Identification of MSTN/*DraI* polymorphism in ten Bulgarian sheep breeds

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Abstract

The success of sheep production depends largely on two important characteristics of sheep – their growth ability and meat quality. The myostatin gene (MSTN) is one of the major regulators of skeletal muscle growth and development and it is defined as a suitable candidate gene for genetic improvement through marker-assisted selection. In this study by means of PCR-RFLP method was identified allelic variants of intron 1 of MSTN gene in ten Bulgarian sheep breeds – Askanian (30 animals), Caucasian (30 animals), Karnobat Merino (60), Local Karnobat (30), Karakachan (60), Breznik (30), Cooper-Red Shoumen (30), Il de France (60), Bulgarian Dairy Synthetic Population (60) and Pleven Black headed (30). Genomic DNA was extracted from 420 blood samples. After PCR amplification, the received 497 bp fragment of the myostatin gene was digested with the restriction enzyme *DraI*. The results showed that this area of the myostatin gene is highly conservative in Bulgarian sheep breeds. All of the investigated ten sheep breeds were monomorphic for the tested locus. Only the B allele and genotype BB were found in all studied animals.

Key words: sheep, polymorphism, MSTN gene, PCR-RFLP method

Introduction

Selection in farm animals depends on productive direction and external traits of the individuals. Conventional breeding strategies by estimating breeding values need a lot of efforts, time and funds. Sheep breeding could be improved by identifying different genes associated with economically important traits. Identification of genetic potential of breeds is required for conservation and development of modern trends in animal husbandry. Improvement of sheep has focused on the selection of breeding individuals with superior phenotypes. Application of molecular markers and polymorphisms at the DNA level could contribute to obtaining animal production with better qualities. The application of candidate genes could be in great use of early selection of animals based on identification of specific genotype to improve the economically important traits (Deb et al., 2012; Yadav et al., 2017; Khalil, 2020).

Growth differentiation factor-8 (GDF-8) or myostatin (MSTN) gene plays an essential role in regulating skeletal muscle growth and lipid metabolism and connects with enlarged skeletal muscle mass in different species of mammals, including sheep (An et al., 2011). It is a negative

regulator of muscle mass deposition (Grade et al., 2019). Animals with MSTN deficiency show an increase in skeletal muscle mass known as double muscling (Aiello et al., 2018). MSTN is a member of the transformational growth factor (TGF) -β superfamily and plays an important role in regulating development, tissue homeostasis and reproduction (Du et al., 2007). The sheep myostatin gene (MSTN) is expressed both in developing and adult skeletal muscles. Whole gene size is 6757 bps (GenBank no NC_040253.1), whereas transcript consists of 1128 bps (Zerbino et al., 2018; Grochowska et al., 2020). It is located at the end of the long arm (2q32.2 locus) on a chromosome 2 of the sheep genome (Boman et al., 2009). The ovine GDF-8 gene consists of three exons and two introns and its exons encode a 375-amino acid precursor protein (Jeanplong et al., 2001; Bellige et al., 2005). Aiello et al. (2018) summarized 77 reported SNPs in the myostatin gene in different sheep breeds, most of which are located in non-coding regions of the gene except for 1-bp deletion (MSTN: c.960delG) in Norwegian white sheep and 1-bp insertion (c.120insA) in NZ Romney. European sheep breeds have not been extensively studied for myostatin polymorphisms, especially in intron 1, so it is important to determine which mutations are present in which breed and what the effects of such mutations are. It is necessary to study each breed separately to determine the effect of polymorphism in it (Kolenda et al., 2019).

The aim of present study was to study and determine the genetic diversity in intron 1 of MSTN gene in 10 sheep breeds in Bulgaria.

Materials and methods

Animals

The research team tested a total of 420 animals belonging to 10 different sheep breeds as follows: Askanian (AS) – 30 animals, Breznik (BR) – 30 animals, Caucasian (CA) – 30 animals, Karnobat Merino (KTM) – 60 animals, Local Karnobat (LK) – 30 animals, Karakachan (KK) – 60 animals, Cooper-Red Shoumen (CRS) – 30 animals, Il de France (IdF) – 60 animals, Bulgarian Dairy Synthetic Population (BDSP) – 60 animals and Pleven Black Headed (PBH) – 30 animals. For the purpose of the investigation, a blood sample was taken from each individual from the jugular vein into a vacuum tube containing EDTA as an anticoagulant agent.

DNA extraction

The experimental work was conducted in the laboratory of genetics, part of the Agronomy Faculty at University of Forestry, Sofia, Bulgaria. DNA was extracted and purified from whole blood by manual commercial kit for DNA purification according to the manufacturer’s instruction (Illustra Blood GenomicPrep DNA Purification Kit, GE Healthcare, US) by technique described by Miller et al. (1988). The DNA concentration and purity of each sample was determined by spectrophotometer Biodrop. The quality of the obtained about 10–50 ng DNA was tested on 1% agarose (Bioline, UK) gel prepared with TAE buffer (Jena Bioscience, Germany).

PCR amplification

The polymerase chain reaction analysis was carried out in total volumes of 10 μl, containing 4 μl DNA template, 0.2 μl dd H_2O, 0.4 μl of each primer and 5 μl of 2 × (1.5 mM $MgCl_2$) MyTaq TM HS Red Mix 2x (Bioline, UK). Li et al. (2008) suggested the primer set (Thermo, US): forward primer: 5′-TGGCGTTA CTCAAAAGCAAA -3′ and reversed primer: 5′-AACAGCA GTCAGCAGAGTCG-3′.

PCR reactions were accomplished by thermal cycler QB-96 (Quanta Biotech) under the following conditions: initial denaturation at 95 °C/5 min, followed by 30 cycles of: denaturation at 95 °C/30 s, annealing at 58 °C/45 s, extension at 72 °C/1 min and final elongation at 72 °C/10 min.

RFLP analysis

The genotypes of all tested animals were identified using restriction fragment length polymorphism analysis (RFLP). All PCR products were digested separately in 10 μl final volume, containing 6 μl PCR product, 2.5 μl dd H_2O, 10 U/μl restriction speed enzyme *DraI* and dilution buffer (Jena Bioscience, Germany). The specific

endonuclease recognizes the following palindrome sequence:

5' ---TTT↓AAA--- 3'
3' ---AAA↑TTT--- 5'.

The digestion reactions were carried out at 37 °C for 10–20 min in thermal block. The fragment sizes were determined on 2.8 % agarose (Bioline) gel stained by RedGel Nucleic Acid Stain (Bioline, UK). The fragment sizes were established by DNA Ladder 50 bp (Thermo, US). The obtained results were visualized under UV light.

Results and discussion

After DNA purification were received 420 samples with extracted genomic DNA. The quality and quantity of the probes were tested on 1% agarose gel and by spectrophotometer. The obtained results were shown on Figure 1.

PCR-RFLP approach was suitable tool for identification of allele variant in MSTN sheep gene. For all tested samples were received PCR products of ovine MSTN gene with expected length of 497 bp.

When the 497 bp fragment of MSTN intron 1 obtained by amplification is treated with the restriction endonuclease Dra1, it is possible to identify on the agarose gel two allelic variants – allele A – with one uncut 497 bp fragment and allele B – with two visible fragments (427 bp and 70 bp).

Fig. 2 clearly showed identified fragments from the tested animals. It is obvious that all samples revealed the same allelic pattern. In all investigated breeds it was observed only allele B and only genotype BB, respectively. This genotype was characterized with two visible fragments on the agarose gel (427 bp and 70 bp) (Figure 2, Table 1).

Similar results were obtained by Al-Barzinji et al. (2020) in a study of a commercial flock of 52 Awassi ewes. The results obtained by them show only one genotype in all samples, with only allele B and genotype BB present. Dehnavi et al. (2012) in 200 (190 ewes and 10 rams) animals from Iranian sheep breed Zel also observed monomorphism of intron 1 in the myostatin gene.

The results obtained by another research team with randomly selected 60 individuals from 3 Egyptian sheep breeds – Oseimi, Barki and Rahmani, differ significantly from those obtained by us. They found the presence of both allelic variants – A and B and two genotypes – heterozygous AB and homozygous AA in all three breeds. Genotype AA was not identified in this study. Differences in the frequency of alleles were found in different breeds – allele A has the highest frequency in the breed Barki – 0.23, in Rahmani is 0.17, and in Osseimi is 0.07 (Shafey et al., 2014).

In our previous studies, animals of Bulgarian sheep breeds were studied for the genetic diversity of exon 3 of the myostatin gene (Dimitrova et al., 2017; Dimitrova et al., 2019; Bozhilova-Sakova et al., 2022), but monomorphism in the study area was also found. These results show the high degree of conservatism of the myostatin gene in Bulgarian sheep breeds.

Conclussions

The present study showed that this site in intron 1 of the ovine myostatin gene is highly conservative in Bulgarian sheep breeds. All of the investigated ten sheep breeds were monomorphic for the tested locus. Only the B allele

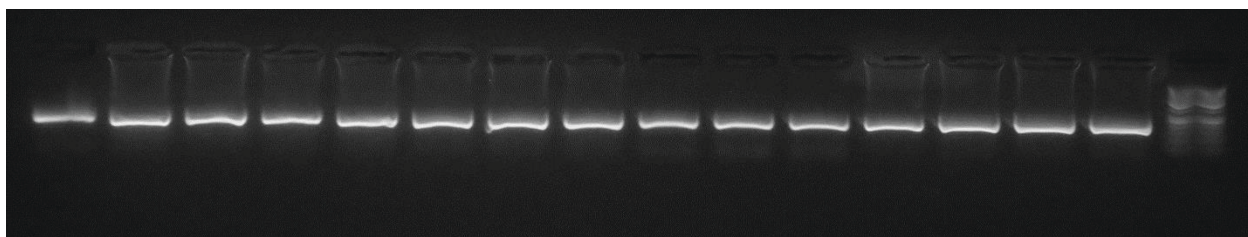

Fig. 1. Extracted DNA samples tested on 1% agarose gel

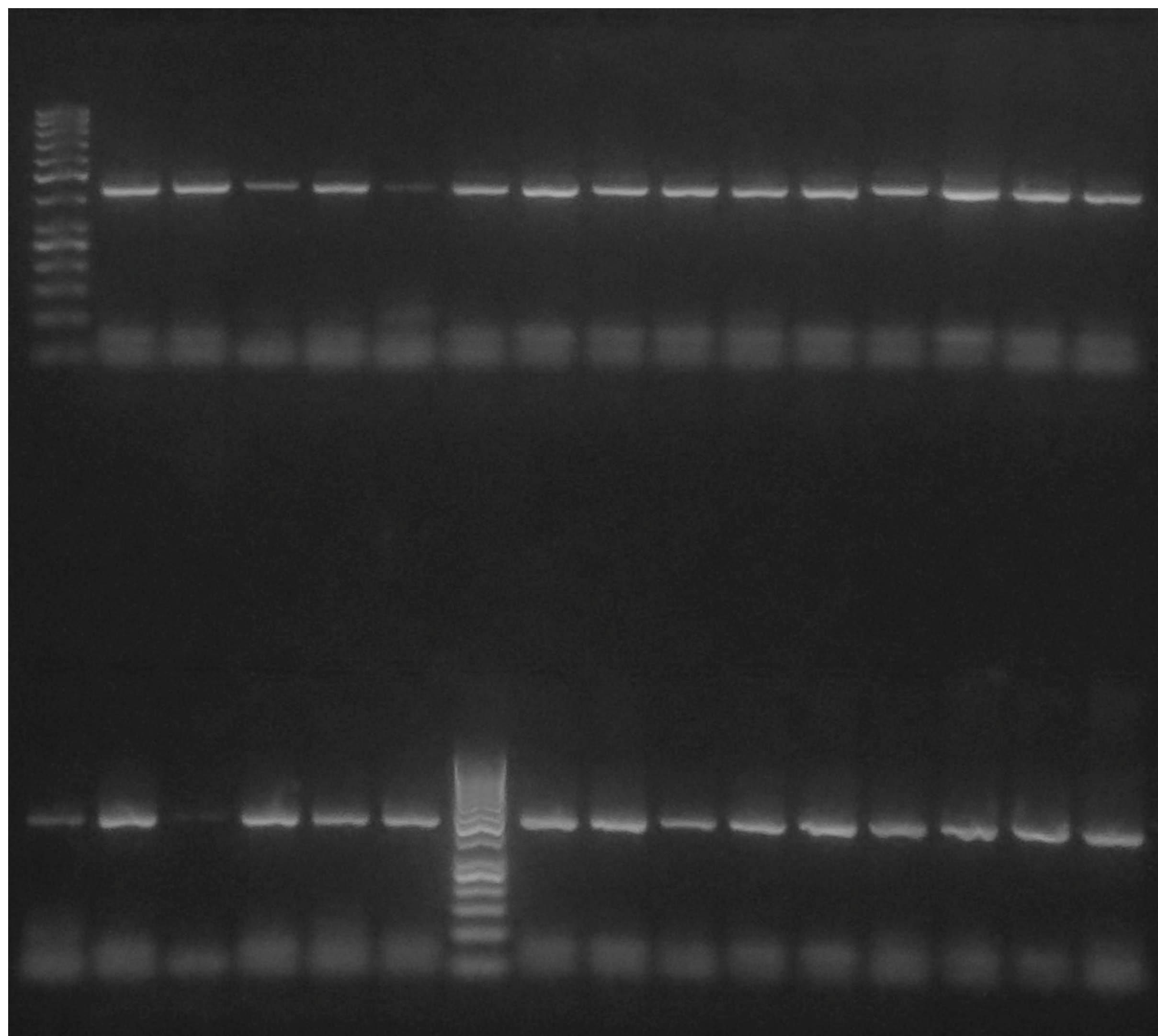

Fig. 2. Restriction fragments of the tested breeds visualized on 2,8 agarose gel under UV light

Table 1. Tested breed, allele and genotype frequencies, heterozygosity and p-value

Breed	n	Allele frequencies		Genotype frequencies			Heterozygosity		P value
		A	B	AA	AB	BB	Ho	He	
AS	30	0.00	1.00	0.00	0.00	1.00	0.000	0.000	
BR	30	0.00	1.00	0.00	0.00	1.00	0.000	0.000	
CA	30	0.00	1.00	0.00	0.00	1.00	0.000	0.000	
KTM	60	0.00	1.00	0.00	0.00	1.00	0.000	0.000	
PBH	30	0.00	1.00	0.00	0.00	1.00	0.000	0.000	
LK	30	0.00	1.00	0.00	0.00	1.00	0.000	0.000	NS
KK	60	0.00	1.00	0.00	0.00	1.00	0.000	0.000	
CRS	30	0.00	1.00	0.00	0.00	1.00	0.000	0.000	
IdF	60	0.00	1.00	0.00	0.00	1.00	0.000	0.000	
BDSP	60	0.00	1.00	0.00	0.00	1.00	0.000	0.000	

and genotype BB were found in all studied animals.

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