

Polymorphism of Insulin-Like Growth Factor I Gene among Chicken Breeds in Egypt

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The insulin-like growth factor I (IGF-I) regulates growth, protein synthesis, and cell proliferation and differentiation in vertebrates. Polymorphisms of IGF-I gene transcripts of three breeds of chicken were assessed. The associations of these polymorphisms with the growth rate of the studied breeds were also evaluated. Total RNA was isolated from chicken livers, and the IGF-I gene was amplified from each breed RNA by RT-PCR using specific primers flanking a certain region of the gene. The amplified RT-PCR products were formed to identify the transcripts and to correlate them to the phenotype of growth, by performing single stranded conformation polymorphism (SSCP) analysis for genotype identification. In this report, we describe how SSCP analysis of RT-PCR products can be used to evaluate the transcript expression pattern of avian IGF-I polymorphism, and their effect on the growth traits of chickens.

Key words: IGF-I Gene, Polymorphism, Chicken Breeds

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 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) has 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 the polypeptide hormone family, preproinsulin, which is consisted of proinsulin, IGF-I, IGF-II and C peptide with multiple metabolic and anabolic functions. It has great importance during postnatal growth and is mainly produced by the liver under the influence of growth hormone and nutritional conditions. Hepatic IGF-I is secreted into the circulation, acting in an endocrinological manner on its target tissues. Most of the extrahepatic tissues pro-

duce IGF-I that functions 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 mediate the IGF-I biological actions (Scanes *et al.*, 1989). Six exons spanning over a large region of the chromosome from 73 to 85 kb constitute the IGF-I gene in humans and rats (LeRoith and Roberts, 1991). An alternative splicing pattern leads to numerous transcripts; the best known model is that of rats (Musaro *et al.*, 2001) where all transcripts must contain exons 3 and 4 that give the mature IGF-I peptide. Different signal peptides encode by alternative transcription of either exon 1 or 2; the hepatic transcript comprises exon 2 while the extrahepatic one uses exon 1.

Regarding the avian IGF-I gene, it is more compact and occupies about 48 kb of chromosome one. A simpler pattern of avian IGF-I transcripts exists as it appears to comprise four exons related to exons 1, 3, 4 and 6 of rats (Kajimoto and Rotwein, 1991). Mature IGF-I is spanning 210 bp and encodes a single-chain polypeptide of 70 amino acids. The structure of the IGF-I gene is variable among chicken breeds, but the association of this variability with the phenotypic variation is not yet

clear (Klein *et al.*, 1996; Amills *et al.*, 2003). Different chicken breeds with variable growth rates are available now. 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) making the IGF-I 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; Tomas *et al.*, 1998).

The objective of the present study was to identify genetic polymorphisms of IGF-I gene transcripts and to find a correlation between the genotypic polymorphisms and the phenotype of growth rate of some chicken breeds in Egypt (Sasso, Arbor Acres plus, and Cobb 500). This may afford prospective insight into a possible further selective approach aiming to increase the efficiency and make improvements in the production performance.

Material and Methods

Chicken samples

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

RNA isolation

Total RNA was isolated from the liver samples by a total RNA isolation reagent (TRIR) 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 used for RT-PCR was based on the sequence of cDNA of the IGF-I (Genebank accession number M32791) of the chickens (*Gallus gallus*) as shown in Fig. 1. The forward (F) primer sequence was 5'-ACT GTG TGG TGC TGA GCT GG-3' and the reverse (R) one was 5'-AGC GTG CAG ATT TAG GTG GCT T-3' to amplify a 203-bp fragment that spans from base 402 to 604 of cDNA (Fig. 1). The primers were synthesized as commercial order by Sigma-Aldrich Chemie (Steinheim, Germany).

RT-PCR for avian IGF-I gene

The RT-PCR technique was performed using the following reaction mix: 2× RT-PCR Master Mix, 2 µg total RNA template, IGF-I F and R primers (20 pmol/µl each), Reverse-iT™ RTase Blend (50 U/µl), and RNase-free water up to 50 µl (ABgene). The reaction conditions were 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 s, annealing at 57 °C for 1 min and extension at

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1   gctgtttctt gtctacagt tctgtgtaat gtagataaat gtgaggattt tctctaaatc
61  cctcttctgt ttgtctaaatc tcactgtcac tgctaaaatc agagcagata ggcctgctgc
121 aatggaataa agtcctcaat attgaaatgt gacattgctc tcaacatctc acatctctct
181 ggatttcttt tttctcatca ttactgctaa caaattcatt tccagacttt gcacttttaa
241 gaagcaatgg aaaaaatcaa cagtctttca acacaattag ttaagtgtctg cttttgtgat
301 ttcttgaagg tgaagatgca cactgtgtcc tacattcatt tcttctacct tggcctgtgt
361 ttgcttaact taaccagttc tgctgtgtcc ggcccagaaa cactgtgtgg tgctgagctg
421 gttgtatgctc ttcagttcgt atgtggagac agaggcttct acttcagtaa gcctacaggg
481 tatggatcca gcagtagacg cttacaccac aagggaaatag tggatgagtg ctgcttccag
541 agttgtgacc tgaggaggct ggagatgtac tgtgctccaa taaagccacc taatctgca
601 cgctctgtac gtgctcagcg ccacactgat atgccaaaag cacaaaagga agtgcatttg
661 aagaatacaa gtagagggaa cacaggaaac agaaactaca gaatgtaaga tcatgccatc
721 cacaagaatg aagaatgaat gtgccatctg cacagtagctt tgctgtaaat aaattatttg
781 ttaaacattg gaagact

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Fig. 1. cDNA sequence of chicken insulin-like growth factor I. Bold, italics, underlined letters denote the position of the forward and reverse primers.

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 ethidium bromide, and the expected correct size was confirmed in relation to the 50-bp molecular ladder (Jena Bio-science Laboratory) as a standard size marker.

Single stranded conformation polymorphism (SSCP) analysis

A mixture of 5 μ l of amplified RT-PCR product and 10 μ l denaturing buffer (5 \times loading dye in formamide in the ratio 1:4) was prepared. The mixture was heated to 95 °C for 7 min and then cooled on ice for 15 min. Thereafter the mixture was loaded onto 15% polyacrylamide gel with 20% formamide and electrophoresed in 1 \times 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 deionized water for 15 min. Gels were visualized under a UV transilluminator and photographed using a digital camera.

Results and Discussion

Records from local farms for the different chicken breeds clearly demonstrated that the Cobb 500 breed was the fastest growing one with the best feed conversion rate (FCR) among the studied breeds, whereas the Sasso breed was the slowest growing one as shown in Table I.

Amplicons of the expected sizes were obtained from RT-PCR of different avian RNA samples. These were approx. 203 bp in size as predicted by the published avian sequence (Fig. 2). These amplicons exhibited polymorphism upon SSCP analysis and three unique banding patterns could be identified for the IGF-I gene of the studied chicken breeds as shown in Fig. 3.

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 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

Table I. The growth rates of the different chicken breeds.

Breed	Average body weight at day 49 [g]	Average daily gain [g bird ⁻¹ d ⁻¹]	Feed conversion rate
Sasso	1918	39.3	2.06
Arbor Acres plus	2553.9	51.28	1.9
Cobb 500	3177.1	64.8	2.81

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 (Fig. 3), highlighting the sensitivity of the technique. SSCP analysis 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 migrate differently.

Given that all of the RT-PCR products are of the order 203 bp, the migration pattern of the fragment in these (polyacrylamide) gels demonstrates to which extent conformation, rather

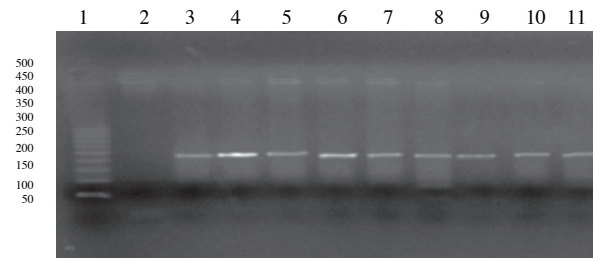


Fig. 2. RT-PCR products of avian IGF-I. Lane 1, 50-bp ladder run as size standard; lane 2, control negative; lanes 3–5, RT-PCR products of Sasso breed; lanes 6–8, Arbor Acres plus breed; lanes 9–11, Cobb 500 breed.



Fig. 3. SSCP analysis of RT-PCR products of avian IGF-I. Lanes 1–3, Sasso breed; lanes 4–6, Arbor Acres plus breed; lanes 7–9, Cobb 500 breed.

than molecular weight, is the major determinant of gel mobility. Using SSCP analysis, three non-overlapping SSCP patterns were found indicating the 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; 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 we have found in this gene coding for IGF-I hint at the possibility of exploring this approach for the search of genetic markers located in these chicken breeds and producing SSCP markers for gene mapping (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 a valuable genetic resource for the improvement of these chicken breeds.

It is known that the genes of the growth axis play crucial roles in the regulation of growth, development and differentiation. An important as-

sociation exists between meat quality and growth and body composition. For this reason, the genes of the growth axis probably affect the meat quality traits of animals (Lei *et al.*, 2007).

Many studies revealed that IGF-I polymorphisms are related to some growth traits. There are associations between IGF-I promoter polymorphism, average daily gain and feed efficiency found in two genetically diverse Black Penedencense chicken strains by Amills *et al.* (2003). These authors also reported that the IGF-I gene is 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, 2004). Moreover, it was suggested that single nucleotide polymorphism (SNP) of IGF-I encountered by Lei *et al.* (2007) could affect the chicken muscle fiber growth. In addition, they found that C51978309T SNP of the IGF-I gene was significantly linked with the transversal area of the leg muscle between Xinghua and white Plymouth Rock chickens.

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

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