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Association of Melatonin Receptor 1A Gene Polymorphisms with Production and Reproduction Traits in Zandi Sheep

Research Article

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ABSTRACT

Melatonin regulates some major physiological processes such as maturation and function of reproductive system, pubertal development, seasonal reproduction and adaptation. The activation of melatonin hormone is mediated by melatonin receptor. Previous studies showed melatonin receptor 1A gene (MTNR1A) is highly polymorphic in seasonally breeding species. The aim of the present study was detection of MTNR1A polymorphism and its association with production (body weights at birth, 1, 3, 6, 9 and 12 month of age) and reproduction (litter size) traits in Zandi sheep using PCR-RFLP methodology. Hundred blood samples were randomly collected and genomic DNA was isolated using modified salting out method. A large fragment of exon 2 of MTNR1A gene was amplified by PCR using specific primer pairs. After amplification, the PCR product was digested with *MnlI* endonuclease. Restricted digestion allowed the determination of two alleles (M, m) and two genotypes (MM, Mm) with frequencies of 0.91, 0.09 and 0.82, 0.18, respectively. Least square means showed that MM individuals had higher body weight at one month of age (BW1) than Mm individuals ($P<0.05$). No associations were found between observed genotypes and other studied traits. In the subsequent studies using a large number of samples along with the other important parameters such as sire effect and the lamb health status which may influence lamb body weight is recommended.

KEY WORDS MTNR1A, PCR-RFLP, Zandi sheep.

INTRODUCTION

Melatonin, the pineal hormone, plays an important role in regulation of seasonal reproduction and circadian rhythms. Its effects are mediated via high affinity melatonin receptors, located on cells of the pituitary pars tuberalis (PT) and suprachiasmatic nucleus (SCN), respectively.

There are two subtypes of mammalian melatonin receptors, the MT1 (Mel1a) and the MT2 (Mel1b) melatonin receptor subtypes. Both subtypes are members of the seven-transmembrane G protein-coupled receptor family (Gall *et al.* 2002).

The melatonin is secreted from the pineal gland during the hours of darkness and acts as a hormonal message of the photoperiod in vertebrates. In mammals, melatonin has two major physiological functions:

1. Melatonin is critical for the regulation of seasonal changes in various aspects of physiology and neuroendocrine function (Bartness *et al.* 1993; Malpaux *et al.* 2001).
2. Melatonin affects the phase of circadian rhythms by a direct action on the biological clock that resides within the hypothalamic suprachiasmatic nucleus (Gall *et al.* 2002).

The MTNR1A gene has been mapped on chromosome 26 of sheep (Messer *et al.* 1997) consists of two exons divided by a large intron (Reppert *et al.* 1994). The exon one shows low degree of polymorphism (Trecherel *et al.* 2010). The exon 2 of MTNR1A gene coding for the ovine MT1 receptors is known to be highly polymorphic (Barrett *et al.* 1997;

Messer *et al*. 1997; Pelletier *et al*. 2000) and the differences occurring in the structure of receptors result from changes in the second exon (Barrett *et al*. 1997). Exon 2 of the gene encoding the MT1 receptor in sheep has two sites of restriction fragment length polymorphism (RFLP), one for *MnlI* and the second for *RsaI* enzymes. The MTNR1A/*MnlI* site is characterized by a mutation leading to the absence (– or M) of the specific *MnlI* cleavage site at position 605 of the coding sequence, which leads to a characteristic pattern of digestion by this enzyme (Messer *et al*. 1997; Hernandez *et al*. 2005). The effect of melatonin on lamb body weight has been analyzed in previously studies. Lincoln and Ebling (1985) showed the influence of melatonin implantation on overall body weight in ram lamb. Administration of exogenous melatonin had significant effects on the milk levels of solids, protein, fat and lactose and on the fatty acid content of sheep milk (Edyta *et al.* 2011). As a result, the changes of ewe's milk composition can affect the body weight of lamb during growth period. The association between allelic polymorphism of *MnlI*-RFLP and sheep prolificacy have been reported in different study (Pelletier *et al*. 2000; Notter *et al.* 2003; Chu *et al*. 2003; Mura *et al.* 2010; Teyssier *et al.* 2010; Trecherel *et al.* 2010). The objective of the present study was firstly to detect the *MnlI*-RFLP polymorphism of the exon 2 of the MTNR1A and investigate the associations between observed polymorphism and production and reproduction traits in Zandi sheep.

MATERIALS AND METHODS

Genomic DNA preparation, amplification and digestion

One hundred Zandi sheep were randomly selected from Khojir Zandi sheep breeding station. All the ewes had seasonal reproduction records and the nutrition status was similar for all of sampled individuals. 10 mL of blood were collected from the jugular vein in EDTA coated tubes. Genomic DNA was extracted by modified salting out method and then kept at −20 ˚C until was used for polymerase chain reaction (PCR). The MTNR1A/*MnlI* polymorphism was identified using the PCR-RFLP method. A DNA fragment with the size of 824bp from exon 2 of ovine MTNR1A gene was amplified with following primer pair:

Forward: 5'-TGT GTT TGT GGT GAG CCT GG-3'
Reverse: 5'-ATG GAG AGG GTT TGC GTT TA-3'

The amplification reactions were carried out in 25 µL tube containing 2.5 µL 10X PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.0), 0.1% Triton X-100), 2.5 mmol/L $MgCl_2$, 0.5 mmol/L each dNTP, 2 µmol/L each primer, 50 ng ovine genomic DNA, and 1U *Taq* DNA polymerase. The PCR conditions were carried out by an early denaturation at 95 ˚C for 5 minutes, followed by 45 PCR cycles with Touchdown program (Table 1), extension at 72 ˚C for 1 min, with a final extension at 72 ˚C for 10 min. The PCR products were separated by electrophoresis on 1.5% agarose gel. After amplification, the 7µL of PCR product was digested with 2 unites *MnlI* endonuclease at 37 ˚C for 4 hours, followed by a deactivation process at 65 ˚C for 20 minutes. For genotyping of studied samples, the digested fragments were electrophoresed on 3% agarose gel and stained with ethidium bromide. The allelic and genotype frequencies and test of Hardy Weinberg (HW) equilibrium were done using Pop Gene software version 1.32.

Table 1 Cycle numbers and temperature for Touchdown PCR

Temperature (˚C)	Cycle number	Time
63	5	45 s
62	5	45 s
61	10	45 s
60	10	45 s
59	10	45 s
58	5	45 s

Marker trait association study

The GLM procedure of the SAS package (SAS, 2000) was used to estimate the effect of MTNR1A/*MnlI* genotypes on production (body weight at birth, 1, 3, 6, 9 and 12 months of age) and reproduction trait (litter size) of Zandi ewes with following statistical model:

$$Y_{ijk} = \mu + G_i + e_{ij}$$

Where:
Y_{ijk}: phenotypic value of interested traits.
µ: overall population mean.
G_i: the effect of i^{th} genotype.
e_{ij}: residual error.

RESULTS AND DISCUSSION

PCR-RFLP analysis of exon 2 of MTNR1A gene

An 824 bp fragment of exon 2 of ovine MTNR1A gene was amplified with a quality which could be directly analyzed by RFLP (Figure 1). Digestion of amplified fragment with *MnlI* produced nine fragments of 303, 236, 218, 135, 82, 67, 36, 28 and 22 in Zandi sheep, which separated on a 3% agarose gel (the fragments of 36, 28 and 22 bp were not separated on agarose gel) (Figure 2). The fragment of 286bp and 236bp were polymorphic (Messer *et al.* 1997) but in our study only the fragment of 303 bp was polymorphic. Absence of the fragment of 303 bp was referred to allele M and the presence of this fragment was referred to allele m. Two genotypes MM and Mm were detected in studied Zandi population with frequencies of 0.82 and 0.18,

respectively (Figure 2 and table 2). The mm genotype was not detected in Zandi sheep.

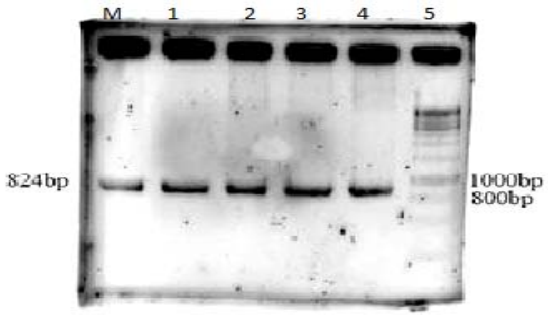

Figure 1 Electrophoresis pattern of PCR product of exon 2 of MTNR1A gene in Zandi sheep (1.5% agarose gel)
Lanes 1-5: PCR amplification product and M: DNA molecular marker

The allelic frequencies of 0.9109 and 0.0891 were observed for M and m alleles of MTNR1A locus. The X^2 test confirms that studied Zandi sheep population was in HW equilibrium.

Marker trait association study

Association analysis showed that MTNR1A/*MnlI* marker site had significant effect (P>0.05) on BW1, as ewes with Mm genotypes had higher BW1 than MM individuals (Table 3). No significant association was observed between MTNR1A/*MnlI* marker site and other studied traits (Table 3).

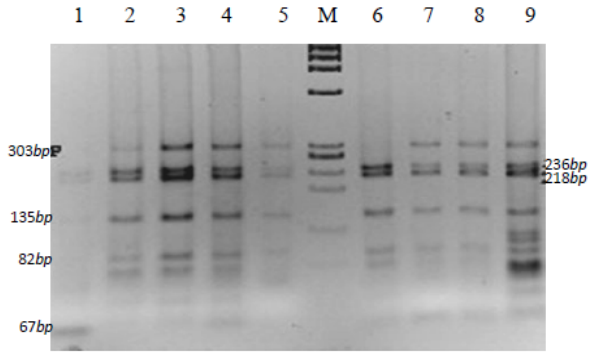

Figure 2 Restriction analysis of PCR products for exon 2 of MTNR1A gene on 3% agarose gel
Lanes 1 and 6: MM genotype; Lanes 2, 3, 4, 5, 7, 8 and 9: M / m genotype; M: 50 bp DNA molecular weight marker

The MTNR1A gene can be one of the possible candidate genes controlling ovine reproductive seasonality. The homozygous genotype for the presence (m) of a polymorphic *MnlI* site at position 605 of exon 2 of MTNR1A gene was associated with year-round estrus in ewes, and the homozygous genotype for the absence (M) of this site was associated with seasonal and ovulatory activity in ewes (Pelletier *et al.* 2000; Notter *et al.* 2003; Chu *et al.* 2003; Chu *et al.* 2007). The MTNR1A locus showed two genotypes of Mm and MM in Zandi sheep population. The mm genotype was not observed in genotyped samples which can be due to the low sample size and / or for the lowest frequency of m alleles in the present study.

Table 2 Allelic and genotype frequencies of MTNR1A gene in Zandi sheep

Breed	N	*MnlI*				
		Allele frequency		Genotype frequency		
		M	m	MM	Mm	mm
Zandi	100	0.919	0.081	0.82	0.18	0.0

Table 3 Least squares means for production Traits of Zandi ewes

Traits[a]	P-value	Genotype	
		MM	Mm
BW (kg)	0.2983^{ns}	4.4889	4.2952
BW1 (kg)	0.0414*	9.6216	10.8000
BW3 (kg)	0.9915^{ns}	21.2542	21.2444
BW6 (kg)	0.7687^{ns}	34.3916	34.1056
BW9 (kg)	0.8061^{ns}	35.301	35.044
BW12 (kg)	0.8740^{ns}	36.9944	36.8373
Litter size	0.8892^{ns}	1.22170	1.22234

[a] BW: birth weight; BW1, 3, 6, 9 and 12: body weight at 1, 3, 6, 9 and 12 months of age.
* P<0.05.

According with our results, the M allele in Small Tailed Han sheep (Chu *et al.* 2003), Shall and Karakul sheep (Ghiasi *et al.* 2006), Sarda sheep (Mura *et al.* 2010), and Merinos d'Arles sheep (Teyssier *et al.* 2010) had higher frequency than m allele. The m allele may lead to a reproductive activity less linked to photoperiod. Kaczor *et al.* (2006) investigated the polymorphism at the MTNR1A locus in prolific Olkuska sheep, Polish mountain sheep, Suffolk and in sheep F1 crosses (Merino-Romanov). A high frequency of the M allele was found in sheep with seasonal sexual activity: prolific Olkuska sheep (0.643), Polish mountain sheep (0.684) and Suffolk (0.60). In aseasonal F1 (Merino-Romanov) sheep, a higher proportion of the m allele was found (0.795). The frequencies of MM genotype was 0.529, 0.474, 0.6 and 0.205 in prolific Olkuska sheep, Polish mountain sheep, Suffolk and F1 (Merino-Romanov) crosses, respectively.

The RFLP analysis showed nine cleavage sites of *MnlI* enzyme were presented in exon 2 of MTNR1A marker site. However, only one of those was shown to be polymorphic. The polymorphic site was at fragment 303 bp. Results of variance analysis indicated that there was no association between the MTNR1A/*MnlI* marker site and litter size in Zandi sheep (Table 3). Chu *et al.* (2003) showed that the marker site for *MnlI* endonuclease on MTNR1A gene had no significant effect on litter size in both the first parity and the second parity in Small Tail Han sheep (P>0.05). Reports on the effect of genotype at the MTNR1A loci on the

sheep litter size indicated that genetic factors had no significant effect on this productive trait despite the fact that slightly larger litters were observed in sheep with one copy of the m allele (Notter *et al.* 2003; Chu *et al.* 2003).

The effect of MTNR1A/*MnlI* genotypes on production traits was found in Zandi sheep. The MTNR1A/*MnlI* genotypes were significantly associated with BW1 ($P<0.05$). Least square analysis indicated that Mm genotypes had higher BW1 than MM genotype (Table 3). No significant differences ($P<0.05$) were detected between MTNR1A/*MnlI* genotypes and other studied traits (Table 3).

CONCLUSION

Two genotypes of MM and Mm were found in MTNR1A/*MnlI* marker site in Zandi sheep. No mm genotype was detected in the present study. Marker trait association analysis showed the significant effects of the MTNR1A/*MnlI* polymorphism on BW1. In the subsequent studies using a large number of samples along with the other important parameters such as sire effect and the lamb health status which may influence lamb body weight is recommended.

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