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POLYMORPHISM OF INSULIN LIKE GROWTH FACTOR-I GENE AMONG CHICKEN BREEDS IN EGYPT

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SUMMARY

Insulin like growth factor I (IGF-I) regulates growth, protein synthesis, and cell proliferation and differentiation in vertebrates. Polymorphisms of insulin-like growth factor-1 (IGF-1) gene transcript alleles of 3 different breeds of chicken (Sasso as a native breed, Arbor Acres plus and Cobb 500) were assessed. The associations of these polymorphisms with growth rate of the studied breeds were also evaluated. Total RNA were isolated from chicken livers and IGF-1 gene was amplified from each breed by RT-PCR using specific primers flanking a certain region of the gene. The amplified products were then subjected to SSCP analysis for genotype identification. Three different banding patterns were obtained for the three breeds with a unique pattern for the Cobb 500 (the fastest growing breed). In this report, we describe how SSCP analysis of RT-PCR products can be used to evaluate the transcript expression pattern of avian IGF-1 polymorphism and their effect on the growth traits of chickens.

Key words: IGF-I gene, polymorphism, chicken breeds, RT-PCR /SSCP and growth rate.

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INTRODUCTION

Some understanding of the genetic architecture of quantitative traits may be gained by systematically analyzing genetic markers in major metabolic pathways (**Li *et al.*, 2008**). Recently, mapping of some quantitative trait loci for growth were studied by many investigators (**Duclos, 2005; Stearns *et al.*, 2005 and Yan *et al.*, 2003**). In this context, the major endocrine pathway mediated by liver and other tissues that produce insulin like growth factor-I (IGF-I) have been recognized, indicating that this hormone and other growth correlated hormones together with their receptors and binding proteins, provide a complex regulatory network that coordinates a

multitude of quality traits (**Harvey and Hull, 1997**).

IGF-I is a member of polypeptide hormone family, preproinsulin, which is consisted of pro-insulin, IGF-I, IGF-II and C peptide with multiple metabolic and anabolic functions. It has a great importance during postnatal growth, and is mainly produced by the liver under the influence of growth hormone and nutritional conditions. The hepatic IGF-I is secreted into circulation, acting in an endocrinological manner on their target tissues. Most of the extrahepatic tissues produce IGF-I that function as an autocrine and/or paracrine growth stimulator. IGF-I mediates the majority of growth hormone functions. A family of cell surface receptors, insulin receptor, IGF-I receptor and IGF-II receptor mediates IGF-I biological actions (**Scanes *et al.*, 1989**).

Six exons spanning over a large region of the chromosome from 73 to 85 kilo-base constitute IGF-I gene in human and rat (**LeRoith and Roberts, 1991**). Alternative splicing pattern leads to numerous transcripts, the best known model is that of rat (**Musaro *et al.*, 2001**) where all transcripts must contain exons 3 and 4 that give the mature IGF-I peptide. Different signal peptides encoded by alternative transcription of either exon 1 or 2, the hepatic transcript comprises exon 2 while extra-hepatic one uses exon 1.

Regarding avian IGF-I gene, it is more compact, occupy about 48 kilobase of chromosome one. A simpler pattern of avian IGF-1 transcripts is existed as it appears to comprise 4 exons related to exons 1, 3, 4 and 6 of rat (**Kajimoto and Rotwein, 1991**). Mature IGF-I is spanning 210 bp that encodes a single chain polypeptide of 70 amino acids. The structure of IGF-1 gene is variable among chicken breeds, but the association of this variability with the phenotypic variation is not yet clear (**Klein *et al.*, 1996 and Amills *et al.*, 2003**). There are also lines of evidence indicating that the genetic polymorphism of IGF-I is associated with body weight and height characteristics (**Ballard *et al.*, 1990**) and making the IGF-1 gene a very suitable target for genetic manipulation (**Goddard and Boswell, 1991**).

Therefore, it is considered as an attractive candidate gene to search for DNA polymorphisms, both in humans (**Lee *et al.*, 1989**) and in domestic species (**Jones and Clemmons, 1995; Oudin *et al.*, 1998 and Tomas *et al.*, 1998**).

The objective of our study was to identify genetic polymorphisms of IGF-1 gene transcripts and to find the correlation between the genotypic polymorphisms to the phenotype of growth rate of some chicken breeds in Egypt (Sasso, Arbor Acres plus and Cobb 500). This may afford prospective

insight about possible further selective approach aiming to increase efficiency and make improvements in production performance.

MATERIALS AND METHODS

Materials

Total RNA isolation reagent (TRI reagent): RT-PCR kit and DNA ladder from Abgene®, UK.Oligonucleotide primers were synthesized as commercial order from Sigma. All other chemicals were obtained in pure grade from Sigma.

Chicken samples

Three chicken breeds with different growth-rates, Sasso as a native breed, Arbor Acres plus and Cobb 500 as fast growing chicken breeds were used in the current study. Liver samples were collected from these breeds from local farms in Egypt. The samples were kept in containers containing liquid nitrogen just after rapid evisceration. The records of the growth rates of the chicken breeds were also delivered.

RNA isolation:

Total RNA was isolated from obtained liver samples by total RNA isolation reagent (TRI reagent) from ABgene, UK according to the manufacturer's protocol. The quantity and quality of the isolated RNA were assessed by measuring the optical density spectrophotometrically at 260 and 280 nm.

Primer design:

The design of the primer that used for RT-PCR was based on the sequence of cDNA of IGF1 (Gene bank accession is M32791) of the chicken (Gallus gallus) as shown in figure 1.

The forward primer sequence is: 5'-ACT GTG TGG TGC TGA GCT GGT T-3' and the reverse one is: 5'-AGC GTG CAG ATT TAG GTG GCT T-3' to amplify 203 bp fragment that span from 402 to 604 base of cDNA (figure 1). The primers were synthesized as commercial order from Sigma.

1 gctgtttcct gtctacagtg tctgtgtaat gtagataaat gtgaggattt tctctaaatc

61 cctcttctgt ttgctaaatc tcactgtcac tgctaaaatc agagcagata gagcctgcgc

121 aatggaataa agtcctcaat attgaaatgt gacattgctc tcaacatctc acatctctct

181 ggatttcttt tttctcatca ttactgctaa caaattcatt tccagacttt gcacttttaa

241 gaagcaatgg aaaaaatcaa cagtctttca acacaattag ttaagtgctg cttttgtgat

301 ttcttgaagg tgaagatgca cactgtgtcc tacattcatt tcttctacct tggcctgtgt

361 ttgcttacct taaccagttc tgctgctgcc ggcccagaaa cactgtgtgg tgctgagctg

421 gttgatgctc ttcagttcgt atgtggagac agaggcttct acttcagtaa gcctacaggg

481 tatggatcca gcagtagacg cttacaccac aagggaatag tggatgagtg ctgcttccag

541 agttgtgacc tgaggaggct ggagatgtac tgtgctccaa

taaagccacc taaatctgca

601 cgctctgtac gtgctcagcg ccacactgat atgccaaaag cacaaaagga agtgcatttg

661 aagaatacaa gtagagggaa cacaggaaac agaaactaca gaatgtaaga tcatgccatc

721 cacaagaatg aagaatgaat gtgccatctg cagagtactt tgctgtaaat aaattatttg

781 ttaaacattg gaagact

Fig. (1): cDNA sequence of chicken insulin like growth factor-1, bold italic underlined letters denotes the position of the forward and reverse primers.

RT-PCR for avian IGF1gene:

RT-PCR technique was performed using the following reaction mix: 2x RT-PCR Master Mix, 2 µg total RNA template, IGF1 F & R primers (20 pmol/µl each), Reverse-iT™ RTase Blend (50 U/µl) and RNase-free water up to 50ul (ABgene, UK). The reaction conditions were proceeded as follows: first strand synthesis at 47°C for 30 min for one cycle, RNA, blend inactivation and initial denaturation at 94°C for 2 min for one cycle, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 1 min and extension at 72°C for 1 min. The program was terminated with a final extension step at 72°C for 7 min.

PCR products were detected by electrophoresis on 2% agarose gel containing ethedium bromide and the expected correct size were confirmed in relation to 50 bp molecular ladder (Jena Bioscience Laboratory) as a standard size marker.

Single Strand Conformational Polymorphism (SSCP) analysis:

A mixture of 5 µl of amplified RT-PCR product and 10 µl denaturing buffer (5x loading dye in formamide in ratio of 1:4) was prepared. The mixture was heated at 95°C for 7 min and then cooled on ice for 15 min before loading onto the gel. The mixture was loaded into 15% polyacrylamide gel with 20% formamide and electrophoresed in 1x TBE buffer at 150 V for 5 min, then at 80 V at room temperature until the blue dye reached the bottom of the gel **(Gouda *et al.*, 2008).** The gel was stained with ethidium bromide (1 mg/ml) for 2 min and then destained in water for 15 min. Gels were visualized under a UV trans-illuminator and photographed using a digital camera.

RESULTS

Records from local farms for the different chicken breeds clearly demonstrated that Cobb breed was the fastest growing with best feed conversion rate (FCR) among the studied breeds, whereas the Sasso breed was the slowest one as shown in table (1).

Table (1): The growth rates of the different chicken breeds

Breed	Average body weight/g at 49 day	Average Daily gain g/bird/day	Food conversion rate
Sasso	1918	39.3	1.9
ArborAcres	2553.9	51.28	2.06
Cobb500	3177.1	64.8	2.81

Amplicons of the expected sizes were obtained from RT-PCR of different avian RNA samples. These were approximately 203 bp in size as predicted from the published avian sequence (Fig. 2).

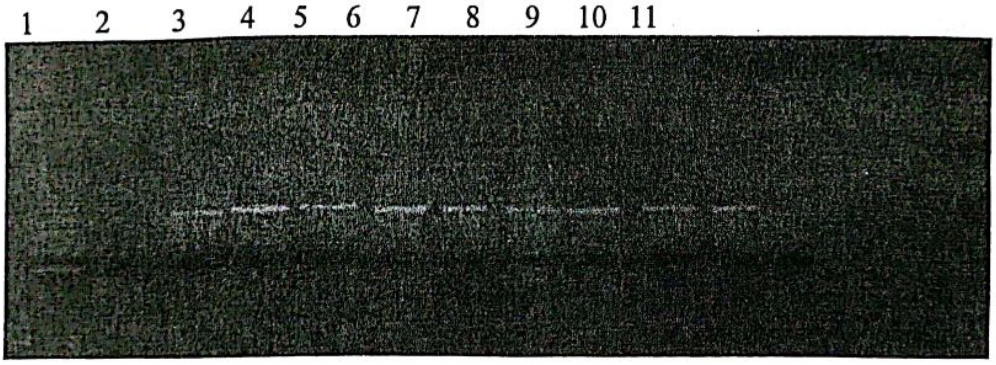

Fig. (2): RT-PCR products of avian IGF-1: Lane 1, 50 bp ladder was run as size standard, lane 2, control negative, lanes 3-5 RT-PCR products of Sasso breed, lanes 6-8, Arbor Acres plus and lanes, 9-11 Cobb 500 breed

These amplicons exhibited polymorphism upon SSCP analysis and three distinct banding patterns with a unique one for C0bb 500 chicken breed could be identified for IGF-I gene of the studied chicken breeds as shown in figure (3).

1 2 3 4 5 6 7 8 9

Fig. (3): SSCP analysis of RT-PCR products of avian IGF-1 , Lanes 1- 3, Sasso breed, lanes 4-6, Arbor Acres plus and lanes, 7- 9 Cobb 500 breed.

DISCUSSION

The study of applicant genes is one of the primary methods to determine whether specific genes are related to economic traits in farm animals (**Zhou et *al.*, 2005**). SSCP approaches can be extended to characterize and quantitate the relative contribution of specific homologous gene pairs to the mRNA pool. As it is predicted, electrophoresis of RT-PCR products on 2% agarose gel was unable to distinguish between the allelic pairs of fragments (fig. 2). The allelic sequences of fragments might be differed from one another by 1 to 6 nucleotides (**Ortf *et al.*, 1997**), but in each case the SSCP profiles were very distinct (figure 2), highlighting the sensitivity of the technique. SSCP is a simple and reliable technique, based on the assumption that changes in the nucleotide sequence of a PCR product affect its single strand conformation. Molecules differing by as little as a single base substitution should have different conformers under non-denaturing conditions and migrated differently. Given that all of the RT-PCR products are of the order of 203 bp, the migration pattern of the fragment in the polyacrylamid gels demonstrates the extent to which conformation, rather than molecular weight, is the major determinant of gel mobility. Using SSCP analysis, three non-overlapping SSCP patterns were found indicating successful development of breed-diagnostic markers across the studied breeds (fig. 3). The cobb 500 breed showed a unique SSCP pattern with four bands.

As mentioned in several studies (**Bodenes *et al.*, 1996** and **Tokue *et al.*, 1995**), it is sometimes possible to detect multiple bands for some fragments under electrophoretic conditions. Theoretically, in a SSCP gel, a maximum of four single strands for heterozygous samples could be detected. Besides, the presence of multiple bands was evident in many studies. **Orita *et al.* (1989)**, **Hayashi (1991)** and **Tokue *et al.* (1995)** supposed that occasionally, a single strand can be separated in two or more bands, although the sequence is the same. This suggests that strands with the same sequence may have different molecular

conformations, originating multiple bands under some electrophoretic conditions (**Bastos *et al.*, 2001**).

The SSCP polymorphisms we have found in this gene coding for IGF-I hint to the possibility of exploring this approach for the search of genetic markers located in these chicken breeds and produce SSCP markers for genemapping (**Slabaugh *et al.*, 1997**). Indeed, our sample is too small and our analysis limited to the gene transcript. If it is possible to specifically define haplotypes at these candidate genes that can be associated with growth traits it would be rendered available a valuable genetic resource for improvement of these chicken breeds.

The genes of the growth axis played crucial roles in the regulation of growth, development and differentiation. Important association existed between meat quality and growth and body composition, for this reason, the genes of growth axis probably affect the meat quality traits of animals (**Lei *et al.*, 2007**).

The distinctive SSCP pattern observed for Cobb 500 breed which had the highest rate of growth are in line with many studies, revealed that IGF-I polymorphisms are related to some growth traits. There were associations between IGF-1 promoter polymorphism, average daily gain (ADG) and feed efficiency found in two genetically diverse Black Penedesenca chicken strains (**Amills *et al.*, 2003**). They added also that the IGF-I gene was associated with body weight, breast weight and breast yield. Myofiber numbers and myofiber densities were related to body weight, breast weight and breast yield (**Scheuermann *et al.*, 2003 and 2004**).

Moreover, it was suggested that single nucleotide polymorphism (SNP) of IGF-I encountered by **Lie *et al.* (2007)** could affect chicken muscle fiber growth. In addition, they found that C51978309T SNP of IGF-I gene was significantly linked with the transversal area of the leg muscle between Xinghua and white Plymouth Rock chicken.

The current study thus identifies IGF-I gene as a candidate gene of quantitative trait loci (QTL) for growth, which may be used to increase growth rate or market weight in marker-assisted selection programs.

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