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SHORT COMMUNICATION

Polymorphism in Melatonin Receptor 1A *(MTRN1A)* Gene in Chios, White Karaman and Awassi Sheep Breeds [1]

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Summary

The aim of this study was to investigate polymorphism of the melatonin receptor 1A gene *(MTNR1A)* in Chios, White Karaman and Awassi sheep breed. A large fragment of the exon II of the *MTNR1A* gene was amplified and uniform fragment of 824 bp was obtained in 138 ewes (48 Chios, 40 White Karaman and 50 Awassi) of three breeds. The PCR products was digested with restriction endonuclease *Mnl*I and *Rsa*I , and genetic polymorphism was detected by PCR-RFLP. Biallelic polymorphism was found with restriction endonuclease *Mnl*I and two genotypes were detected *Mm* (303bp, 236bp/67bp) and *MM* (236bp/67bp, 236bp/67bp). Allelic frequency for Chios, White Karaman and Awassi breeds was 0.90, 0.80 and 0.84 respectively for *M* allele; 0.10, 0.20 and 0.16 respectively for *m* allele, while genotypic frequency was 0.80, 0.60 and 0.68 for *MM* and 0.20, 0.40 and 0.32 for *Mm* respectively. No polymorphism at the *Rsa*I cleavage sites was detected in three sheep breeds.

Keywords: *Turkish sheep breeds, Melatonin receptor 1A gene, Polymorphism*

Sakız, Akkaraman ve İvesi Koyun Irklarında Melatonin Reseptör 1A *(MTRN1A)* Gen Polimorfizmi

Özet

Bu çalışma Sakız, Akkaraman ve İvesi ırkı koyunlarda melatonin reseptör 1A *(MTNR1A)* gen polimorfizminin incelenmesi amacıyla yapılmıştır. Çalışmada *MTNR1A* geni ekzon II bölgesi çoğaltılmış ve toplam 138 örnekte (48 adet Sakız, 40 adet Akkaraman ve 50 adet İvesi) 824 bç'lik tek PZR ürün elde edilmiştir. Elde edilen PCR ürünleri *Mnl*I ve *Rsa*I enzimleri ile kesilmiştir. *Mnl*I enzim kesimi sonucu iki allel belirlenmiştir. İncelenen ırklarda *Mm* (303bp, 236bp/67bp) ve *MM* (236bp/67bp, 236bp/67bp) olmak üzere iki genotip saptanmıştır. Sakız, Akkaraman ve İvesi ırkları için allel frekansları; *M* alleli için sırasıyla 0.90, 0.80 ve 0.84; *m* alleli için sırasıyla 0.10, 0.20 ve 0.16 bulunmuştur. Genotipik frekanslar ise *MM* genotipi için sırasıyla 0.80, 0.60 ve 0.68 bulunurken *Mm* genotipi için 0.20, 0.40 ve 0.32 bulunmuştur. *Rsa*I enzim kesimi için her üç ırkta da polimorfizm saptanmamıştır.

Anahtar sözcükler: *Yerli koyun ırkları, Melatonin reseptör 1A geni, Polimorfizm*

INTRODUCTION

The reproductive activity of ovine species in temperate latitude follows a seasonal pattern, influenced by annual variation in day length [1]. In sheep, this mechanism is thought to be due to melatonin, which acts in hypothalamus. Melatonin is an important hormone in animal physiology both for its role in the regulates circadian rhythms and seasonal reproduction. Other actions of the hormone are the inhibition of dopamine release from retina, vaso-regulator activity, immune modulatory roles, and effects on cell growth. Melatonin secretion is rhythmic, with peak levels occurring during night in all vertebrates examined, independent of whether the animal is diurnally or nocturnally active [2].

Photoperiod varies in its regulatory effect on the reproductive activity, depending on the species. Sheep and

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goats, maintained at temperate latitudes naturally show increased seasonal breeding activity in autumn, to give birth in spring [3]. Photoperiod is the principal environment factor which affects the succession of reproductive periods. Light signal perceived by the retina is translated into hormonal message by pineal gland through melatonin secretion [4]. Melatonin shows a low blood-concentration during daylight and high concentration during darkness [5]. High melatonin levels, typical during autumn, have a positive influence on reproduction in small ruminants [3]. Melatonin functions through specific receptors located in different areas of the central nervous system (CNS), including nuclei which regulate reproduction [3,6].

In the ovine species, ovulatory activity of ewes is generally inhibited for several consecutive months of the year, referred to as the anestrous season, which occurs in spring. In Mediterranean latitudes, great variability exists between breeds and within breeds in terms of the presence and duration of anestrous. Some ewes completely cease ovulatory activity, whereas others show isolated ovulations during anoestrus or continue to cycle throughout the year [7]. In the Turkish sheep breeds, presence of spontaneous sexual activity during autumn.

Investigation of major genes or quantitative trait loci affecting the control of seasonal reproduction in sheep and goat may be an important factor in understanding those neurophysiological processes. One of the those genes is melatonin receptor 1A (*MTNR1A*) gene [8]. The melatonin receptor 1A (*MTNR1A*) gene has been maped to ovine chromosome 26 and represents two RFLP polymorphic sites, one for *Mnl*I enzyme and one for *Rsa*I enzyme [9]. There are eight sites identified by the *Mnl*I enzyme within the amplified sequence [9-11]. Some studies on meat sheep breeds point out that the presence of the cleavage sites for *Mnl*I and *Rsa*I enzyme in position 606 and 612 respectively and investigated the effects of these genotypes on the reproductive activity [7,10]. Notter et al.[10] reported that by means of the implied relationship between allelic versions of the gene and reproductive performance of sheep, the genotype at the *MTNR1A* gene locus might become one of the markers used in studying the sexual activity of sheep.

The Chios sheep breed has a high milk yield and an outstanding prolificacy. The average litter size is 2.3. The Awassi is principally a milk breed, but meat production from this breed is also important and the twinning rate is 10-20%. The White Karaman is a breed native to Turkey with a twinning rate of 20-30% [12]. It is well known that Chios sheep are highly prolific in comparison with many other breeds.

The aim of the present study was to reveal the genetic polymorphism of *MTNR1A* gene in high prolificacy Chios and White Karaman and Awassi sheep breeds.

MATERIAL and METHODS

Animal Resources and DNA Isolation

Jugular blood samples (2 ml per ewe) were collected from 48 Chios sheep, 40 White Karaman sheep, 50 Awassi sheep using EDTA as an anticoagulant. These ewes were chosen at random. Genomic DNA was extracted from the whole blood using the phenol-chloroform method and then it was dissolved in 10mM Tris-HCl (pH 8.0) buffer.

The study was approved by the Ethical Committee of Faculty of Medicine, Firat University. 09/02, 04.10.2007.

DNA Amplification and Genotyping

Primers for PCR of Messer et al.[9] were employed corresponding to positions 285-304 (sense primer 5′-TGTGTTTGTGGTGAGCCTGG-3′) and 1108-1089 (antisense primer 5′- ATGGAGAGGGTTTGCGTTTA-3′) of the sequence (GeneBank U14109) of exon II of the ovine *MTNR1A* gene from Reppert et al.[13]. The expected amplification fragment size was 824 bp. PCR reaction was carried out in 50 µl of total volume, containing 10X PCR Buffer (50 mM/l KCl, 10 mM/l Tris-HCl (pH 8.0), 0.1% Triton X-100), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 10 pM/l of each primer, 50 ng genomic DNA and 1 U *Taq* DNA polymerase (Eppendorf AG, Hamburg Germany). PCR conditions were as follows: denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min, on Mastercycler® (Eppendorf AG, Hamburg, Germany). The PCR products were separated by electrophoresis on 2% agarose gel (Promega, Madison,WI,USA) in paralel with a 100 bp DNA marker.

PCR product of 5 µl were digested separately with 2U *Mnl*I and (Fermentas GmbH, St. Leon-Rot, Germany) and 2U *Rsa*I (Takara Bio Inc, Shiga, Japan) at 37°C overnight. PCR products of digestion were resolved by electrophoresis on a 4% agarose gel (Promega, Madison,WI,USA).

Statistical Analysis

Direct counting was used to estimate phenotype and allele frequencies of *MTNR1A* gene *MnlI* genetic variants. The Chi-Square Test (χ^2) was used to evaluate whether the populations were Hardy-Weinberg equilibrium. All calculations and χ^2 analyses were carried out using PopGene32 software [14].

RESULT

In the present study, the primers for the exon 2 of ovine *MTNR1A* gene were used for amplification genomic DNA three Turkish sheep breeds and uniform fragment was obtained after 2% agarose gel electrophoresis. The results showed that amplification fragment size 824 bp was

consistent with the target one and had good specificity, which could be directly analyzed by RFLP.

The results of this study indicated the presence of seven *MnlI* cleavage sites (221, 254, 324, 560, 582, 610 and 693) within the exon II sequence, but only one (324) was shown to be polymorphic. Allele *M* contained the restriction site for *Mnl*I and resulted in 236 and 67 bp fragments, whereas the absence of the restriction site in the *m* allele resulted in a single 303 bp fragment. Allelic and genotypic frequencies of *MTNR1A* gene for *Mnl*I site in three Turkish sheep breeds were presented in *Table 1*. As a result, a biallelic polymorphism was found with restriction endonuclease *Mnl*I and two genotypes were detected *Mm* (303bp, 236bp/67bp) and *MM* (236bp/67bp, 236bp/67bp). Allelic frequency for Chios, White Karaman and Awassi breeds was 0.90, 0.80 and 0.84 respectively for allele *M*; 0.10, 0.20 and 0.16 respectively for allele *m*, while genotypic frequency was 0.80, 0.60 and 0.68 for *MM* and 0.20, 0.40 and 0.32 respectively for *Mm*.

Table 1.*Gene and genotype frequency of the exon II of MTNR1A gene for MnlI site in three Turkish sheep breeds*

Tablo 1.*Türkiye'deki üç yerli koyun ırkında MTNR1A geninin MnlI enzim kesimi sonucu elde edilen allel ve genotip frekansları*

Breed	n	Genotype Frequency			Allele Frequency		$(\chi^2)^a$
		Mm	MM	mm	M	m	
Awassi	50	0.32	0.68	0	0.84	0.16	1.6867*Ns*
W. Karaman	40	0.40	0.60	0	0.80	0.20	2.3214*Ns*
Chios	48	0.20	0.80	0	0.90	0.10	0.5786*Ns*

a *Test of Hardy-Weinberg equilibrium;* ***Ns,*** *not significant*

The results of this study indicated the presence of four *Rsa*I cleavage sites (56, 323, 346 and 757) within exon II sequence, but none of these was shown to be polymorphic. Allele *T*, in which the polymorphic restriction sites at position 606 is absent, is characterized by the presence of the largest fragment of 290 bp length. Consequently, only one genotype detected in investigated sheep breeds, which is *TT* (290bp, 290bp).

DISCUSSION

The 824 bp product of exon 2 of the *MTNR1A* gene was digested with restriction endonucleas *Mnl*I and *Rsa*I in three Turkish native sheep breeds. For *Mnl*I site two allele were found and two genotypes were detected *Mm* and *MM*. The results showed that there was a big difference between frequency of alleles *M* and *m*. While the most common genotype was *Mm*, no *mm* homozygotes were detected in all three populations. The reason may be allele *m* could be rare; frequency of genotype *mm* could be so low that *mm* could not be detected or homozygote *mm* did not exist. This result was found the similar by Chu et al.[15]. Also, Chu et al.[16] reported that in Hu and Small Tail Han sheep breeds, *mm* genotype is very low frequencies (0.04 and 0.01 respectively).

The occurrence of *MTNR1A* gene *Mnl*I variants in this study is similar to other sheep breeds studied previously, but there is a difference with respect to gene frequencies. In this study show that the frequency of the M allele in the breeds used in this study was higher than in the other sheep breeds (Dorset, German Mutton Merino, Hampshire, Sarda, Small Tail Han, Suffolk, Soay, Ile-de France, Texel) [1, 16-18].

The results revealed the presence of four *Rsa*I cleavage sites within exon II sequence, but none of these was shown to be polymorphic. The reason for these results may be that the sheep breeds detected were different.

Samples from three sheep breeds were found to be in Hardy-Weinberg equilibrium both the *Mnl*I and *Rsa*I.

In this study, the 824 bp product of exon 2 of the *MTNR1A* gene was digested with restriction endonuclease *Mnl*I and *Rsa*I in studied populations. For *Mnl*I site, Messer et al.[9] and Notter et al.[10] find that the 286 bp and 236 bp fragments were polymorphic, in spite of this Pelletier et al.[7], Chu et al.[15], Chu et al.[16] and Carcangiu et al.[1] showed that the 303 bp and 236 bp fragments were polymorphic. The results of this study were consistent with those of Pelletier et al.[7], Chu et al.[15], Chu et al.[16] and Carcangiu et al.[1].

In conclusion, the present study showed that there was a genetic polymorphism at *MTNR1A* gene in Chios, White Karaman and Awassi sheep breeds for the first time. We can state that a biallelic polymorphism was found with restriction endonuclease *Mnl*I, whereas no polymorphism was found *Rsa*I. However future investigation is required to confirm the link with reproductive activity. Any future research should investigate genotypes and its influence on reproductive seasonality.

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