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Original Article

Screening of *IGF-I* and *IGFBP-3* genes polymorphism in popular Goat breeds in Egypt

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ABSTRACT

The genetic polymorphisms of two functional genes; Insulin-like growth-I (*IGF-I*) and Insulin-like growth factor binding protein-3 (*IGFBP-3*) were investigated in three goat breeds (Barki, Damascus and Zaraibi) by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), for differentiating these breeds. *IGF-I-HaeIII/*RFLP revealed three genotypes (CC, CG and GG) with frequencies; 0.40, 0.10 and 0.50, respectively in Barki. Moreover, the expected heterozygosity (He) value (0.51) was higher than observed heterozygosity (Ho) value (0.10) in Barki. Chi-square ($\chi 2$) value in Barki (13.6) showed a significant deviation from Hardy-Weinberg equilibrium [HWE] ($P<0.05$). *IGFBP-3-XspI/*RFLP revealed three genotypes (CC, CT and TT), with only CT and TT in all breeds. CT frequency was the highest in Barki (0.80), and TT frequency was the highest in Damascus (0.75). Ho value was higher than He value in all breeds. Additionally, Ho, He and $\chi 2$ values were the highest in Barki (0.80, 0.49 and 8.26, respectively) and $\chi 2$ value showed a significant deviation from HWE ($P<0.05$). The polymorphisms demonstrated in these results are recommended as effective markers for genetic differentiation among goat breeds, opening up interesting prospects for goat breeders for future selection programs.

Keywords: Genetic Polymorphism, goat, PCR-RFLP, *IGF-I* gene, *IGFBP-3* gene

INTRODUCTION

Goats in Egypt are almost 3.13 million goats. They are raised mainly in three regions: the Upper Egypt, Nile Delta and in the desert rangelands [1]. Production systems and breeds in the three zones are different. There are about 1.7 million goats, mainly in mixed flocks, with some goats kept as household animals. In the desert rangelands, 1.4 million sheep and goats are kept in extensive systems [2]. There are five indigenous goat breeds: Baladi (local breed in Delta), Barki or Sahrawi (local breed in Desert), Sinaoy (Bedouin), Saidi and Zaraibi (or Egyptian Nubian). They are duple - purpose animals, with does bred for milk and bucks bred for meat [3]**.** The former five breeds are pure whereas, the other breeds are mixtures of these breeds, like the Anglonubian breed, coming from crossbreeding of Egyptian Nubian breed with some English breeds. Goat genetic improvement schemes in Egypt have involved crossbreeding trials with foreign breeds like Damascus goats.

Lately, genetic polymorphisms at candidate genes affecting economic traits (like growth, milk yield, meat production and reproductive traits) have stimulated research interest because genetic polymorphisms are well-considered as an aid to genetic selection and to mark evolutionary relationships in different livestock animal breeds [4]. Genetic polymorphism arises from mutation which ranged from change in one nucleotide base (SNP) to variation in several hundred bases [5]**.**

The *IGFs* signaling system, composed of *IGF-I*, *IGF-II*, *IGF-I* receptor, *IGF-II* receptor and six binding proteins (*IGFBP-1–IGFBP-6*), plays a vital role in growth, development, ageing and reproduction. *IGF-I* gene in goat is located on chromosome five and includes six exons and five introns [6]. It exerts a prime function in growth, milk yield, meat production and reproductive traits. Polymorphism in *IGF-I* gene has been studied in different livestock animals such as in cattle [7]**,** sheep [8]**,** goat [9]**, chicken** [10] and in swine [11].

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Insulin-like growth factor binding protein-3 (*IGFBP-3*) gene encodes a structural protein, *IGFBP-3*, that binds non-covalently to insulin-like growth factors (*IGFs*) system in circulation and responsible for their multiple influences [12]. Thus, it plays a key function in different body functions such as growth, metabolism, reproduction, controlling body weight, immunity etc. Consequently, *IGFBP-3* gene is well-considered as an effective genetic marker in growth, milk yield and meat production traits [13,14] . *IGFBP-3* gene in goat and cattle is located on chromosome four [15]. The full length of the *IGFBP-3* gene is 8.9 kb and contains five exons [16]. Polymorphism in *IGFBP-3* gene has been studied in different livestock animals such as in sheep [17] , buffalo [18], cattle [19] and in goat [20].

The aim of the present study is to screen the genetic polymorphism of two functional genes (*IGF-I* and *IGFBP-3*) in three goat breeds (Barki, Damascus and Zaraibi) via PCR-RFLP method in order to differentiate between these breeds.

MATERIALS AND METHODS

Animals and DNA extraction: A total of 60 healthy does, belonging to three breeds, Barki, Damascus and Zaraibi; 20 samples for each breed. All animals were born and reared in the Agriculture Research Station, belonging to Faculty of Agriculture, Cairo University. Blood samples were collected in tubes containing 2.7% EDTA as anticoagulant and transported to the laboratory under cooled conditions. Genomic DNA was extracted and purified from whole blood collected samples using the salting out technique described by **Miller *et al.*** [21]**.** The DNA concentration was measured using the U.V spectrophotometer at wavelength 260 nm.

Polymerase Chain Reaction (PCR): Two pairs of primers were used for amplifying each of *IGF-I* and *IGFBP-3* loci using primers suggested by **Liu *et al.*** [22] and **Lan *et al.*** [20], respectively. The primer sequences are represented in table (1). Amplification reaction was carried out in a 25 µl volume containing 100 ng genomic DNA, forward and reverse primer (both at concentration 10 pmol/µl), 1U *Taq* polymerase, 2.5 µl Taq polymerase buffer, four dNTPs (each at final concentration of 2.5 mM/µl) and de-ionized double distilled H_2O up to a total volume of 25 µl. Amplification conditions are shown in table (2). The amplicons were analyzed by 1.5 % agarose gel electrophoresis. The gels were stained with ethidium bromide and visualized under ultraviolet light.

Restriction Fragment Length Polymorphism (RFLP): It was carried out in 15 µl of reaction mixture of each sample containing 5 µl of PCR product, 9.5 µl of 10 X buffer and 0.5 µl of fast restriction enzyme (MBI Fermentas, Germany) specific for each gene (Table 1). The reaction mixture was incubated at 37°C for 10 minutes. Digestion products were separated by electrophoresis on 2.5 % agarose gel, stained with ethidium bromide. The bands were visualized under UV light and the gels were photographed using digital gel documentation system (Bio-Rad, USA).

Statistical Analysis: The genotypic and allelic frequencies, the observed and expected heterozygosities and the **$\chi 2$** test for Hardy-Weinberg equilibrium (HWE) were calculated using Pop Gene 32.1 package [23].

RESULTS AND DISCUSSION

Many different studies detected *IGF-I* gene polymorphisms and proved their association with growth traits among different livestock domestic animals [6,8,9,29,30,31]. For example, **Zhang *et al.*** [30] detected a single nucleotide polymorphism (SNP) [G→C] at intron four of the *IGF-I* gene and described its significant association with birth weight, body weight at six and twelve months in Nanjiang Huang goats.

In the current study, *IGF-I*-PCR produced a DNA fragment of 363 bp as shown in figure 1. It includes exon four and part of intron four of *IGF-I [*28]. Digestion of this fragment with the restriction enzyme *HaeIII* produced two alleles; C and G with a complete absence of allele G in both Damascus and Zaraibi breeds. Three genotypes; CC (363 bp), CG (363, 264 and 99 bp) and GG (264 and 99 bp) were found. As shown in table (3), the genotype GG had the highest frequency (0.50) in the Barki breed. Whereas, all goats of both Damascus and Zaraibi breeds showed only CC genotype. The value of expected heterozygosity (0.51) was higher than that of observed heterozygosity (0.10) in the Barki breed. Chi-square ($\chi 2$) value in the Barki breed (13.6) showed a significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$).

These findings were similar to those illustrated previously by **Liu *et al.*** [28], who screened this caprine *IGF-I* gene polymorphism in exon four and part of intron four in two Chinese local goat breeds, via gene sequencing and PCR–RFLP. This discovered genetic polymorphism appeared associated with body weight in cashmere goats. However, this association was not significant. In Iranian Markhoz goats, **Kurdistani *et al.*** **[**24]

detected the polymorphism of *IGF-I* gene intron four and its association with growth traits and yearling fleece weight. Likewise, **Sharma *et al.*** [9] investigated the effect of two detected SNPs (g.4700T > C and g.5524C > T) of *IGF-I* gene in Indian Sirohi goats. Furthermore, **Rasouli *et al.*** [25] investigated caprine *IGF-I* gene polymorphism in Iranian Markhoz goats by sequencing and PCR-SSCP. Sequencing results revealed a G to A transition at position 1617 of the *IGF-I* gene (g. 1617 G>A) in the 5´ flanking region of the *IGF-I* gene.

Several studies showed the *IGFBP-3* gene polymorphism and illustrated its association with growth traits among various livestock animals, cattle [33,32,19,34], buffalo [35,18], goat [9,25] and sheep [37,17]. In the current study, *IGFBP-3*-PCR produced a DNA fragment of 655 bp as shown in figure 3. It includes a part of intron two, exon three and intron three of *IGFBP-3* gene **Lan *et al.*** [20]. Digestion of this fragment with the restriction enzyme *XspI* produced two alleles, C and T, with a higher frequency of allele T in all the three studied breeds. Three genotypes; CC (655 bp), CT (655, 421 and 234 bp) and TT (421 and 234 bp) were found (Figure 4). As shown in table (4), the uncut CC genotype is absent in all the three breeds and only two genotypes AB and BB were revealed with a higher frequency of CT genotype in both the Barki and Zaraibi (0.80 and 0.55, respectively), and a higher frequency of TT genotype (0.75) in Damascus. The value of observed heterozygosity (Ho) was higher than that of expected heterozygosity (He) in all the three studied breeds. In addition, the values of both Ho and He were the highest in Barki breed (0.80 and 0.49, respectively). Chi-square ($\chi 2$) value was the highest and also showed a significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$) in only the Barki breed.

These findings were in consonance with those previously reported by **Lan *et al.*** [20] who detected four mutations of goat *IGFBP-3* gene in some Chinese dairy goat breeds, by PCR-SSCP and DNA sequencing methods. However, they identified three genotypes, not two as in the current study, by *XspI*/PCR-RFLP, genotype X1X1(655 bp), X1X2 (655, 421 and 234 bp) and X2X2 (421 and 234 bp). This difference in genotypic patterns may be due to breed difference. Similarly, **Sharma *et al.*** [9] identified Eight SNPs in the exon two of *IGFBP-3* gene indicating the high level of heterozygosity of this fragment. Moreover, **Rasouli *et al.*** [25] investigated goat *IGFBP-3* gene polymorphism in Markhoz goats by gene sequencing and PCR-SSCP, and their relationship with growth traits. Sequencing results revealed T to C transition at position 58 of exon two of the *IGFBP-3* gene.

On the other hand, no polymorphisms were found in a portion of sheep *IGFBP-3* gene including exon two, intron two, exon three and intron three by PCR-RFLP test **(Kumar *et al.*** 17**).** Moreover, **Shafey *et al.*** [26] found no polymorphism of sheep *IGFBP-3* gene in three Egyptian sheep breeds (Barki, Rahmani and Osseimi), when used *HaeIII*/PCR-RFLP. They found only one restriction pattern including 201, 201, 87, 67, 57, 19, 16 and 7 bp fragments which represent only the allele B. Furthermore, **Ali *et al.*** [27] reported the same finding in the four Egyptian sheep breeds (Rahmani, Osseimi, Barki and Awassi) indicating the homozygosity of *IGFBP-3* gene in the four breeds studied.

CONCLUSION

It is concluded that the *IGF-I* /*HaeIII* and *IGFBP-3/XspI* polymorphisms may be utilized as effective markers for genetic differentiation between goat breeds. So, further studies should be made to sequence the amplified DNA fragments 363 and 655 bp of *IGF-1* and *IGFBP-3*, respectively, for more precise differentiation between these breeds at the level of DNA base pair. In addition, *IGF-1* and *IGFBP-3* gene may be potential molecular markers for various economic traits such as growth, reproduction, meat production and milk yield traits. Thus more studies, with large sample size for each goat breed, are needed for the association analysis between caprine *IGF-1* and *IGFBP-3* polymorphisms and these traits.

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Table 1: Gene, primer sequence (5'→3'), length of PCR product, region and specific restriction enzyme of IGF-I & IGFBP-3 genes

Gene	primer sequence (5'→3')	PCR product length	region	restriction enzyme
IGF-I	F: CACAGCGTATTATCCCAC R: GACACTATGAGCCAGAAG	363 bp	Exon 4 & intron 4	*HaeIII*
IGFBP-3	**F: CCA AGC GTG AGA CAG AAT AC R: AGG AGG GAT AGG AGC AAG TT**	**655 bp**	**Intron 2, exon 3 & intron 3**	***XspI***

F: forward R: reverse

Table 2: PCR conditions

Gene	Primary denaturation in 1st cycle		Denaturation		Annealing		Elongation		Final extension		Number of cycles
	°C	Sec	°C	Sec	°C	Sec	°C	Sec	°C	Sec	
IGF-I	94	300	94	40	56	35	72	35	72	600	35
IGFBP-3	95	240	94	40	55	35	72	35	72	600	30

Table 3: Genotype frequency, allele frequency, observed heterozygosity (Ho), expected heterozygosity (He) and $\chi 2$ values of *IGF-I-HaeIII/* PCR-RFLP

Gene / Restriction Enzyme	Breed	Genotype Frequency			Allele Frequency	
		CC	CG	GG	C	G
IGF-I	Barki	0.40	0.10	0.50	0.45	0.55
	Damascus	1.00	0.00	0.00	1.00	0.00
	Zaraibi	1.00	0.00	0.00	1.00	0.00

Table 4: Genotype frequency, allele frequency, observed heterozygosity (Ho), expected heterozygosity (He) and $\chi 2$ values of *IGFBP-3-XspI* / PCR-RFLP

Gene / Restriction Enzyme	Breed	Genotype Frequency			Allele Frequency		Observed Het. (Ho)	Expected Het. (He)	χ^2
		CC	CT	TT	C	T			
IGFBP-3	Barki	0.00	0.80	0.20	0.40	0.60	0.80	0.49	8.26*
	Damascus	0.00	0.25	0.75	0.12	0.88	0.25	0.22	0.32
	Zaraibi	0.00	0.55	0.45	0.27	0.73	0.55	0.41	2.57

(*) means ($P \leq 0.05$)

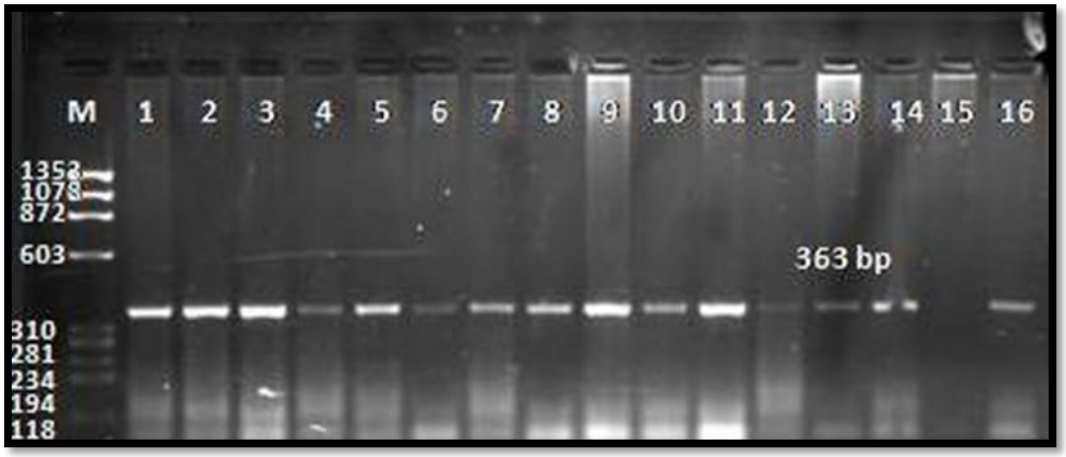

Fig. 1: Agarose gel electrophoresis of *IGF-I* - PCR fragment (363 bp). Lane M, Φ DNA ladder. Lanes (1→6) Barki, (7→12) Damascus and Lanes (13→17) Zaraibi breeds.

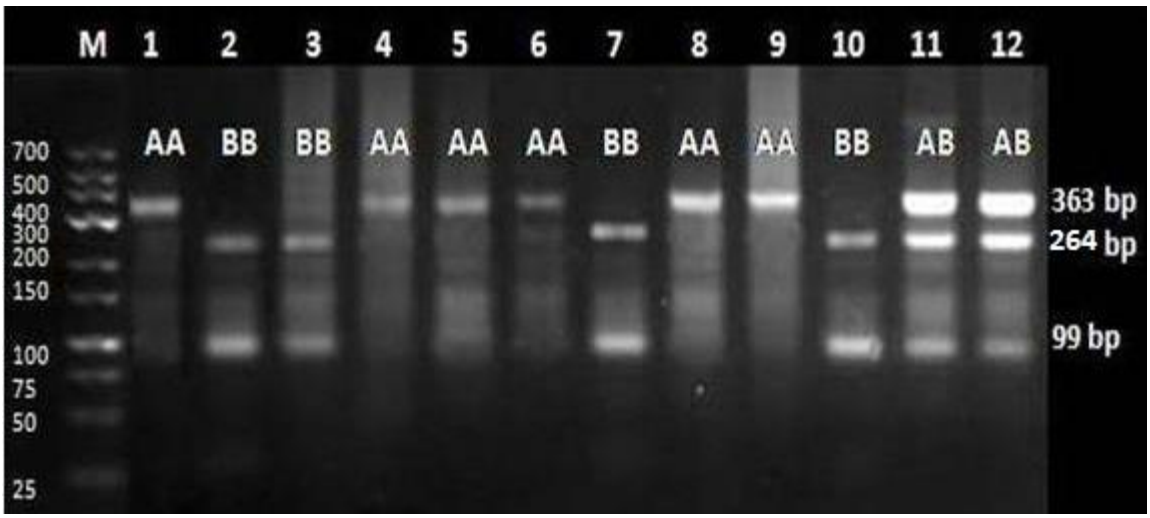

Fig. 2: Agarose gel electrophoresis of *IGF-I -HaeIII* / PCR-RFLP fragments. Lane M, 25bp DNA ladder, lanes (1, 4, 5, 6, 8, 9) Genotype CC (363 bp), lanes (11, 12) Genotype CG (363 , 263 and 99bp) and lanes (2, 3, 7, 10) Genotype GG (264 and 99bp).

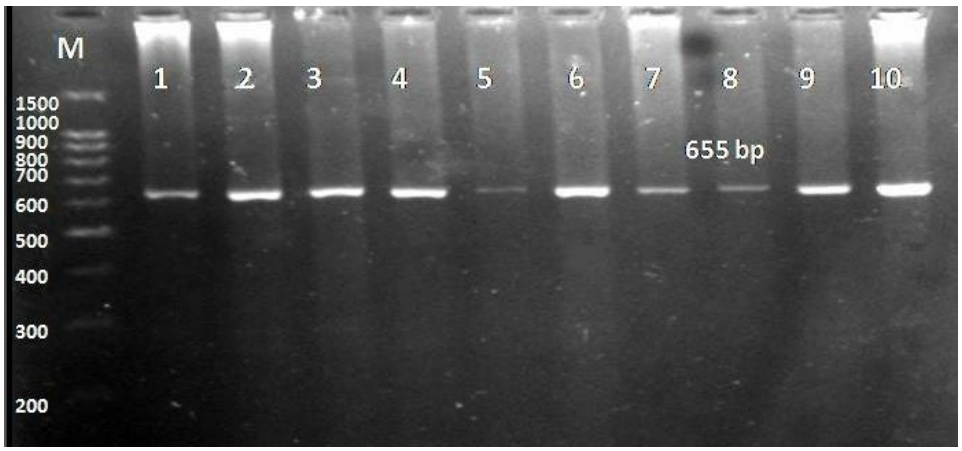

Fig. 3: Agarose gel electrophoresis of *IGFBP-3*- PCR fragment (655 bp). . Lane M, 100 bp DNA ladder. Lanes (1→3) Barki, (4→6) Damascus and Lanes (7→10) Zaraibi breeds.

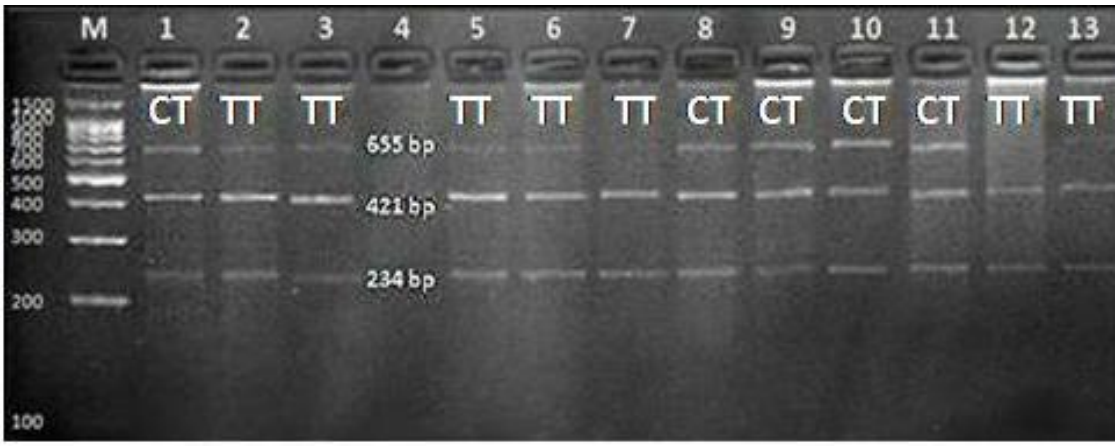

Fig. 4: Agarose gel electrophoresis of *IGFBP-3-XsPI / PCR-RFLP* fragments. Lane M, 100 bp DNA ladder, lanes (1, 8, 9, 10, 11) Genotype CT (422 , 366 and 56bp) and lanes (2, 3, 5, 6, 7, 12, 13) Genotype TT (366 and 56bp).

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