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Genetic Polymorphism of Myostatin and Insulin-Like Growth Factor Binding Protein-3 Genes in Egyptian Sheep Breeds

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Abstract: The genetic polymorphism of some genes related to meat production in three Egyptian sheep breeds (Barki, Rahmani and Osseimi) was studied. The candidate genes were: Myostatinand Insulin-like growth factor. The technique applied was the restriction fragment length polymorphism for the polymerase chain reaction products. Polymorphism was found in the Myostatin gene, while no polymorphism was exhibited by Insulin-like growth factor binding protein-3 gene in the three breeds understudy. Myostatin digested with *DraI* had two genotypes AB and BB. The AA genotype cannot be detected. The highest allelic frequency was for allele B. Insulin-like growth factor binding protein-3 had only one genotype, BB genotype for Insulin-like growth factor binding protein-3 digested with *HaeIII*, therefore this is not recommended in the selection program. The result of Chi-square analysis indicated that the three Egyptian sheep breeds were in Hardy-Weinberg Equilibrium.

Key words: Genetic Polymorphism · Sheep · PCR- RFLP · Myostatin gene · IGFBP3 gene

INTRODUCTION

Sheep is one of the most important domestic animals raised in Egypt. Sheep population in Egypt increased by 66.7 % from 1961 to 2005 [1]. There are almost 5.5 million head of sheep [2]. Sheep are raised mainly for meat production with carpet sheep wool as a secondary product [3].

The three major Egyptian sheep breeds are: Barki, Rahmani and Osseimi, They represent about 65% of the total sheep population in Egypt [4]. The Egyptian sheep are characterized as small-to medium-sized body, fat tailed and fleece is coarse wool breeds [5].

Meat is an integral part of a healthy diet and meat consumption continues to increase steadily, while the world's producers of livestock for red meat production are finding it difficult to cope with the increase in demand [1]. Sheep contribute 6% of the total red meat produced and are considered the second source of red meat in Egypt [6].

At the present time, the consumers demand for sheep meat is not focused on quantitative traits only, but also qualitative traits of meat. Animal breeders could accelerate the rate of genetic improvement attained in carcass composition and meat quality traits through the application of gene-assisted selection (GAS), based simply on incorporating some candidate genes in traditional breeding programs [7].

The allele variation within candidate genes between breeds is warranted to differentiate them on genetic basis. The result of this variation may assist in choosing the best strategy of improvement options for these genetic resources [8].

There are many published articles on different genes associated with meat-related traits in different sheep breeds, among these genes are *MSTN* and *IGFBP-3*.

Myostatin (*MSTN)* gene,(also known as growth and differentiation factor 8, GDF8) is the major regulator of myogenesis and it functions as a negative regulator of muscle growth in mammals. Mutations in *MSTN* are associated with increased skeletal muscle mass (double muscling) in sheep [9, 10]. The regulation of muscularity, adiposity and tendon structure potentially has important implications for sheep meat production. Ovine *MSTN* is located on OAR 2. Myostatin is known to directly influence muscular hypertrophy and carcass conformation [11].

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Insulin-like growth factor binding protein-3 (*IGFBP3*) is a specific carrier protein that binds non-covalently to IGFs in circulation [12]. It acts as a marker for different body functions such as growth, metabolism, reproduction, in controlling body weight, immunity and energy balance [13]. *IGFBP-3* has been localized to OAR21 [14] Polymorphism in *IGFBP3* has been studied in different live stocks. There are a few reports in cattle [15], buffalo [16] and sheep [17], which suggest the polymorphic/non-polymorphic nature of *IGFBP-3* gene.

The aim of the present study is to identify the genetic polymorphism for two genes correlated with meat gain and traits (*MSTN* and *IGFBP-3*) in three Egyptian sheep breeds (Barki, Rahmani and Osseimi). Application of Polymerase Chain Reaction-Restriction Fragment Length polymorphisms (PCR-RFLPs) will be carried out.

MATERIALS AND METHODS

Animals and Blood Collection: A total of 60 animals representing the three Egyptian sheep breeds understudy, Osseimi, Barki and Rahmani were randomly sampled (20 samples per 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 EDTA as anticoagulant and transported to the laboratory under cooled conditions.

DNA Isolation: DNA was extracted and purified from blood samples using the whole blood salting out technique described by Miller *et al.* [18]. DNA concentration and purity were determined using a UV spectrophotometer at optical density of 260 and 280 nm.

Polymerase Chain Reactions (PCR): Reactions were performed using specific primers for each gene under study. Details of the primer sequences are listed 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. The general PCR program for the amplification of the genes included in the current study was: initial denaturation: 95°C for 3 min., 95°C for 15 sec. (denaturation), 58–63°C for 30–60 sec. (annealing depending on the gene) and 72°C for 30 sec. up to 35 cycles, then final extension at 72°C for 5 min. and finally storage at 15°C.

Table 1: List of primer sequences and restriction enzymes of candidate genes

Gene symbol	Primer séquences	Restriction enzyme
MSTN	'TGGCGTTACTCAA AAGCAAA	*Dra I*
	AACAGCAGTCAGCAGAGTCG	
IGFBP-3	CCAAGCGTGAGACAGAATAC'	*HaeIII*
	AGGAGGGATAGGAGCAAGAT	

For PCR optimization the temperature and the time of the annealing temperature were changed. The success of PCR was tested after running some of the products on 2% horizontal agarose gel electrophoresis and staining with ethidium bromide.

Restriction Fragment Length Polymorphism (RFLP): Ten microliters from the PCR products were digested with 5 units of the fast restriction enzyme including specific buffer (Fermentas, Germany) specific for each gene (Table 1) in a final reaction volume of 15 µl. The reaction mixture was incubated at 37°C in water bath for 30 minutes.

After restriction digestion, the restricted fragments were visualized after running in horizontal gel electrophoresis (2-3 % agarose) and staining with ethidium bromide, except *IGFBP*3 reaction mixtures were running in vertical polyacrylamide gel electrophoresis (since the cut fragment size was small). The 100-bp ladder was used as molecular size marker. The bands were visualized under UV light and the gels were photographed using digital gel documentation system (Bio-Rad, USA). The allele sizes were determined using free software named Lab. Image V2.7. It is dispersed free from Proland company (Germany), from the internet through the web page: http://www.labimag-ing.com/servlet/ engine/home/ start.html.

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 [19].

RESULTS AND DISCUSSION

Genetic Polymorphism of *MSTN* Gene Digested with *Dra1*: The ovine *MSTN* gene produced a DNA fragment of 497 bp (Figure 1). Digestion of this fragment by restriction endonuclease *Dra1* generated only two different genotypes, AB and BB, no AA genotype was found. The uncut was related to the A allele, while the cut fragment (427 and 70 bp) was corresponding to the B allele (Figure 2). In general the A allele was characterized

Table 2: Genotypic and allelic frequencies of *MSTN* gene digested with *Dra1*

Locus		Genotypic Frequency			Allelic Frequency	
Restriction enzyme.	Breed	AA	AB	BB	A	B
MSTN (*Dra1*)	Barki	0	0.45	0.55	0.23	0.77
	Rahmani	0	0.35	0.65	0.17	0.83
	Osseimi	0	0.15	0.85	0.07	0.93

Table 3: Observed heterozygosity (Ho), expected heterozygosity (He) and $\chi 2$ estimates of *MSTN* gene digested with *Dra1*

Locus Restriction enzyme.	Breed	Ho	He	$\chi 2$
MSTN (*Dra1*)	Barki	0.45	0.36	1.47
	Rahmani	0.35	0.30	0.75
	Osseimi	0.15	0.14	0.08

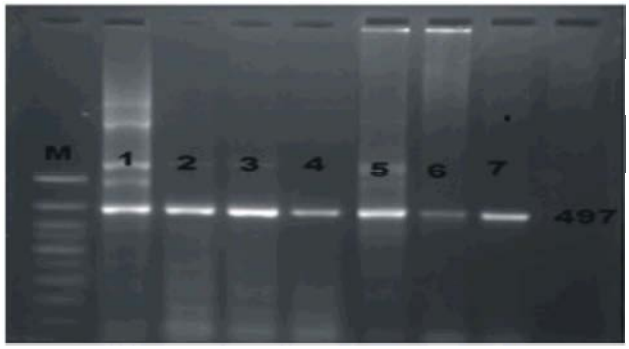

Fig. 1: Detection of PCR products of *MSTN* gene on 2% agarose gel electrophoresis. Allele size for the *MSTN* was about 497 bp. Lane M, 100bp DNA ladder. Lanes (1, 2) Barki, (3→5) Rahmani and (6, 7) Osseimi breeds

Fig. 2: DNA electrophoretic pattern of *MSTN*amplicon after digestion with *Dra1* endonuclease. Lane M, 100bp DNA ladder, lanes (1, 2, 3, 6, 10, 11, 12) genotype AB (497, 427and 70bp) and lanes (4, 5, 7, 8, 9) genotype BB (427 and 70). It was difficult to classify the 70 bp fragment on 2.5% agarose gel since it could not be seen

by the deletion of a small DNA fragment (TTTTA). This deletion was found in 5′UTR of goat *MSTN* gene and causing a significant effect on body weight and size [20].

Xianglong and his colleges [20] reported that such deletion was unique for goats. The present study indicates that this deletion can be also seen in different sheep breeds and not limited to goats.

It was observed that the BB genotype was the more common genotype in all breeds under study; this might be due to the low frequency noticed for the allele A (Table 2).

The values of the observed heterozygosity were higher than the expected ones in all breeds (Table 3). The frequency of the B allele was significantly higher than that of A allele, especially in Osseimi breed. This may explain the lowest heterozygosity in this breed.

Absence of AA genotype and presence of AB genotype in Egyptian sheep's may be due either to a mutation (deletion) in corresponding locus or allele A is naturally exists in the sheep genome. Further studies, especially sequencing of the heterozygous genotype (AB) is recommended.

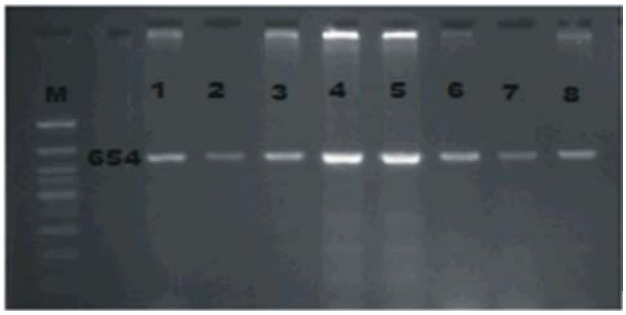

Fig. 3: Detection of PCR products of *IGFBP-3* gene on 2% agarose gel electrophoresis. Allele size for the *IGFBP-3* was about 654 bp. (Lane M, 100bp DNA ladder). Lanes (1→3) Barki, (4→5) Rahmani and (6→8) Osseimi breed

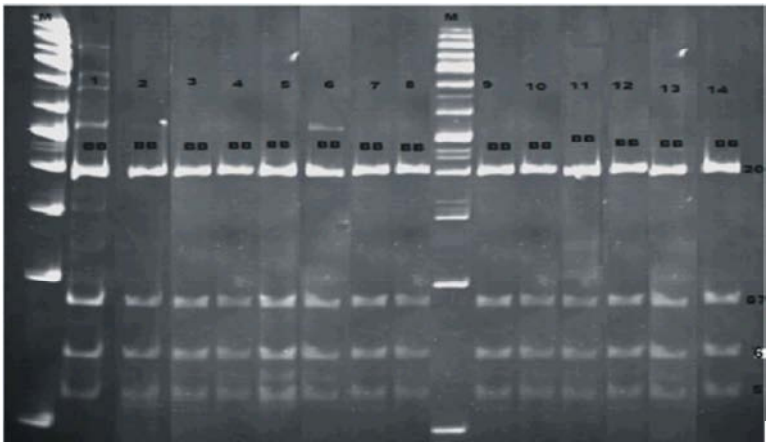

Fig. 4: Polyacrylamide gel electrophoresis for DNA of *IGFBP-3*amplicon after digestion with *HaeIII* endonuclease. Lane M, 50 bp DNA ladder, all lanes (1→14) genotype BB (201, 201, 87, 76 and 57bp). It was difficult to classify the (19, 16 and 8 bp) fragments on polyacrylamide gel

The highest heterozygosity either observed (0.45) or expected (0.36) was in Barki sheep. The lowest values, 0.15 and 0.14 were in Osseimi but Rahmani has moderate value of 0.35 for (Ho) and 0.30 for (He) (Table 3).

HWE of breeds was determined by using χ2-test. The *MSTN* locus *Dra1* had χ2 value of 1.47, 0.75 and 0.085 for Barki, Rahmani and Osseimi respectively. χ2 values indicate non-significant deviation from HWE (P>0.05) (Table 3).

Genetic Polymorphism *of IGFBP-3* Gene Digested With *HaeIII*: The length of the amplified product of ovine *IGFBP-3* gene was 654 bp in sheep (Figure 3). The amplified 654 bp fragment comprises the last part of exon 2, complete intron 2, exon 3 and a part of intron 3. The exon–intron regions were assigned on the basis of the published reports of this gene in cattle [21].

It is clear from *HaeIII* RFLP pattern represented in Figure (4) that, there was no polymorphism among the three Egyptian sheep breeds in respect to *IGFBP-3* gene. Digestion of the PCR product of *IGFBP-3* gene with *HaeIII* revealed only one type of restriction pattern including fragments of sizes 201, 201, 87, 67, 57, 19, 16 and 7 bp (The first five fragments were visible) which represent the allele B only. This result indicates the homozygosity of this gene in the three studied sheep breeds.

The present findings are similar to those reported by Kumar *et al.* [16] who found no polymorphism in *IGFBP-3* gene in five Indian sheep breeds. However, they obtained *HaeIII* restriction pattern of eight fragments of sizes 201, 201, 87, 67, 57, 19, 16 and 7 bp in all animals studied revealing absence of polymorphism in these Indian sheep breeds. Also, the present results showed only one genotype (BB) in the three Egyptian sheep breeds.

Ali *et al.* [22] reported the same result in the four Egyption sheep breeds (Rahmani, Osseimi, Barki and Awassi) indicating the homozygosity of this gene in the four breeds studied. They also reported that all the sheep have intact *HaeIII* restriction site (GG↓CC) indicating the absence of polymorphism at this site.

CONCLUSION

The gene *MSTN* was polymorphic in all breeds examined. Barki sheep breed showed the highest observed heterozygosity for *MSTN* genes. The same breed also showed the highest genotypic frequency (AB) for *MSTN* gene, followed by Rahmani then Osseimi.The *IGFBP3* gene was monomorphic, therefore this is not recommended in the selection program. In order to have sharp molecular markers especially for economically important traits such as gain and meat traits in the Egyptian sheep breeds, studies must be carried out on the candidate genes on a large smaple size from each bread.

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