

## Genetic polymorphism of the *melatonin receptor 1A* locus in Iranian Shall and Karakul sheep

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### Abstract

The genotypes of the melatonin receptor 1A (MTNR1A) were determined by PCR-RFLP in the native Iranian Shall and Karakul breeds of sheep. Blood samples were collected from 60 Karakul and 50 Shall breeds. Genomic DNA was extracted based on the Guanidin Thiocyanate-silica gel method. After PCR reaction, PCR products were digested by the *Mnl1* restriction enzyme. The *MTNR1A* locus had two genotypes with frequencies of 0.7 (+ +) and 0.3 (+ -) in the Karakul breed, 0.58 (+ +) and 0.42 (+ -) in the shall breed. Heterozygosity value for the *MTNR1A* locus in Shall and Karakul breeds were 0.42 and 0.3, respectively. This study provided evidence that sheep breeds, Shall and Karakul have variability in *MTNR1A* locus. Therefore, this locus can be used as a marker for the reproduction trait in selection programs.

**Keywords:** MTNR 1A; Seasonal reproductive; Melatonin; Polymorphism; Shall and Karakul sheep.

Seasonal reproductive activity is a common feature among various mammalian species of the temperate latitudes (Ortavant *et al.*, 1985). Ewes have a breeding season, characterized by succession of 16-18 days long estrus cycles, which usually appear in summer or at the beginning of autumn and finish in late winter or at the very beginning of spring (Thiery *et al.*, 2002). The seasonality of reproductive activity in sheep breeds in temperate latitudes is controlled by the photoperiod (Thiery *et al.*, 2002). The photoperiodic information is conveyed through several neural relays from the retina to the pineal gland where the light signal is translated into the daily cycle of melatonin secretion (Malpaux *et al.*, 1999). Melatonin level is high at night and low

during the day (Ganguly *et al.*, 2002). The length of nocturnal secretion of melatonin reflects the duration of night and it regulates the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Malpaux *et al.*, 1999). Changes in GnRH release induce corresponding changes in luteinising hormone (LH) secretion which are responsible for the alternating presence or absence of ovulation in the female and varying sperm production in the male (Malpaux *et al.*, 1999). Three specific melatonin receptor subtypes have been cloned and characterized in mammals: Mel<sub>1a</sub>, Mel<sub>1b</sub> and Mel<sub>1c</sub> (Migaud *et al.*, 2002). Thus so far only Mel<sub>1a</sub> and Mel<sub>1b</sub> have been found in mammalian species, but three are found in lower vertebrates. Only the Mel<sub>1a</sub> receptor is involved in central control of seasonal reproduction (Pelletier *et al.*, 2000).

In sheep two polymorphic RFLP sites within the ovine melatonin receptor 1a gene (*MTNR1A*) were discovered by Messer *et al.* (1997). A particular allelic form of the Mel<sub>1a</sub> receptor gene characterized by the absence of an *Mnl1* restriction site at position 605 of the coding sequence, leading to the homozygous genotypes (- -) when the corresponding mutation occurs on both chromosomes (Migaud *et al.*, 2002). Pelletier *et al.* (2000) also reported that the *MTNR1A* genotype was associated with seasonal reproduction in Merino Darles ewes. Notter *et al.* (2003) reported that in adult ewes (3 years old and older), fertility of ewes with genotype of mm for polymorphic *Mnl1* site in *MTNR1A* gene is 10 to 11.2% less than that in other ewes. They also this researcher reported that ewe's litter size is not significantly associated with *MTNR1A* genotype, but adult ewes with the mn genotype had approximately 11% fewer lambs per ewe than ewes of other genotypes (Notter *et al.*, 2003). The aim of the

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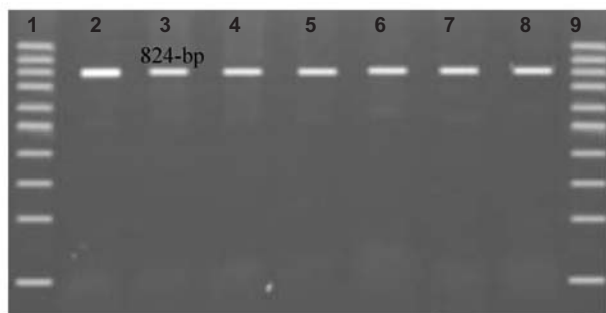
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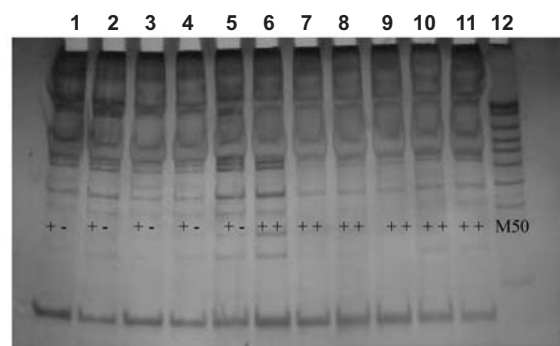
present study was to identify the different genotypes of the *MTNR1A* gene in Iranian Shall and Karakul sheep by the PCR-RFLP method.

Genomic DNA was extracted from blood samples of 60 Karakul sheep from Sarakhs Karakul Breeding Center, Mashhad and 50 Shall sheep from Smael Abad Shall Breeding Center, Ghazvin, Iran. 100 µl blood samples were used for DNA extraction as described by Boom *et al.* (1990). 50 ng of genomic DNA was used for PCR employing the primers of Messer *et al.* (1997), (*MTNR1A*) 5' TGT GTT TGT GGT GAG CCT GG 3'; (*MTNR2A*) 5' ATG GAG AGG GTT TGC GTT TA 3'. Sense primer corresponding to position 1089-1108 was used for amplification of PCR product from exons 2 of the *MTNR1A* gene with a length of 824 bp (Fig. 1). The reaction mixture consisted of 1 µl of taq polymerase, 2.5 µl of PCR buffer, 200 µM dNTPs, 3 µl of each primer and 11 µl ddH<sub>2</sub>O. PCR was performed in 35 cycles (denaturation: 94°C, 1 min; annealing: 59°C, 1 min; extension: 72°C, 2 min) and a final extension step at 72°C for 10 min.



**Figure 1.** PCR products. Lanes 1 and 9: M100 Molecular marker (1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100). Lanes 2-8: PCR products of *MTNR1A* with 824 bp size.

**Genotyping samples:** 6 µl of each PCR product was digested at 37°C using 2U of *MnlI* restriction enzyme. The polymorphic site in exon 2 of *MTNR1A* gene is located at position 605. The presence of the cleavage site for *MnlI* resulted in 236 bp and 67 bp products (allele -), while the loss of this site led to a single 303 bp (allele +). The (+ +) genotype exhibited both frag-



**Figure 2.** Restriction analysis of amplification products of *MTNR1A* in 8% polyacrylamid gel. Lanes 1-5: the (+ -) genotype (236, 67 bp). Lanes 6-11: the (+ +) genotype (303, 236 and 67 bp). Lane 12: M50 Molecular marker (500, 450, 400, 350, 300, 250, 200, 150, 100, and 50 bp).

ments of 236 and 67 bp. The (+ -) genotype exhibited 303, 236, and 67 bp products. The (- -) genotype was not detected in this study. The allele and genotype frequencies and the H-W test were calculate using Popgene software version 1.32. The *MTNR1A* locus was polymorphic in the Iranian Shall and Karakul sheep breeds. the genotype (+ +) and (+ -) for the *MTNR1A* locus were observed but the genotype (- -) was absent in both breeds (Fig. 2).

In this study two genetic variants (+ and -) with allele frequencies of 0.79 and 0.21 and 0.85 and 0.15 were observed in the Shall and Karakul breeds, respectively. Similar results were observed in Merino d'Arles ewes (Pelletier *et al.*, 2000). The most frequent genotype in the Shall and Karakul breeds was the (+ +) genotype with frequencies of 0.58 and 0.7, respectively. Mean observed heterozygosity was higher than mean expected heterozygosity. The deviation from the Hardy-Weinberg equilibrium was not detected in the Shall and Karakul breeds. The  $\chi^2$  test confirms the H-W equilibrium in both populations (Table 1). Polymorphism in the *MTNR1A* locus has been studied by many researchers. Pelletier *et al.* (2000). resulted that there is association between (- -) genotype for site *MnlI* at position 605 and seasonal anovulation activity in Merino Darles. It was shown that the effect of *MTNR1A* genotypes on fertility and litter size was not significant for mating involving ewes of all ages.

**Table 1.** Allele frequencies, observed hetrozygosity and expected hetrozygosity.

Breed	Allele (+)	Allele (-)	Genotype frequencies			Obs-Het	Exp-Het	Nei
			++	+-	--			
Shall	0.79	0.21	29	21	0	0.42	0.3352	1.4966
Karakul	0.85	0.15	42	18	0	0.3	0.2571	1.7928

Obs-Het: observed heterozygosity.  
Exp-Het: expected heterozygosity.

However in adult ewes (3 yr old and older), fertility of ewes of mm genotype was 10 to 11% less than that of other ewes. Also adult ewes with the mm genotype had approximately 0.11 fewer lambs per ewe lambing than ewes of other genotypes (Notter *et al.*, 2003). It has been shown by Pelletier *et al.* (2000) that there is association between the (- -) genotype for site *Mnl1* at position 605 and seasonal anovulation activity. Based on the experimental results of Pelletier *et al.* (2000), the present study indicates that the Shall and Karakul ewes, genetically have the potential to show estrus during short and long days.

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