

Original Article

No polymorphism of melatonin receptor 1A (MTNR1A) gene was found in Markhoz goat

Sargol Abdolahi*, Borhan Shokrollahi* , Nazila Saadati† and Salim Morammazi‡

*Department of Animal Science, Agriculture School, Sanandaj Branch, Islamic Azad University, Kurdistan, Iran, †Department of Biology, Basic Sciences Faculty, University of Kurdistan, Kurdistan, Iran and ‡Department of Animal Science, Faculty of Agricultural and Natural Resources, University of Persian Gulf, Bushehr, Iran

Abstract

Melatonin is the main hormone of seasonal breeding in sheep and goat which has an effect on reproductive organs via its receptors. Studies have shown that mutations in melatonin receptor 1A (MTNR1A) gene are related to litter size as well as the ovulation rate in sheep and goats. In this study, polymorphism of two loci in MTNR1A melatonin receptor gene was studied in order to survey their relationship with litter size in Markhoz goats. PCR primers were employed to mask polymorphisms of MTNR1A in 150 does by PCR-RFLP method. After DNA extraction, the PCR-RFLP was performed using *Eco*3II and *Hpa*I restriction enzymes. Results showed that these loci were not polymorphic. These results show that the fecundity of Markhoz goats is not linked to MTNR1A. No polymorphism in MTNR1A was found in Markhoz goats, therefore, it is essential to test polymorphism of other genes or loci to facilitate marker-assisted selection techniques to improve reproduction traits in Markhoz goats.

Keywords: melatonin, MTNR1A receptor, polymorphism, Markhoz goat.

Correspondence: Borhan Shokrollahi, Sanandaj Branch, Islamic Azad University, Sanandaj, Kurdistan, Iran. E-mail: Borhansh@gmail.com

Introduction

Goat production is an activity mainly for landless laborers and small farmers in developing countries. In goat production systems, high reproduction efficacy and kidding rate are very important. Litter size is affected by various factors in goats (Amoah & Gelaye 1990; Pan *et al.* 2015). Genetic factors, as the most important factors, may have more effect than other factors affecting the litter size. Genetic markers that are associated with litter size are very important to induce improvement of the next generation through the selection of high potential parents.

Identification of the effect of genetic markers on traits is nowadays achieved through the candidate gene approach, which recognizes the genetic polymorphisms causing phenotypic differences. It is employed to accelerate the improvement of reproductive traits (Wu *et al.* 2009; An *et al.* 2010). Physiological control of the reproductive traits is organized

by multiple genes that can be important candidates for unraveling the genetic variation in economically related traits in farm animals (An *et al.* 2010).

Melatonin, which is generated rhythmically by the pineal gland, transmits photoperiod signals (Goldman 2001). The circadian efficacy of melatonin has been shown to be mediated by its receptors in the hypothalamic suprachiasmatic nucleus (the location of circadian and its effects on reproductive functions mediated by its receptors in hypophyseal pars tuberalis (Reppert *et al.* 1994; Jia *et al.* 2012). MTNR1A and MTNR1B, two G-protein coupled melatonin receptors, have been isolated and characterized (Reppert *et al.* 1994; Dubocovich & Markowska 2005). However, MTNR1A is suggested to be the main receptor associated with the control of seasonal reproductive activity (Dubocovich *et al.* 2003). In addition, the central roles of MTNR1A gene in reproductive traits have made it as a robust candidate gene for litter size in animals such as sheep

(Chu *et al.* 2006; Mateescu *et al.* 2009), as well as goats (Carcangiu *et al.* 2009) and pigs (Ramírez *et al.* 2009). Capra MTNR1A gene is situated on chromosome 26 and consists of two exons with one intron (Reppert *et al.* 1994). Studies in pig, sheep and cattle breeds have shown polymorphisms in the MTNR1A gene (Messer *et al.* 1997; Lai *et al.* 2013).

Markhoz (Angora) goats are raised in western Azerbaijan, Kurdistan and Kermanshah provinces of Iran (Shokrollahi 2015; Bahmani 2017). The mohair obtained from these animals has an important cultural role and is used for making of local clothes in Kurdistan (Bahmani 2017). The population of this breed has been decreased to <10% from 30 years ago due to urbanization and lack of a clear breeding policy and genetic improvement of the breed. Therefore, the main problem for Markhoz goats is the reduction in population size resulting in an endangered status. On the other hand, the variation in the kidding size of Markhoz goats makes them an interesting genetic material to study the underlying genetic mechanism of prolificacy (Bahmani 2017).

The aim of this study was the investigation of allelic variation in two loci of MTNR1A gene and their association with litter size in Markhoz goats.

Materials and methods

Animals

Whole blood samples of 150 Markhoz does at the goat research station in Sanandaj city, Kurdistan province, were collected. There were 281 breeding females but a different number of sires (15–20) were used per year in the flock. The required information about the animals including litter size, season of kidding and parity (2–3) were provided.

Genomic DNA preparation

About 10 mL of blood was collected aseptically from the jugular vein into sodium citrate. All samples were taken back to the laboratory and stored at -20°C until DNA extraction. The genomic DNA was extracted from white blood cells by the salting-out process (Miller *et al.* 1988). The DNA samples were dissolved in TE buffer (pH = 8.0) and stored at -20°C for use.

Primer sequences

Primer sequences and product size of its amplicons, as well as restriction enzymes, are given in Table 1. The primers were used to amplify the interested regions according to Lai *et al.* (2013). The primers were manufactured by CinnaGen Co., Ltd. The restriction endonucleases (RE) were purchased from Fermentas Co., Ltd.

Polymorphism screening and detection

PCR-RFLP was used to identify two *Eco3II* and *HpaI* mutations based on methods described by Lai *et al.* (2013). PCR amplification of MTNR1A gene was carried out in 25 μl reaction mixture, including 50 ng of genomic DNA template, 0.5 μM each primer, 1 X PCR buffer, 1U Taq polymerase, 2 mM MgCl_2 and 200 μM of each dNTPs. The thermal cycling conditions were as follows: 95°C for 5 min and 35 cycles at 94°C for 30 s, annealing temperature for 40 s and with temperature according to table 1, and 72°C for 30 s followed by 72°C for 10 min. Following the digestion with both enzymes overnight at 37°C , the outcome products were segregated by electrophoresis with 3%

Table 1. Primer sequence, annealing temperature and restriction enzyme.

Mutation	Primer sequence (5'–3')	Annealing temperature	Region of the gene	Restriction enzyme
MTNR1A1	Forward: GCCTGGCAGTTGCAGACCTG	57	175–1030	<i>Eco3II</i>
	Reverse: CATTITTAACGGAGTCCACC		Acc. No: AB716764.1	
MTNR1A2	Forward: AGCTCAGCCTACACGATCGC	52	522–725	<i>HpaI</i>
	Reverse: CCAGCAAATGGCAAAGAGGAC		Acc. No: AB716764.1	

agarose gel and visualized by ethidium bromide. The MTNR1A possible genotypes were identified according to Lai *et al.* (2013) research. A point genetic variation was set to create a restriction site *Eco3II* (^GTCTC^) in the products of PCR. The strand of wild-type of PCR products was sliced with *Eco3II* restriction digestion generates a 279 and 577 bp at the same time non-carrier products stayed uncut at 856 bp. Also, to produce a restriction site for *HpaI* enzyme (G^GGCC^C) a point mutation was set in the PCR products. With *HpaI* restriction digestion of PCR products, the wild-type strand was sliced and constructed a 185 bp and 83 bp even though non-carrier products continued uncut at 268 bp.

Results

In this study, the MTNR1A1 and MTNR1A2 mutations were explored in the Markhoz goat breed. Genomic DNA of goats was amplified using MTNR1A1 and MTNR1A2 primer pairs. Then products of PCR were digested by restriction enzymes (*Eco3II* and *HpaI*) to survey the existence of genetic variations. The digested PCR products were run on electrophoresis with 3% agarose gel, which bands with 856 bp and 268 bp in length were observed for the two loci in all 150 female goats, respectively (Figs 1, 2). In general, these loci in Markhoz goats were wild homozygous, as a result, no one of the samples accepted the genetic variations in MTNR1A gene.

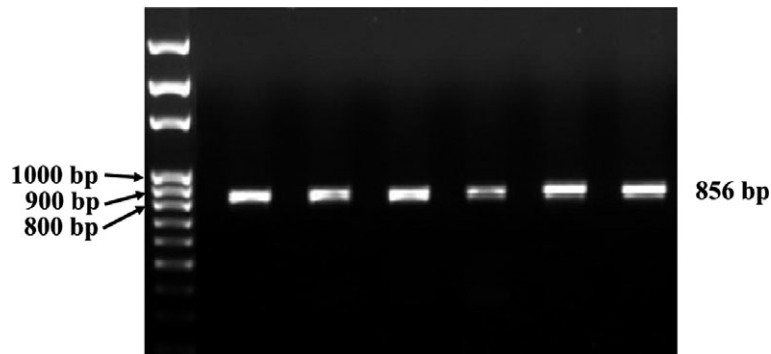


Fig. 1 An agarose gel electrophoretogram for MTNR1A1 locus product digested with *Eco3II* showing genotypes in Markhoz goats. All individuals show wild type allele (uncut) with 856 bp in length.

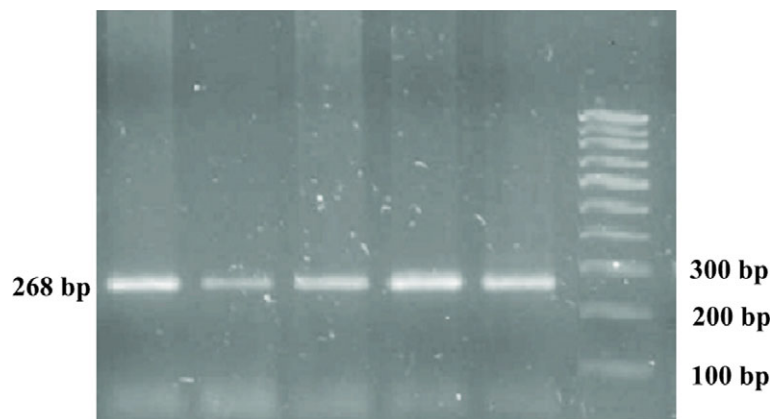


Fig. 2 An agarose gel electrophoretogram for MTNR1A2 locus product digested with *HpaI* showing genotypes in Markhoz goats. All individuals show wild type allele (uncut) with 268 bp in length.

Discussion

Reduction in reproductive seasonal activity and changes in kidding rate are significant economic issues in the goat rearing industry. To date, many studies have been performed to improve the kidding rate. However, small heritability and notable impacts of epigenetic factors on reproductive traits reduced improvement of phenotypic variations within goat populations. Therefore, animal breeders are exploring the genes associated with major effects on reproductive traits to use them for marker-assisted selection and gene introgression to improve such traits. Genetic variations in *MTNR1A* play an important role in modulating melatonin effects because any change in the protein receptor sequence could affect its binding to the ligand and alter the cAMP signal transduction pathway (Pan *et al.* 2015). In this regard, some studies revealed that some SNPs in melatonin receptor gene led to alteration of the amino acid sequence of the receptor (Mazna *et al.* 2005). This study aimed to investigate the polymorphisms in *MTNR1A* gene in Markhoz goat, the results showed that the genotypes of 150 Markhoz does were all identical and monomorphic for two *MTNR1A* gene loci. There is an agreement between our results and others that declare no existence of cleavage sites for the restriction enzyme in *MTNR1A* (Migaud *et al.* 2002; Chu *et al.* 2006; Carcangiu *et al.* 2009). However, some authors have identified extensive variations among species in allele frequency and the effect of *MTNR1A* loci genotype on reproductive traits (Carcangiu *et al.* 2009, 2011). In sheep, it has been shown that the polymorphisms at with *RSAI* and *MNLI* loci of *MTNR1A* gene were associated with seasonal reproduction and litter size (Chu *et al.* 2006; Carcangiu *et al.* 2009; Meena *et al.* 2013). Moreover, Luridiana *et al.* (2012) reported that polymorphisms in the *MTNR1A* gene were rigorously bound with reproductive activity in Italian buffaloes. Carcangiu *et al.* (2009) suggested that a polymorphism in *MTNR1A* gene was linked to reproduction traits in Sarda goat. Also, Lai *et al.* (2013) demonstrated that there was a genetic variation in *MTNR1A* gene in Gulin Ma goat but no relationship with seasonal reproduction was found.

Previous DNA genotyping studies revealed moderate genetic diversity in Markhoz goat (Simaei-Soltani *et al.* 2016). Shokrollahi (2015), Khani *et al.* (2015), and Shokrollahi & Morammazi (2018) reported a very little genetic variation among the Markhoz goat. Results from this study pointed out no existence of polymorphism in *MTNR1A1* and *MTNR1A1* loci. Further investigations are needed to find polymorphic sites in *MTNR1A* gene and other genes that may affect litter size in Markhoz goat.

Acknowledgements

This work was financially supported by Sanandaj Branch, Islamic Azad University.

Conflict of interest

The authors declare no conflicts of interest

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

References

- Amoah E. & Gelaye S. (1990) Reproductive performance of female goats in South Pacific countries. *Small Ruminant Research* **3**, 257–267.
- An X.-P., Dan H., Hou J.-X., Guang L., Wang Y.-N., Ling L. *et al.* (2010) Polymorphism of exon 2 of *FSHβ* gene and its relationship with reproduction performance in two goat breeds. *Agricultural Sciences in China* **9**, 880–886.
- Bahmani H. (2017) The status of Markhoz goat rearing in Kurdistan province. Proceedings of the World goat day symposium, Karaj, Iran, 2017.
- Carcangiu V., Mura M.C., Vacca G.M., Pazzola M., Dettori M.L., Luridiana S. & Bini P.P. (2009) Polymorphism of the melatonin receptor *MT1* gene and its relationship with seasonal reproductive activity in the Sarda sheep breed. *Animal Reproduction Science* **116**, 65–72.

- Carcangiu V., Luridiana S., Vacca G.M., Daga C. & Mura M.C. (2011) A polymorphism at the melatonin receptor 1A (MTNR1A) gene in Sarda ewes affects fertility after AI in the spring. *Reproduction, Fertility, and Development* **23**, 376–380.
- Chu M.X., He Y.Y., Cheng D.X., Ye S.C., Fang L. & Wang Y.Y. (2006) Association between expression of reproductive seasonality and alleles of melatonin receptor 1A in goats. *Animal Reproduction Science* **101**, 276–284.
- Dubocovich M.L. & Markowska M. (2005) Functional MT1 and MT2 melatonin receptors in mammals. *Endocrine* **27**, 101–110.
- Dubocovich M.L., Rivera-Bermudez M.A., Gerdin M.J. & Masana M.I. (2003) Molecular pharmacology, regulation and function of mammalian melatonin receptors. *Frontiers in Bioscience* **8**, 1093–1108.
- Goldman B.D. (2001) Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. *Journal of Biological Rhythms* **16**, 283–301.
- Jia L., Chu M.X., Di R., Cao G., Feng T., Chen H., *et al.* (2012) Polymorphism and structure of exon 2 of caprine melatonin receptor 1b gene and its relations to fertility and seasonal estrus. *Animal Science Papers and Reports* **30**, 169–179.
- Khani K., Abdolmohammadi A.R., Foroutanifar S. & Zebarjadi A. (2015) Association of myostatin gene exon 3 polymorphism with twinning trait in Markhoz goat. *Journal of Agricultural Biotechnology* **7**, 105–117.
- Lai P., Wang P.Q., Chu M.X., Song W.J. & Cai B.J. (2013) Polymorphism of the melatonin receptor genes and its relationship with seasonal reproduction in the Gulin Ma goat breed. *Reproduction in Domestic Animals* **48**, 732–737.
- Luridiana S., Mura M.C., Pazzola M., Paludo M., Cosso G., Dettori M.L. *et al.* (2012) Association between melatonin receptor 1A (MTNR1A) gene polymorphism and the reproductive performance of Mediterranean Italian buffaloes. *Reproduction, Fertility, and Development* **24**, 983–987.
- Mateescu R., Lunsford A. & Thonney M. (2009) Association between melatonin receptor 1A gene polymorphism and reproductive performance in Dorset ewes. *Journal of Animal Science* **87**, 2485–2488.
- Mazna P., Berka K., Jelinkova I., Balik A., Svoboda P., Obsilova V. *et al.* (2005) Ligand binding to the human MT2 melatonin receptor: the role of residues in transmembrane domains 3, 6, and 7. *Biochemical and Biophysical Research Communications* **332**, 726–734.
- Meena A.S., Kumar R., Kumari R., Jyotsana B., Prince L.L.L. & Kumar S. (2013) Genetic polymorphism of melatonin receptor 1A (MTNR1A) gene in Indian sheep breeds. *Indian Journal of Animal Research* **83**, 1284–1287.
- Messer L.A., Wang L., Tuggle C.K., Yerle M., Chardon P., Pomp D. *et al.* (1997) Mapping of the melatonin receptor 1a (MTNR1A) gene in pigs, sheep, and cattle. *Mammalian Genome* **8**, 368–370.
- Migaud M., Gavet S. & Pelletier J. (2002) Partial cloning and polymorphism of the melatonin1a, (Mel1a) receptor gene in two breeds of goat with different reproductive seasonality. *Reproduction* **124**, 59–64.
- Miller S.A., Dykes D.D. & Polesky H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**, 1215.
- Pan S., Biswas C.K., Majumdar D., Sengupta D., Patra A., Ghosh S. & Haldar A. (2015) Influence of age, body weight, parity and morphometric traits on litter size in prolific Black Bengal goats. *Journal of Applied Animal Research* **43**, 104–111.
- Ramírez O., Tomàs A., Barragan C., Noguera J., Amills M. & Varona L. (2009) Pig melatonin receptor 1a (MTNR1A) genotype is associated with seasonal variation of sow litter size. *Animal Reproduction Science* **115**, 317–322.
- Reppert S.M., Tsai T., Roca A.L. & Sauman I. (1994) Cloning of a structural and functional homolog of the circadian clock gene period from the giant silkworm *Antheraea pernyi*. *Neuron* **13**, 1167–1176.
- Shokrollahi B. (2015) Investigation of BMP15 gene polymorphisms associated with twinning in Markhoz goat. *Biharean Biologist* **9**, 1–4.
- Shokrollahi B. & Morammazi S. (2018) Polymorphism of GDF9 and BMPR1B genes and their association with litter size in Markhoz goats. *Reproduction in Domestic Animals* **53**, 971–978.
- Simaei-Soltani L., Abdolmohammadi A., Zebarjadi A. & Foroutanifar S. (2016) Genetic diversity and distance of Iranian goat breeds (Markhoz, Mahabadi and Lori) compared to the Beetal breed using inter-simple sequence repeat (ISSR) markers. *Archives Animal Breeding* **59**, 477–483.
- Wu W., Hua G., Yang L., Wen Q., Zhang C., Zoheir K.M. & Chen S. (2009) Association analysis of the INHA gene with litter size in Boer goats. *Small Ruminant Research* **82**, 139–143.