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Research Article

IGF-1 Gene polymorphisms in Uttara fowl Analysed by Using PCR-RFLP Techniques

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Abstract

Chicken insulin-like factor 1 gene (IGF1) is a candidate gene for the investigation of growth, body composition, and carcass traits, and is also a positional candidate gene for growth and fat deposition in chickens. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test was performed to investigate the allele frequencies of the insulin-like growth factor 1(IGF-1) in 50 Uttara fowl. A 813 bp fragment of IGF-1 gene was amplified followed by restriction digestion with Hinf I enzymes. In this study, we observed occurrence of two types of alleles A and B and three types of genotypes AA, AB and BB for the IGF-1genes. The results indicated that the frequency of occurrence of B allele (0.69) was higher than A allele (0.31) in Uttara fowl. The findings of the present study can be used for selection of poultry breeds with superior growth performance.

Keywords: IGF-1 gene, PCR-RFLP, Uttara Fowl, Allele frequency

Introduction

Uttara fowl is the newly registered eighteenth breed of the poultry in the country and first registered breed of the Uttarakhand state which is found in the Nainital and Pithoragarh districts of Uttarakhand state. These birds possess different phenotypes from the other registered breeds of the country. Uttara fowl is generally reared under backyard system and two types of populations are found in the state. One is Shank feathered found in Pithoragarh district and another having crown like structure on their head, found in Nainital district of the state. Uttara fowls are hardy, good foragers and resistant to many diseases as well as thrive well under the harsh climate of the state. This is slow growing breed and 625.40±11.80 kg body wt. at 12 weeks of age and Age at first egg is 183.96±0.99 days while egg number is 106 annually. Growth and carcass traits are economically important traits in livestock breeds and are controlled by multiple genes (Singh et al. 2014). Genomic selection is the state of the art today and MAS could be implemented in the genomic selection. The studies of genetic marker applied to animal breeding and production is focused mainly on analyses of mutations located within candidate genes of quantitative traits (Singh et al. 2015). SNP analysis is a well-established tool for the identification of genes associated with traits of economic interest in livestock populations (He et al.2009; Lai et al. 2009; Singh et al. 2013). The insulin-like growth factor (IGF) system controls the prenatal and postnatal growth development, is composed of IGF-1, IGF-2, IGF receptors and IGF binding protein. Growth is a

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complex process that involves the regulated coordination of a wide diversity of neuroendocrine pathways. Leptin, IGF, and their receptors play essential roles in this biological process by modulating intermediary metabolism and cell proliferation. Chicken IGF-1 and IGF-2, are polypeptidic hormones structurally associated with insulin with multiple metabolic and anabolic functions (Zhou et al. 1995). The IGF-1 gene may play important roles in growth of multiple tissues, including muscle cells (myocyte differentiation cell multiplication), cartilage (chondrocyte colony formation, alkaline phosphatase activity), and bone (osteoblast division and proliferation) (Zapf and Froesch 1999) and it is important component of somatotropic axis, Insulin like growth factor-1 (IGF-1) is believed to stimulate anabolic process such as cell proliferation, skeletal growth, and protein synthesis (Sharma et al. 2013). Several studies have reported high concentrations of IGF-1in fast-growing broiler strains than in slow growing strains (McGuinness and Cogburn 1990; Beccavin et al. 2001).

Variations in the genes of somatotropic axis could function as candidates for the evaluation of their effects on animal growth and development traits. In humans, mutations at important regulatory sites of the IGF1R gene were associated with growth. Such mutations resulted in the failure of processing of proIGF1R to mature IGF1R and caused dysfunction and short stature of IGFR. These variations affected partly the expression and physiological functions of the IGF1R gene, and subsequently affected growth. However, few studies on somatotropic axis genes (IGF-1, IGF-2, GH and GHR) with growth and carcass traits were reported in chickens (Li et al. 2008).

Uttara fowl is a unique and newly explored strain of poultry which is found in few pockets of Nainital and Pithoragarh districts of Uttarakhand state of India (Phangchopi et al. 2014). This strain is totally different from the 17 defined breeds of domestic fowl (*Gallus domesticus*) in India. But no information is available in the literature about genetic makeup of this important genetic resource of the Uttarakhand state. IGFs stimulate growth rate in a number of animal species and are likely to contribute to genetic variations of growth potential. Therefore, the present study was planned to study genotype frequencies of IGF-1 gene in Uttara fowl.

Materials and Methods

Present study was carried out on 50 randomly selected Uttara fowl maintained at Instructional Poultry Farm, G. B. Pant University of agriculture and Technology, Pantnagar (Uttarakhand). About 1 ml of blood was collected from wing vein using 0.5M EDTA as an anticoagulant. DNA from the collected blood was extracted using standard protocol (Sambrook et al. 1989). Working dilutions of extracted DNA were prepared for each individual at a concentration of 50 ng/µg. PCR amplification of IGF-1 gene was performed by the gene specific primers (Forward: 5′-GAC TAT ACA GAA AGA ACC CAC-3′, Reverse: 5′-TAT CAC TCA AGT GGC TCA AGT-3)′ (Nagaraja et al. 2000). The reaction mixture consisted of 200mM of each dNTPs, 1.5 mM $MgCl_2$, 50 pmol primer, 0.5 U *Taq polymerase* (Bangalore Genei Pvt Ltd., Bangalore, India) and Taq buffer. The PCR reaction cycle was accomplished by denaturation for 95°C for 5 min then 35 cycles of 95°C for 1 min, 58°C for 45 s, and 72°C for 1 min, with a final extension step for 10 min at 72°C. The 813 bp PCR products were subsequently digested by *Hinf I* at 37°C overnight. Individual PCR-RFLP fragment sizes in each sample were determined with respect to standard DNA molecular weight markers for each gene based on the banding pattern observed under UV light. The restriction fragments were separated using 2.0% agarose gel in 1×TAE buffer at a constant current of 50 mA. The gels were stained with ethidium bromide and the fragments were visualized using a UV transilluminator.

Results and Discussion

In present study, screening of polymorphisms in Uttara fowl of Kumaun region of Uttarakhand revealed the presence of different polymorphic sites in IGF-1 gene.

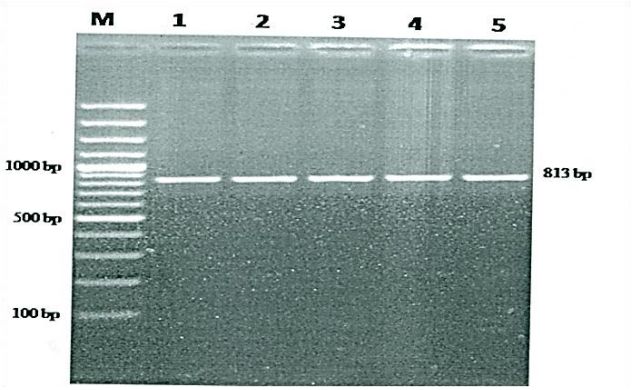

Fig 1. Amplification of PCR product, Lane M: 100bp DNA ladder, Lane 1-5: PCR product

The 813 bp amplified product was digested with *Hinf I* and polymorphism of IGF-1 gene was observed in agarose gel (Fig.1). The length of each fragment generated by *Hinf I* restriction enzyme digestion was compared with 100 bp DNA ladder. Based on the polymorphic pattern, each bird was assigned a specific genotype. The restriction pattern having 191 bp, 244 bp and 378 bp was assigned as a AA genotype, genotype BB was assigned to the pattern having 191 bp and 622 bp fragments and AB to those having 191 bp, 244bp, 378bp and 622 bp bands (Fig. 2).

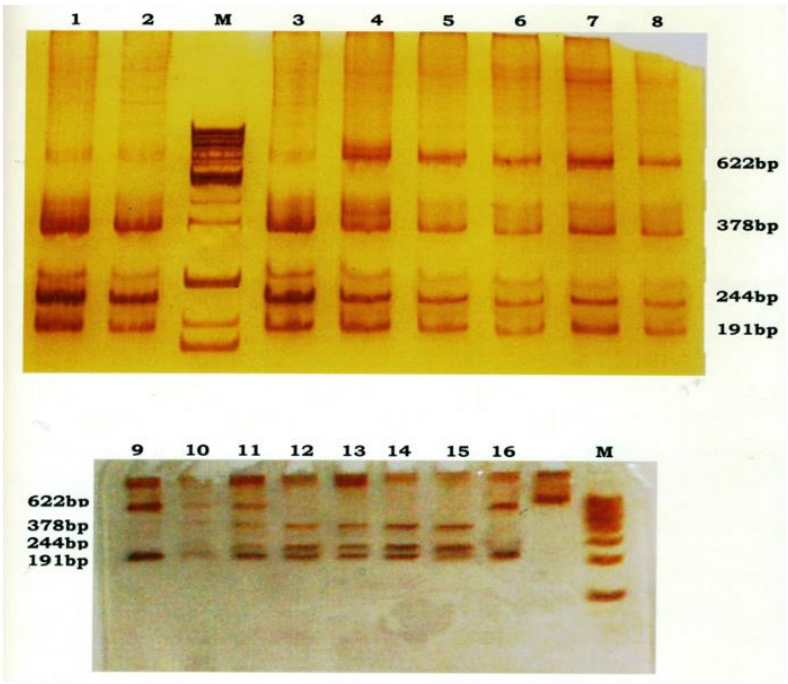

Fig 2. *Hinf I* –RFLP geneotypes of 813bp fragment of IGF-1 gene in Uttara Fowl; Lane 1-2 and 12-15: *Hinf I* digested fragmented showing 3 bands designated as AA genotype; Lane 4-8 and 10 and 11: *Hinf I* digested fragmented showing 4 bands designated as AB genotype; Lane 9 and 16: *Hinf I* digested fragmented showing 2bands designated as BB genotype; Lane M: Molecular size marker (100bp ladder)

The results of present study are similar to that observed by (Zhou et al. 2005), who performed restriction enzyme digestion with *Hinf I* and obtained fragments of 622bp and 191bp length for the 2 inbreed lines, whereas the boiler lines had fragment sizes of 378bp, 244bp and 191bp. In another study, (Li et al. 2009) also found similar observations. They found 621 bp fragment of 5'-UTR (5'-untranslated region) for IGF-1 in Wenchang chicken and the restriction enzyme digestion by *Pst I* produced fragments of 257 bp, 364 bp for the BB genotype and 257 bp, 364 bp, 621bp for the BB genotype and 621 bp (no digestion) for BB genotype. The gene and genotype frequencies have been shown in table 1 and 2. Furthermore, (Khadem et al. 2010) reported allelic frequencies of 0.39 and 0.61 for A and B allele respectively and genotype frequencies of 0.18, 0.42 and 0.40 for AA, AB and BB genotypes, respectively, for IGF-1 loci in Mazandaran native fowls. In this study, we identified two alleles (A and B) and three genotypes (AA, BB, &AB) in Uttara fowl on the basis of restriction digestion patterns. The frequencies of A and B alleles were estimated as 0.31 and 0.69, respectively and for AA, BB and AB genotypes as 0.089, 0.444 and 0.467, respectively. Similar observation has also been reported by (Gouda and Essawy 2010) who analyzed the polymorphism of IGF-I gene among Egypt chicken breeds and found its significant effect on the growth traits of the chicken. Similar results have been also reported by (Shah et al. 2012) in broiler chickens.

Conclusions

The present study on Uttara fowl will be new information to elucidate the possible role of IGF1 gene of the somatotropic-axis genes. Furthermore, IGF1 SNP may be used as a specific candidate gene for future marker assisted selection with concerned growth and production traits and ultimately this genetic information may be helpful for future conservation strategies of the new poultry germplasm.

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Conflict of interest

There is no Conflict of interest.

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