Journal of Genetic Engineering and Biotechnology xxx (2017) xxx–xxx

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Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Original Article

Genetic variability of myostatin and prolactin genes in popular goat breeds in Egypt

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ARTICLE INFO

Article history:
Received 12 February 2017
Received in revised form 25 September 2017
Accepted 5 October 2017
Available online xxxx

Keywords:
Genetic polymorphism
Goat
PCR-RFLP
Myostatin
Prolactin
SNPs

ABSTRACT

The genetic polymorphisms of two functional genes named: myostatin (*MSTN*) and prolactin (*PRL*) were investigated in three goat breeds (Barki, Damascus and Zaraibi) using Sanger nucleotide sequence and restriction fragment length polymorphism (RFLP) methods, in order to differentiate between these breeds. Nucleotide sequencing of 337 bp *MSTN* gene detected five SNPs in Barki breed, two SNPs in Damascus breed, while the Zaraibi breed did not show any SNPs. Moreover, *MSTN-HaeIII*/PCR-RFLP gave a single Genotype BB was found in all the studied breeds. Meanwhile, Nucleotide sequencing of 196 bp *PRL* gene showed two SNPs in Damascus breed, one SNPs in Zaraibi breed, while the Barki breed did not show any SNPs. Moreover, *PRL-Eco24I*/PCR-RFLP showed three genotypes (AA, AB and BB). The genotype AB showed the maximum frequency in all the studied breeds (0.75, 0.85, and 0.90 for Damascus, Barki and Zaraibi breeds, respectively). Observed heterozygosity (Ho) value was higher than expected heterozygosity (He) value all studied breeds. In addition, the values of both Ho and He were the highest in Zaraibi breed (0.90 and 0.51 respectively). Chi-square (χ^2) value revealed a significant variation Hardy-Weinberg equilibrium ($P < .05$) in the three studied breeds. It is the highest in Zaraibi goats and lowest in Damascus breed. The results demonstrated that the *PRL-Eco24I*/PCR-RFLP polymorphism may be utilized as effective marker for genetic differentiation between goat breeds, but *MSTN-HaeIII*/PCR-RFLP revealed no polymorphism or variation, thus it is not recommended in the selection program. Moreover, these results open up interesting prospects for future selection programs, especially marker assisted selection. In addition, the results established that PCR-RFLP method is a suitable tool for calculating genetic variability.

1. Introduction

Genetic variations at candidate genes touching economic traits (like growth, milk yield, meat production and reproductive traits) have stimulated research interest because they stayed well-considered as an aid to genetic selection and to mark evolutionary relationships in different livestock animal breeds [1]. In this aspect, myostatin (*MSTN*) as well as prolactin (*PRL*) are important potential genes due to their positive effect on growth enactment and meat quality traits.

Myostatin (*MSTN*) gene, also named as growing and differentiation factor-8 (*GDF-8*) gene, is coding for converting growth factor-beta (*TGF-β*) super-family (one of the largest protein groups). This gene was physically mapped to goat chromosome 2q11-q12 [2,3], and consists of two introns and three exons [4]. Additionally, it is a powerful candidate gene, needful for growth and development of domestic animals due to its key function in muscularity, and its potential applications in animal farming [5]. Mutations in *MSTN* gene can quiet its expression or create a non-functional protein, which causes undesired muscularity (i.e. dramatic rise in both muscle fiber quantity [hyperplasia] and mass [hypertrophy]) or the "double-muscling" phenomenon in various species [6], such as dogs [7], pig [8], goat [Boer goat] [9] and, sheep [10].

Prolactin (*PRL*) gene plays a key role in changeable growth, variation and lactation, the hair growth cycle [11]. Many researchers recorded that *PRL* polymorphisms are related to wool or cashmere traits in goat and sheep [12–14]. Others studied the association polymorphism of *PRL* with dairy traits like benefit yield besides

Peer review under responsibility of National Research Center, Egypt.
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https://doi.org/10.1016/j.jgeb.2017.10.005
1687-157X/

the yield of protein milk in cattle breeds [15–17]. Furthermore, many researcher suggested the significance of *PRL* gene in improving prolificacy in different sheep breeds [18,19].

Goat remains one of main important livestock kinds and affords a variation of products, such as fiber, milk, meat, and hides. Furthermore, goats are used as a model for biomedical studies [20,21]. Goats in Egypt are almost 3.13 million goats; they are extended essentially in three regions: Upper Egypt, Nile Delta besides in the desert rangelands [22]. 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 breed intended for milk and bucks bred for meat [23].

The aim of the present study is to screen the genetic polymorphism of two functional genes (*MSTN* and *PRL*) in three goat breeds (Barki, Damascus and Zaraibi) via nucleotide sequence and PCR-RFLP methods in order to differentiate between these breeds.

2. Materials and methods

2.1. Animals

A total of 60 healthy goats, belonging to the three breeds understudy: Barki, Damascus and Zaraibi; 20 samples from each breed. All animals were born and reared in the Agriculture Research Station, belonging to Faculty of Agriculture, Cairo University.

2.2. DNA extraction

Blood samples were collected in tubes containing 0.5 M 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 [24]. The DNA concentration was measured using the U.V spectrophotometer at wavelength 260 nm.

2.3. Polymerase Chain Reaction (PCR)

Two pairs of primers were used for amplifying each of *MSTN* and *PRL* loci using primers suggested by [25,26], 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 sterile de-ionized double distilled H_2O up to a total volume of 25 µl. Amplification conditions are shown in Table 2. The gels were stained with ethidium bromide and visualized under ultraviolet light.

2.4. Restriction Fragment Length Polymorphism (RFLP)

It was carried out in15 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 digest restriction enzyme (MBI fermentas, Germany) specific for each gene (Table 1). The reaction mixture was incubated at 37 °C in water bath for a certain time as demonstrated in Table 1. 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 images were captured using digital gel documentation system (Bio-Rad, USA).

2.5. DNA sequencing

The PCR products representing different patterns and alleles of tested genes were purified and sequenced by Macrogen Incorporation (Seoul, South Korea) to identify the SNPs between different patterns and alleles. Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite.

2.6. Statistical analysis

The genotypic and allelic frequencies, the observed and expected heterozygosity and the χ^2 test for Hardy-Weinberg equilibrium (HWE) were calculated using Pop Gene 32.1 package [27].

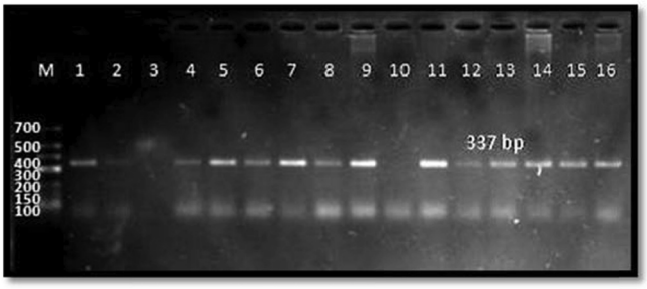

Fig. 1. Agarose gel electrophoresis of *MSTN*-PCR fragment (337 bp). Lane M, 25 bp DNA ladder. Lanes (1, 2, 4, 5, 6), Barki (7, 8, 9, 11), Damascus and Lanes (12, 13, 14, 16) Zaraibi breed.

Table 1
Gene, primer sequence (5′ → 3′), length of PCR product, region and specific restriction enzyme of MSTN *and PRL* genes.

Gene	Primer sequence (5′ → 3′)	PCR product length	Region	Restriction enzyme	Incubation	References
MSTN	F: CCG GAG AGA CTT TGG GCT TGA R: TCA TGA GCA CCC ACA GCG GTC	337 bp	Exon 3	HaeIII	37 °C for 10 minutes	Azari et al. [25]
PRL	F: ATTCCTGGAGCCAAAGAG R: TGTGGGCTTAGCAGTTGT	655 bp	Exon 5	Eco24I	37 °C overnight	Lan et al. [26]

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	
MSTN	94/240	94	60	55.5	35	72	120	72	240	35
PRL	95/300	94	30	5635		72	30	72	600	35

Please cite this article in press as: Abdel-Aziem SH et al. Genetic variability of myostatin and prolactin genes in popular goat breeds in Egypt. Journal of Genetic Engineering and Biotechnology (2017), https://doi.org/10.1016/j.jgeb.2017.10.005