Genetic polymorphism of the melatonin receptor (MTNR1A) gene in populations of fine-fleece sheep breeds in Bulgaria

Milena Bozhilova-Sakova[1*], Ivona Dimitrova[2]

[1]*Agricultural Academy, Institute of Animal Science, Kostinbrod, Bulgaria*

[2]*University of Forestry, Sofia, Bulgaria*

[*]E-mail: bojilova_milena@abv.bg

Citation: Bozhilova-Sakova, M., & Dimitrova, I. (2022). Genetic polymorphism of the melatonin receptor (MTNR1A) gene in populations of fine-fleece sheep breeds in Bulgaria. *Zhivotnovadni Nauki*, *59*(3), 16-20 (Bg).

Abstract

In the present study was investigated the single nucleotide polymorphism in exon II of the melatonin receptor (MTNR1A) gene in a total of 90 individuals representing three fine fleece sheep breeds: Caucasian Merino, Ascanian Merino and Karnobat Merino. By means of PCR-RFLP analysis two allele variants of MTNR1A gene (*C* and *T*) and three genotypes (*CC*, *CT* and *TT*) have been identified. The obtained genotype frequencies of alleles *C* and *T* in the investigated sheep breeds were: 0.60 and 0.40 in Caucasian Merino sheep, 0.47 and 0.53 in Askanian Merino sheep, 0.60 and 0.40 in Karnobat Merino sheep, respectively. The genetic diversity at MTNR1A gene was with the highest value in the population of Ascanian Merino (H_o = 0.533 и H_e = 0.498). The lowest coefficient of inbreeding (F_{is} = -0.070) was also observed in this group and it was in correspondence with HWE equilibrium (p = 0.4). The frequency of mutant allele *T* was higher (0.53) than the wild allele *C* (0.47). In Caucasian and Karnobat Merino breeds the allele frequencies were the same – 0.60 and 0.40 for allele *C* and allele *T*, respectively. Despite the same allelic frequencies, the two breeds had different distributions in the genotype frequencies, with the most significant difference in the heterozygous genotype *CT* – 0.40 for Caucasian Merino and 0.27 for Karnobat Merino. The population of Karnobat Merino sheep breed was not consistent with HWE with p = 0.02. The results in this study showed polymorphism in exon II of the ovine MTNR1A gene in all tested breeds. Due to that fact, it could be concluded that this gene is suitable for genetic marker.

Key words: sheep, reproduction, PCR, RFLP, MTNR1A gene, polymorphism

Introduction

Sheep fertility is an economically important complex trait influenced by many environmental and genetic factors. Composite traits such as reproduction are not affected by traditional selection and do not result in desirable genetic improvement. It is known that sheep reproduction is highly influenced by seasonality, which is a major obstacle to improving animal fertility. The work in this direction is aimed at extending the breeding season. Changes in the duration of the day can significantly influence the breeding abilities of sheep which have maximum reproductive activity during short days (Giantsis et al., 2016).

Melatonin is called the "hormone of darkness" because it is synthesized by the pineal gland during the night. The study of the mechanism of seasonal reproduction in sheep is extremely important, as species with a clearly defined circadian rhythm. Short photoperiods have a positive effect on increasing melatonin levels.

Higher melatonin levels stimulate the secretion of follicle-stimulating hormone and luteinizing hormone from the pituitary gland. Melatonin acts through two specific receptors called 1A and 1B. In sheep, the A1 receptor has been shown to be the major receptor that mediates reproductive and the circadian cycle. Polymorphisms in the MTNR1A gene have been associated with seasonal breeding in different sheep breeds (Ahmad et al., 2015; Fathy et al., 2018).

In the present study was investigated the single nucleotide polymorphism in exon II of the melatonin receptor (MTNR1A) gen in a total of 90 individuals representing three fine fleece sheep breeds: Caucasian Merino, Ascanian Merino and Karnobat Merino.

Materials and methods

The experiment was conducted in laboratory of Genetics, part of the Agronomy faculty at University of Forestry, Sofia, Bulgaria. The object of the investigation were 90 ewes belonging to three fine fleece sheep breeds – 30 from Caucasian Merino, 30 from Ascanian Merino and 30 from Karnobat Merino. Approximately 3 mL of peripheral blood were collected from each individual in vacuum tubes containing anticoagulant EDTA. The samples were transported to the laboratory and stored at -20 °C until the experimental part.

Genomic DNA was extracted from whole blood taken from *vena jugularis* using a genomic DNA purification kit (Illustra Blood Genomic Prep DNA Purification Kit, GE Healthcare) according to the manual instructions. Spectrophotometric (spectrophotometer Biodrop) and agarose gel (1% agarose gel) electrophoresis methods were used to determine DNA quality and quantity. The DNA concentration were about 12–15 ng/µl. PCR reactions were prepared in total volume of 10 µl containing 40 ng genomic DNA, 0.2 µl dd H_2O, 20 pM of each primer and 5 µl of ready-to-use 2×(1.5 mM MgCl2) MyTaqTM HS Red Mix (Bioline). A PCR fragment of exon 2 of the ovine MTNR1A gene (GenBankU14109) was amplified with specific primers as described by Messer et al. (1997) with the following sequences:
forward 5'-TGTGTTTGTGGTGAGCCTGG-3'
reverse 5'-ATGGAG AGGGTTTGCGTTTA-3'

The following PCR conditions were used for amplification of MTNR1A gene: primary denaturation 94 °C / 5 min; 30 times - Denaturation 94 °C / 30 s, Annealing 62 °C / 45 s, Elongation 72 °C / 1 min; Final elongation 72 °C / 10 min.

The PCR products of the MTNR1A gene were digested by fast *RsaI* enzyme (Jena Bioscience) according to Saxena et al. (2015 a). The reaction was conducted in 10 µL volume containing 6 µL of amplicon, 0.5 µL of enzyme, 1 µL of 10 buffer and 2.5 µL of ddH_2O. The incubation was performed at thermal block for 15–20 min at 37 °C.

The fragments after restriction analysis were separated by agarose gel electrophoresis using 50 bp DNA Ladder (Thermo) on 2,8% agarose gel (Bioline) stained by 10000x RedGelTM Nucleic Acid Stain (Biotuim) and 1x TBE buffer (Jena Bioscience). The results were visualized under UV light.

Results and discussion

After DNA extraction from the whole blood were received 90 samples with DNA with concentration about 12–15 ng/µl (Figure 1).

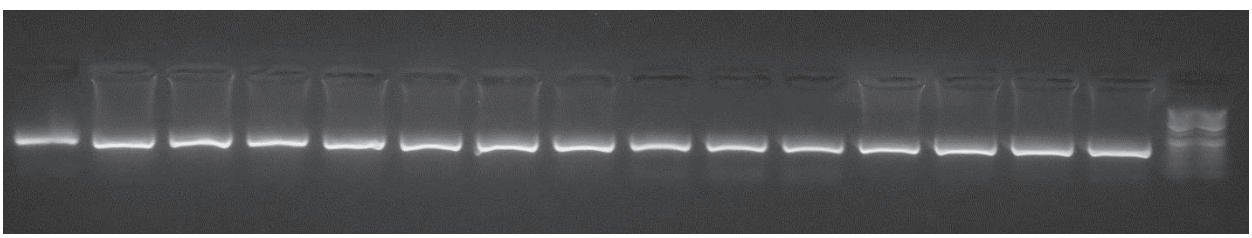

Fig. 1. Extracted DNA samples tested on 1% agarose gel

PCR fragments with expected sizes of 824 bp, corresponding to exon 2 of the MTNR1A gene, was obtained from sheep DNA using specific primers.

By means of PCR-RFLP and *RsaI* enzyme digestion analysis two allele variants of MTNR1A gene (*C* and *T*) and all three genotypes have been identified – *CC* (411 bp, 267 bp), *CT* (411 bp, 290 bp, 267 bp) and *TT* (411 bp, 290 bp) (Figure 2). The obtained genotype frequencies of alleles *C* and *T* in the investigated sheep breeds were: 0.60 and 0.40 in Caucasian Merino sheep, 0.47 and 0.53 in Askanian Merino sheep, 0.60 and 0.40 in Karnobat Merino sheep, respectively. The genetic diversity of MTNR1A gene was with the highest value in the population of Ascanian Merino (H_o = 0.533 и H_e = 0.498). The lowest coefficient of inbreeding (F_{is} = -0.070) was also observed in this group and it was correspondence with HWE equilibrium (p = 0.4). The frequency of mutant allele *T* was higher (0.53) than the wild allele *C* (0.47). In Caucasian and Karnobat Merino breeds the allele frequencies were the same – 0.60 and 0.40 for allele *C* and allele *T*, respectively. Despite the same allelic frequencies, the two breeds had different distributions in the genotype frequencies, with the most significant difference in the heterozygous genotype *CT* – 0.40 for Caucasian Merino and 0.27 for Karnobat Merino. The population of Karnobat Merino sheep breed was not consistent with HWE with p = 0.02. The results in this study showed polymorphism in exon II of the ovine MTNR1A gene in all tested breeds. The allelic and genotypic frequencies of the MTNR1A were shown in Table 1. Due to that fact, it could be concluded that this gene is suitable for genetic marker in sheep.

1 – DNA Ladder 50 bp; 2, 3, 4, 5, 6, 7, 12 – heterozygous genotype *CT* (411, 290, 267 bp); 8, 9, 10, 15 – homozygous genotype *TT* (411, 290 bp); 11, 13, 14, 16 – homozygous genotype *CC* (411, 267 bp)

Limited genetic information using RFLP markers is available on sheep breeds in Bulgaria. Gencheva (2019), studied the single nucleotide polymorphism in exon II of the melatonin receptor (MTNR1A) gene on a total of 177 individuals representing four local Bulgarian sheep breeds: Copper-red Shumen, Karakachan, Pleven Blackhead and Sofia (Elin-Pelin). Two genetic variants of MTNR1A gene (*C* and *T*) and three genotypes (*CC*, *CT* and *TT*) have been identified

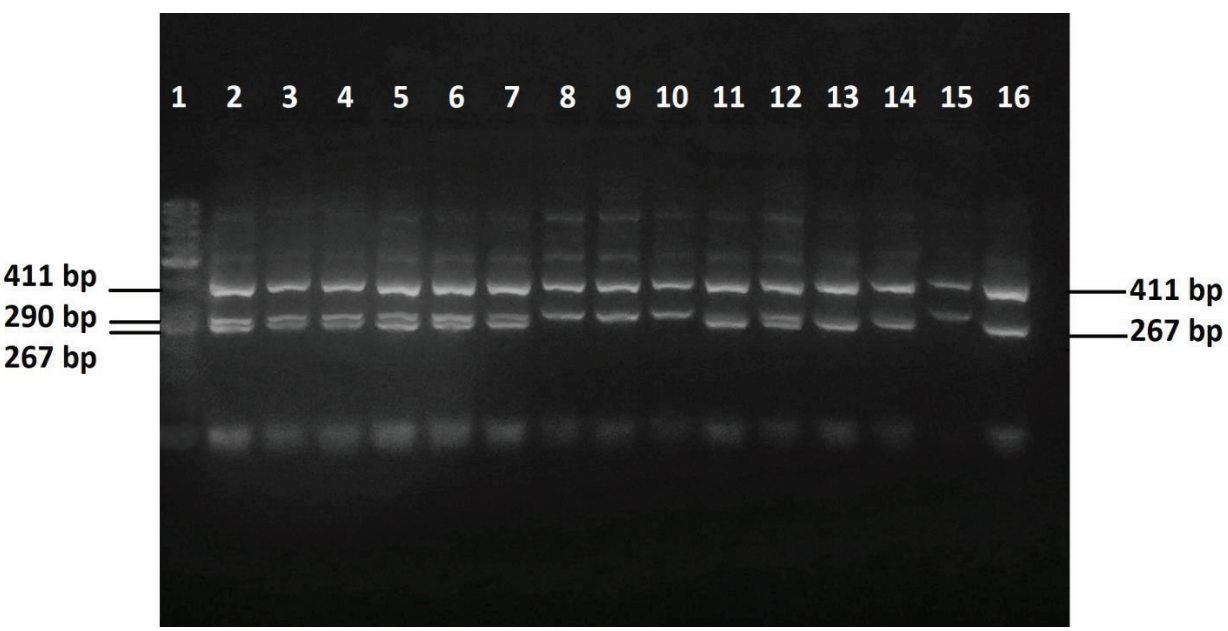

Fig. 2. Agarose gel electrophoresis (2.8%) showing PCR RFLP of exon 2 of MTNR1A using the *RsaI* enzyme

Table 1. Allele and genotype frequencies, heterozygosities, coefficient of inbreeding, chi-square value and p-value of the investigated sheep breeds

Breed	n	Allele frequency		Genotype frequencies			Heterozygosity		Fis	X^2	p
		C	T	CC	CT	TT	Ho	He			
CA	30	0.60	0.40	0.40	0.40	0.20	0.400	0.480	0.166	0.57	0.4*
AS	30	0.47	0.53	0.20	0.53	0.27	0.533	0.498	-0.070	0.21	0.6*
KM	30	0.60	0.40	0.46	0.27	0.27	0.266	0.480	0.445	5.19	0.02**

*$p > 0.05$ – *statistically insignificant differences in HWE equilibrium*
**$p < 0.05$ – *statistically significant differences, not consistent with HWE*

using PCR-RFLP analysis. The obtained gene frequencies of MTNR1A *C* and *T* in two investigated sheep breeds, were contrary: 0.694 and 0.306 in Pleven Blackhead sheep, versus 0.267 and 0.733 in Copper-red Shumen sheep, respectively. Similar allele C/T frequencies were found in the other sheep breed: 0.589 and 0.411 in Karakachan and 0.605 and 0.395 in Sofia sheep, respectively. The genetic diversity at MTNR1A gene was with the highest value in Sofia sheep population (H_o = 0.512 and H_e = 0.489), low coefficient of inbreeding (F_{is} = -0.070) and in correspondence with HWE equilibrium (p = 0.701). An average level of genetic diversity was established in the populations Copper-red Shumen, Karakachan and Pleven Blackhead (H_e = 0.391, 0.489 and 0.428), high coefficient of inbreeding (F_{is} = 0.432, 0.495, 0.258) and HWE deviation (p = 0.002, 0.006 and 0.049).

Worldwide two polymorphic regions in MTNR1A gene have been studied by means of PCR-RFLP technique. Thirty Istrian sheep were tested for polymorphisms at the locus 606 and 612 of the MTNR1A gene by PCR-RFLP method. All three genotypes were determined in both loci (606: *CC* 0.17; *CT* 0.40; *TT* 0.43; 612: *GG* 0.64; *GA* 0.33; *AA 0.03*) and allele frequencies were: *C* 0.37; *T* 0.63; *G* 0.80 and *A* 0.20. In investigated sample of Istrian sheep high frequency of genotype *GG* and allele *G* that are characteristic for out-off-season lambing breeds was determined. Although Istrian sheep were characterized by a genetic predisposition for out-off-season lambing, they are more difficult to achieve by using current technological procedures subordinated to milk production (Držaić et al., 2020).

Saxena et al. (2015) investigated 101 animals of the Chokla breed raised in an experimental farm of the Central Sheep and Wool Research Institute (C.S.W.R.I.), Avikananagar, Rajasthan, India. The aim was to determine the polymorphism in exon-2 of MTNR1A at positions C606T and G612A from the gene locus using the two specific endonucleases *RsaI* and *MnlI*, as well as to search for new mutations with influence on seasonal estrus and reproductive period in sheep.

Cosso et al. (2021) investigated the genetic variation in the coding and promoter region of MTNR1A, in 165 sheep aged 5.2 and 1.5 years, of the Awassi breed and its association to animal fertility. Thirty-one single nucleotide polymorphisms (SNPs) have been identified, five of which are missense mutations, some of which influenced phenotypic parameters.

This gene was characterized with high genotype variation in different sheep breeds, which identified it as suitable molecular marker for investigation of genetic basis of seasonal reproduction in sheep.

Conclusions

The results in this study showed the presence of all three possible genotype of MTNR1A gene in the three tested breeds – Ascanian, Caucasian and Karnobat Merino. Due to that fact it could be concluded that this gene is suitable for genetic marker and it could be implemented in marker assisted selection (MAS).

Acknowledgements

Research was part of the project ДФНИ КП-06-H26/9 (2018–2021) ”Investigation of DNA markers associated with production in sheep breeds raised in Bulgaria” financed by NSF – the Ministry of Education, Youth and Science, Republic of Bulgaria.

References

Fathy, H. A., Gouda, E. M., Gafer, J. A., Galal, M. K., & Nowier, A. M. (2018). Genetic polymorphism in melatonin receptor 1A and arylalkylamine N-acetyltransferase and its impact on seasonal reproduction in Egyptian sheep breeds. *Archives Animal Breeding*, *61*(4), 505-516.

Messer, L. A., Wang, L., Tuggle, C. K., Yerle, M., Chardon, P., Pomp, D., ... & Rothschild, M. F. (1997). Mapping of the melatonin receptor la (MTNR1A) gene in pigs, sheep, and cattle. *Mammalian genome*, *8*(5), 368-370.

Saxena, V. K., Jha, B. K., Meena, A. S., Narula, H. K., Kumar, D., & Naqvi, S. M. K. (2015). Melatonin receptor 1A (MTNR1A) gene sequence characterization and SNP identification in tropical sheep breeds of India. *Receptors & Clinical Investigation*, *2*. 1–5.

Ahmed, A. M. (2008). Biological evaluation of Barki sheep under two different breeding seasons. *Egyptian Journal of Animal Production*, *45*(1), 15-24.

Giantsis, I. A., Laliotis, G. P., Stoupa, O., & Avdi, M. (2016). Polymorphism of the melatonin receptor 1A (MNTR1A) gene and association with seasonality of reproductive activity in a local Greek sheep breed. *Journal of Biological Research-Thessaloniki*, *23*(1), 1-4.

Držaić, V., Ramljak, J., Kasap, A., Širić, I., Antunović, Z., & Mioč, B. (2020). MTNR1A gene polymorphisms in Istrian sheep population. *55. hrvatski i 15. međunarodni simpozij agronoma, 2020 godine, Vodice, Hrvatska, 16.-21. veljače 2020. Zbornik radova*, 424-428.

Gencheva, D. (2019). Genetic polymorphism of melatonin receptor (MTNR1A) gene in populations of local sheep breeds. *Zhivotnovadni Nauki, 56*(1), 21-32 (Bg).

Cosso, G., Nehme, M., Luridiana, S., Pulinas, L., Curone, G., Hosri, C., ... & Mura, M. C. (2021). Detection of Polymorphisms in the MTNR1A Gene and Their Association with Reproductive Performance in Awassi Ewes. *Animals*, *11*(2), 583-590.