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Evaluation of insulin-like growth factor-I gene polymorphism in Egyptian small ruminant breeds

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The genetic improvement of production traits can be developed through marker assisted selection. Insulin-like growth factor I (*IGF-I)* is a member of the somatotrophic axis which has a remarkable variation of its biological effect including protein synthesis and skeletal growth. This study aimed to detect the genetic polymorphism of *IGF-1* in different Egyptian sheep and goat breeds. The amplified fragments at 320-bp were digested with *Hae*III endonuclease and the results show the presence of three different genotypes: CC (15.71%), CG (29.29%) and GG (55.0%). The nucleotide sequence analysis of C and G alleles declared the presence of a single nucleotide polymorphism (C→G) at position 282. The nucleotide sequences of alleles C and G in sheep and goat were submitted to GenBank with the accession number: KX432965, KX432966, KX432967 and KX432968, respectively. In conclusion, a nucleotide substitution (C→G) was detected in *IGF-I* gene in Egyptian sheep and goat breeds resulting in the presence of three different genotypes; CC, CG and GG. The association of *IGF-I* polymorphism with different growth trait parameters were reported at significant levels, so, the genetic and SNP variations in *IGF-I* gene may be a potential molecular marker for growth traits in different Egyptian sheep and goat breeds.

Key words: *IGF-1*, polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP), DNA sequencing, sheep, goat.

INTRODUCTION

Molecular genetics techniques are of great interest in the identification of genetic variations in genetic markers which are associated with different production and reproduction traits in farm animals (Jiang et al., 2002; Arora and Bhatia, 2006; Missohou et al., 2006). These genetic variations affect the physiological pathways that consequently lead to quantitative variations in different phenotype characteristics (Schwerine et al., 1995; Lan et al., 2007). In quantitative genetics, there are number of single genes associated with mammary or muscle growth, development and function which were studied as excellent candidates for linkage relationships with quantitative traits of economic importance.

Growth is a process in which the interaction between different neuroendocrine pathways is done and expressed in this phenotype trait. The somatotrophic axis (GH/IGF-I axis) is involved in these pathways and it is considered as the key in postnatal growth and

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metabolism in different mammals including farm animals (Shoshana et al., 2000; Burkhard et al., 2005). One of the most important member of the somatotrophic axis is insulin-like growth factor I (*IGF-I*) which has a remarkable variation of its biological effect like protein synthesis and skeletal growth (Froesch et al., 1985; Baxter, 1985; Clemmons et al., 1987).

In Egypt, there is a shortage in meat production comparing to the nutritional requirements, and there is an increasing gap between dairy products produced domestically and the amount consumed. Production improvements can be achieved by using new genetic technology and linear type appraisal for better selection of heritable traits.There are several indigenous sheep and goat breeds. The common sheep breeds include Barki, Rahmani and Ossimi, while goat breeds include Baladi, Barki and Zaraibi (Galal et al., 2005). The contribution of both species to the total red meat produced in Egypt is about 9.1% (6.4% for sheep and 2.7% for goats). The latest count of slaughtered sheep and goat numbers, represent 41.7% of all slaughtered livestock (27.4% for sheep and 14.3% for goats) (MoA, 2004).

Due to lack of knowledge about the genetic characterization and nucleotide sequence variations of *IGF-1*gene in Egyptian sheep and goat breeds, this study aimed to detect the genetic polymorphism of *IGF-1*/*Hae*III in different small ruminant breeds reared in Egypt and to identify the single nucleotide diversity in different *IGF-1* genotypes.

MATERIALS AND METHODS

Animals and DNA extraction

The blood samples were collected from 140 animals belonging to three sheep breeds; Barki (32 animals), Ossimi (28 animals) and Rahmani (22 animals) in addition to three goat breeds; Baladi (16 animals), Barki (20 animals) and Zaraibi (22 animals). Genomic DNA was extracted from the whole blood according to the method described by Miller et al. (1988) with minor modifications. Briefly, blood samples were mixed with cold 2x· sucrose-triton and centrifuged at 5000 rpm for 15 min at 4°C. The nuclear pellet was suspended in lysis buffer, sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water bath at 37°C. Nucleic acids were extracted with saturated NaCl solution. The DNA was picked up, washed in 70% ethanol and was dissolved in 1X TE buffer. The DNA concentration was determined, using Nano Drop1000 Thermo Scientific spectrophotometer, and then diluted to the working concentration of 50 ng/µl, which is suitable for polymerase chain reaction.

Polymerase chain reaction (PCR)

The DNA fragment of the studied gene was amplified using polymerase chain reaction technique developed by Mullis et al. (1986). This amplified fragment spans from intron 3 to intron 4 and cover exon 4 of *IGF-1*gene in sheep and goat. A PCR cocktail consists of 1.0 mM primers ((forward primer: 5' - GCT GGG TGT AGC AGT GAA CA -3' and reverse primer: 5' - GTT GCT TCA GCC GCA TAA CT-3'; Zhang et al., 2008), 0.2 mM dNTPs and 1.25 U of Taq polymerase. The cocktail was aliquot into PCR tubes with 100 ng of sheep or goat DNA. The reaction was cycled with the following conditions; initial denaturation for 5 min at 94°C followed by 35 cycles of denaturation at 94°C, annealing at 60°C and extension at 72°C, each step for 1 min and the final extension for 5 min at 72°C. The amplification was verified by electrophoresis on 2% agarose gel in 1x TBE buffer using GeneRuler^{TM} 100-bp ladder as a molecular weight marker for confirmation of the length of the PCR products. The gel was stained with ethidium bromide and visualized on UV trans-illuminator.

Restriction fragment length polymorphism (RFLP)

Ten microliter of PCR product were digested with 1 µl of FastDigest *Hae*III restriction enzymes at 37°C for 5 min. The restriction fragments were subjected to electrophoresis in 2 % agarose/ethidium bromide gel (GIBCO, BRL, England) in 1× TBE buffer (0.09 M Tris-boric acid and 0.002 M EDTA). Gels were visualized under UV light and documented in FX Molecular Imager apparatus (BIO-RAD).

Sequence analysis

The PCR products for each genotype of the tested gene were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite and the results of the endouclease restriction were carried out using FastPCR. The nucleotide sequences of the tested gene in Egyptian sheep and goat were submitted to GenBank (NCBI, BankIt).

RESULTS AND DISCUSSION

In quantitative genetics, there are number of single genes associated with mammary or muscle growth, development and function which were studied as excellent candidates for linkage relationships with quantitative traits of economic importance. Among them, a somatotropic axis (SA) contains the most promising candidates (Szewczuk et al., 2012). Insulin growth factor-1 (*IGF-1*) gene is one important gene belonging to the somatotropic axis.

IGF-I has a variety of biological effects which plays an essential role in embryonic and postnatal growth. *IGF-I* concentration is related with fetal size in different species (Baker et al., 1993; Gluckman, 1995; Breier, 1999). *IGF-I* has an important role in growth of fetal organs and skeletal muscles (Lok et al., 1996). *IGF-I* is considered as a key factor in animal growth through its effect on longitudinal bone growth, muscle growth and cartilage growth (Duclos et al., 1999; Zapf and Froesch, 1999; Yakar et al., 2002). *IGF-1* plays an important role in mammalian fertility through the regulation of many hormones which are critical for reproductive system (Miller and Gore, 2001; Velazquez et al., 2008). Due to its important role in growth and reproduction traits, *IGF-1* gene is considered as a candidate marker for these traits and its genetic polymorphism identification is of great interest in Egyptian small ruminant breeds.

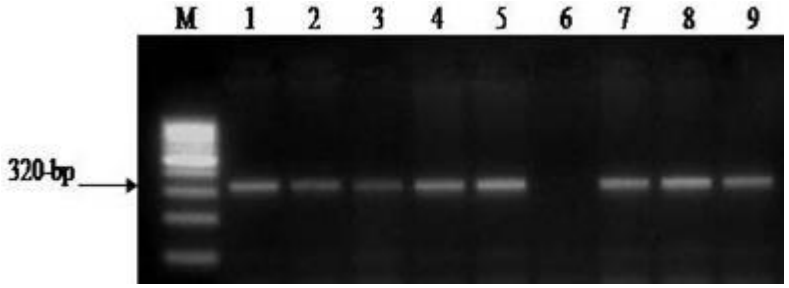

Figure 1. Ethidium bromide-stained gel of PCR products representing amplification of *IGF-1g*ene in Egyptian sheep and goat animals. Lane M, 100-bp ladder marker; lanes 1-5 and 7-9, 320-bp PCR products amplified from sheep and goat DNA; lane 6, negative control.

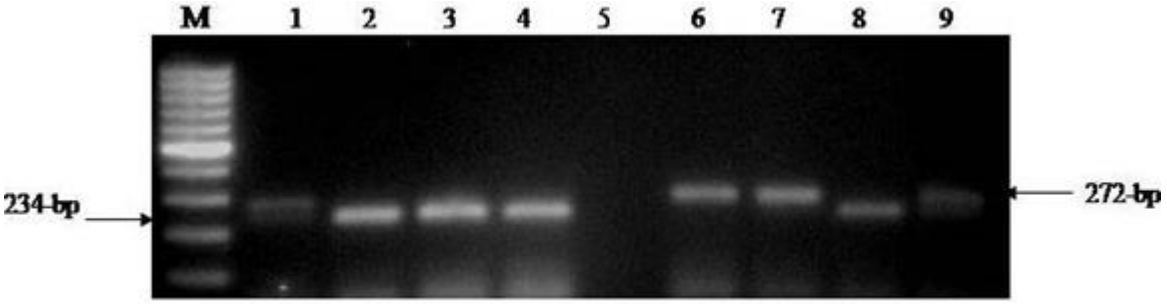

Figure 2. The electrophoretic pattern obtained after digestion of PCR amplified fragment of *IGF-1* gene with *Hae*III restriction enzyme. Lane M, 100-bp ladder marker; lanes 1 and 9: CG heterozygous genotype with four fragments at 272-, 234, 48- and 38 bp; lanes 2-4 and 8: GG homozygous genotype with three fragments at 234, 48 and 38 bp; lanes 6 and 7: CC homozygous genotype with two fragments at 272- and 48-bp; lane 5; negative control * Small sized fragments at 48 and 38 bp are not show in the figure.

The primers used in this study flanked a 320-bp fragment spanning from intron 3 to intron 4 covering exon 4 of *IGF-1*gene in sheep and goat. The amplified fragments obtained from all tested sheep and goat DNA gave the expected fragment at 320-bp (Figure 1).

These PCR amplified fragments (320-bp) were digested with *Hae*III restriction enzyme. Depending on the presence of one or two restriction sites (GG^CC) at positions 48^49 and/or 282^283, the results showed the presence of 3 different genotypes: CC with two digested fragments at 272- and 48-bp, GG with three digested fragments at 234, 48 and 38 bp and CG with four digested fragments at 272, 234, 48 and 38 bp (Figure 2).

The frequencies of GG, CG and CC genotypes were53.125, 37.5 and 9.375% in sheep Barki animals (32 animals), 57.14, 28.57 and 14.29% in sheep Ossimi animals (28 animals) and 59.09, 27.27 and 13.64% in sheep Rahmani animals (22 animals), respectively with the total frequencies of 53.66, 30.49 and 15.85% for GG, CG and CC genotypes, respectively in 82 tested sheep animals for this gene. In tested goat animals, the frequencies of GG, CG and CC genotypes were 56.25, 31.25 and 12.5% for Baladi (16 animals), 55.0, 30.0 and 15.0% for Barki (20 animals) and 59.09, 22.73 and 18.18% for Zaraibi (22 animals), respectively with total frequencies of 56.89, 27.59 and 15.52% for GG, CG and CC genotypes, respectively in 58 tested goat animals for this gene. The total frequencies of G and C alleles in all tested animals (140 animals) were 69.64 and 30.36%, respectively (Table 1).

These three detected genotypes resulted from the presence of two different alleles C and G. The sequence analysis of these two alleles represented a single nucleotide polymorphism (C→G) at position 282 which is responsible for the presence of 2nd restriction site at position 282^283 in the allele G (Figures 3 and 4). The nucleotide sequences of alleles C and G in sheep and goat were submitted to GenBank with the accession nos: KX432965, KX432966, KX432967 and KX432968, respectively.

Several polymorphisms of *IGF-I* gene and their associations with production traits were reported in goats. Zhang et al. (2008) reported the polymorphism in intron 4 of the *IGF-I* gene and its association with growth traits in the Nanjiang Huang goat. The associations of g.5752 G>C and g.1617 G>A mutations with milk yield and body

Table 1. The genotype and allele frequencies of *IGF-1* gene in Egyptian sheep and goat breeds.

Species	Breeds	Number of animals	Genotype frequencies						Allele frequencies	
			GG		CG		CC		G	C
			Number	Frequency (%)	Number	Frequency (%)	Number	Frequency (%)	Frequency (%)	Frequency (%)
Sheep	Barki	32	17	53.125	12	37.5	3	9.375	71.875	28.125
	Ossimi	28	16	57.14	8	28.57	4	14.29	71.43	28.57
	Rahmani	22	13	59.09	6	27.27	3	13.64	72.73	27.27
	Sub-total	82	44	53.66	25	30.49	13	15.85	68.9	31.1
Goat	Baladi	16	9	56.25	5	31.25	2	12.5	71.875	28.125
	Barki	20	11	55.0	6	30.0	3	15.0	70.0	30.0
	Zaraibi	22	13	59.09	5	22.73	4	18.18	70.45	29.55
	Sub-total	58	33	56.89	16	27.59	9	15.52	70.69	29.31
	Total	140	77	55.0	41	29.29	22	15.71	69.64	30.36

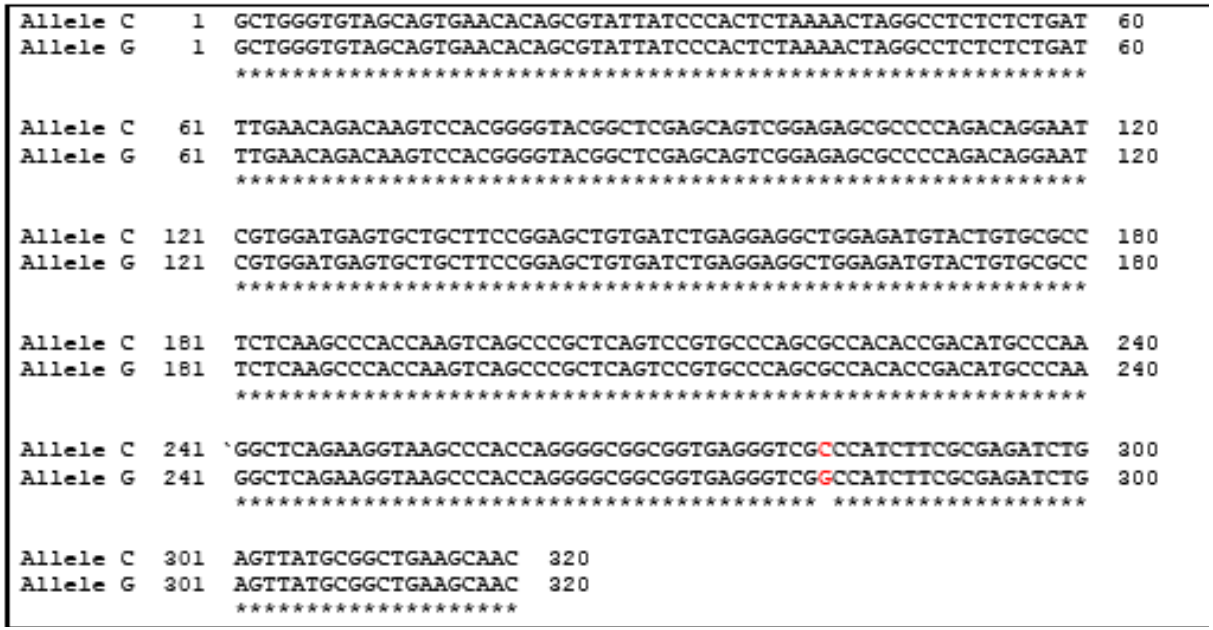

Figure 3. The nucleotide sequence alignment between the two different alleles C and G. Single nucleotide polymorphism (C/G) at position 282 in red.

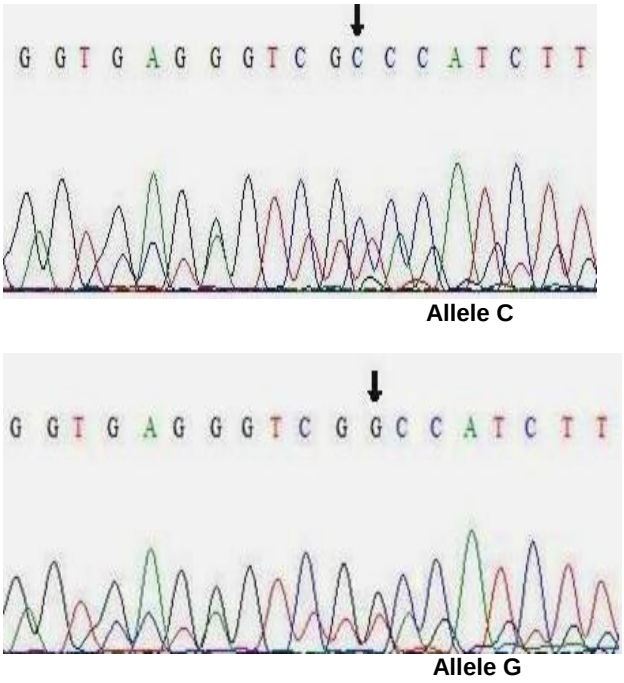

Figure 4. Single nucleotide polymorphism (C→G) at position 282 in alleles C and G.

size in Chinese dairy goats were reported with a significant effect of these variations on the examined productive traits (Deng et al., 2010).

Qiong et al. (2011) evaluated the relation between *IGF-1* variation and cashmere production traits as well as body weight in three Chinese goat breeds. A novel SNP was detected in exon 4 and it is significantly associated with cashmere production traits. The cashmere fineness of BB genotype animals was higher than those of AB and AA genotype individuals.

PCR–SSCP analysis of goat *IGF-I* gene was used to detect the polymorphisms in two Iranian goat breeds (Karimi Kurdistani et al., 2013) which revealed novel G to A transition (g.1617 G >A). Same authors used PCR-RFLP analysis of a part of intron and exon 4 of goat *IGF-I* gene to identify the associations between *IGF-I* /HaeIII polymorphism and growth trait. This polymorphism was significantly associated with different growth parameters which include yearling weight, post-weaning average daily gain and first shearing fleece weight. Animals which possess GG genotype in this site appeared potentially more favorable for these mentioned traits. The frequencies of GG, CG and CC in Iranian goats were 0.61, 0.29 and 0.10% respectively. Our results agree with the findings of this study where, the frequencies of different genotypes of *IGF-1* gene in Egyptian goat breeds were 56.89, 27.59 and 15.52% for GG, CG and CC, respectively. These results also declared the dominance of G allele (70.69%) over C allele (29.31%) in all tested Egyptian goat animals.

The same technique PCR-SSCP in Iranian Makoei sheep breed, Moradian et al. (2013) determined the genetic polymorphism at exon 1 of the *IGF-I* gene and the results reveal the presence of three genotypes; AA (52.0%), AG (42.0%) and GG (6.0%). He et al. (2012) examined the polymorphism of *IGF-1* gene in four Chinese sheep breeds which show the association of different genotypes in ewes with their lams at significant levels.

In conclusion, a nucleotide substitution (C→G) was detected in *IGF-I* gene in Egyptian sheep and goat breeds. Three different genotypes; CC, CG and GG were observed due to the presence of two alleles; C and G. The association of *IGF-I* polymorphism with different growth trait parameters were reported at significant levels. So, the genetic and SNP variations in *IGF-I* gene may be a potential molecular marker for growth traits in different Egyptian sheep and goat breeds and could be used in molecular marker-assisted selection for small ruminant programs.

Conflicts of Interests

The authors have not declared any conflict of interests.

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