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

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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 EcoRI and HpaI 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.

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 hypothalamic 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 Eco31I 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 MgCl2 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% agarose gel.

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

| Mutation   | Primer sequence (5′–3′)                                                                 | Annealing temperature | Region of the gene | Restriction enzyme |
|------------|----------------------------------------------------------------------------------------|-----------------------|--------------------|-------------------|
| MTNR1A1    | Forward: GCCCTGGCAGTTGCAGACCTG                                                       | 175–1030              | Acc. No: AB716764.1 | Eco31I            |
|            | Reverse: CATTTTTAAACGGAGTCCACC                                                        |                       |                    |                   |
| MTNR1A2    | Forward: AGCTCAGCTACAGCTAGCTGC                                                       | 522–725               | Acc. No: AB716764.1 | HpaI              |
|            | Reverse: CCAGCAAATGGCAGAAGGGACC                                                      |                       |                    |                   |

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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 Eco31I (^GTCTC^) in the products of PCR. The strand of wild-type of PCR products was sliced with Eco31I 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 (Eco31I 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 Eco31I 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 & Moramazzi (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.

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**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.

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