucleotide variability of IGF-1 in CB compared with original sequence (sequence from which primer was designed for present study)

regions of promoter as well as coding regions of IGF-1 gene (Amills *et al*. 2003; Zhou *et al*. 1995; Li *et al*., 2010;)

Association of polymorphism and body weight

IGF-1 promoter was observed to be monomorphic therefore; no association was reported with the body weight. This observation was in contrast to various studies which reported significant association of polymorphism with body weight and other economic traits (Amills *et al*. 2003; Zhou *et al*. 2005; Li *et al*., 2010; Bhattachrya *et al.* 2013)

CONCLUSION

IGF-1 promoter was found to be monomorphic and there was no association between body weight and polymorphism of IGF-1 promoter in fast growing chicken

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