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Association of melatonin receptor 1A gene polymorphisms with production and reproduction traits in Zandi sheep

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Abstract
The aim of the present study was to detect the *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 a Zandi sheep flock. Blood samples were randomly collected from 100 individuals from Khojir Zandi sheep breeding station 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 and the PCR product was digested with *Mnl*I endonuclease. Restricted digestion allowed the determination of two alleles (M, m) and two genotypes (MM, Mn) with frequencies of 0.91, 0.09 and 0.82, 0.18 for *MTNR1A* marker site in Zandi sheep population, respectively. Least square means showed that MM individuals had higher body weight at one month of age (BW1) than Mn individuals ($p<0.05$). No any associations were found between observed genotypes and other studied traits.

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 and suprachiasmatic nucleus, respectively. There are two melatonin receptor, the MT1 (Mel1A) and the MT2 (Mel1B) 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). it 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). 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 the low degree of polymorphism (Trecherel et al., 2010), but the exon II 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). Therefore, the differences occurring in the structure of receptors result from changes in the second exon (Barrett et al., 1997). Exon II of the gene encoding the MT1 receptor in sheep has two sites of restriction fragment length polymorphism (RFLP), one for *Mnl*I and the second for *Rsa*I enzymes. The *MTNR1A/Mnl*I 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 association

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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 II of the *MTNR1A* and then 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 sheep were randomly selected from Khojir Zandi sheep breeding station. Ten millilitres 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 used for polymerase chain reaction (PCR). The *MTNR1A/Mnl*I Polymorphism was identified using the PCR-RFLP method as described by MESSER et al. (1997). A fragment with the size of 824 bp from exon II of *MTNR1A* gene were amplified with a specific primer (forward: 5'-TGT GTT TGT GGT GAG CCT GG-3', reverse: 5'-ATG GAG AGG GTT TGC GTT TA-3') pairs. The amplification reactions were carried out in 25µl tube containing 2.5µl 10× 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, 2µmol/l each primer, 50 ng ovine genomic DNA, and 1U of *Taq* DNA polymerase. PCR conditions were carried out with an early denaturation at 95°C for 5 minutes, followed by 45 cycles of denaturation with touchdown method as described in 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 products was digested with 2 U of *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 POPGENE software, version 1.32.

Table 1: Cycle number 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 Ver. 8.1, 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} is phenotypic values of interested traits, μ is overall population mean, G_i is effect of genotype, and e_{ij} is random errors.

Results

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PCR-RFLP analysis of exon II of MTNR1A gene

Part of exon II (824 bp) from ovine *MTNR1A* gene was amplified with a quality which could directly analyzed by RFLP (figure. 1). Digestion of amplified fragment with *MnlI* produced six fragments of 286, 236, 216, 137, 82, and 67 bp in Zandi sheep, which separated on a 3% agarose gel (figure. 2). Absent of the fragment of 286bp was referred to allele M and the present of this fragment was referred to allele m. Two genotypes of MM and Mm were detected in studied Zandi population with frequencies of 0.82 and 0.18, respectively (Figure 2 and table 1). The mm genotype was not detected in Zandi sheep.

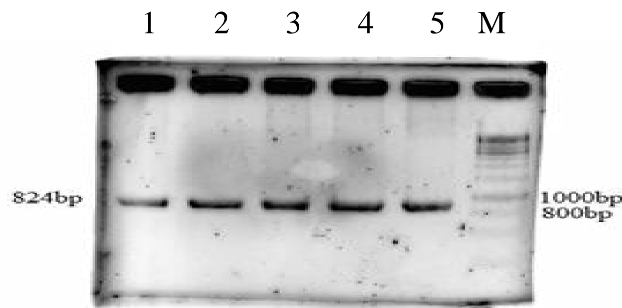

Figure 1. Electrophoresis pattern of PCR product of exon II of *MTNR1A* gene in zandi sheep (1.5% agarose gel). Lanes 1–5: PCR amplification product. M: DNA molecular marker.

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

Marker-trait association study

Marker-trait association analysis showed that *MTNR1A/MnlI* locus had significant association (p>0.05) with BW1, as ewes with Mm genotypes had higher BW1 than MM individuals (table 2). There were no significant association between *MTNR1A/MnlI* marker site and studied traits (table 2).

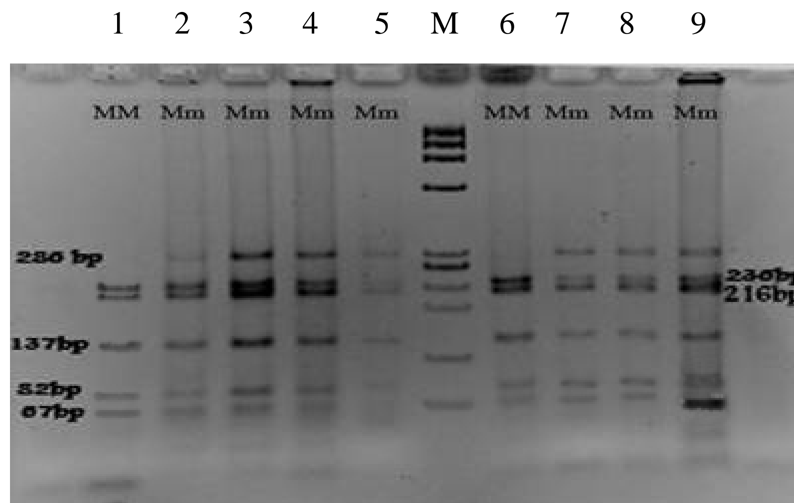

Figure 2. Restriction analysis of PCR product for exon II of *MTNR1A* gene on 3% agarose gel). Lanes 1 and 6: MM genotype. Lanes 2,3,4,5,7,8 and 9: M/m genotype. M: 50 bp DNA molecular marker

Discussion

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 *Mnl*I site at position 605 of exon II of *MTNR1A* gene was associated with year-round estrus in ewes (Pelletier et al., 2000; Notter et al., 2003; Chu et al., 2003, 2007). Two genotypes of

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Mm and MM were found in Zandi sheep population in the present study. The mm genotype was not observed in studied population which it can be due to low sample size and also the low frequency of m allele (table 2).

Table 1. Allelic and genotype frequencies of *MTNR1A* gene in Zandi sheep

Breed	N	*Mnl*I				
		Allele frequency		Genotype frequency		
		M	m	MM	Mm	mm
Zandi	100	0.919	0.0891	0.82	0.18	0.0

In consistency with other studies, 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 (Teyysier et al., 2010) had higher frequency than m allele. The m allele leads 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 the F1 crosses between Merino and Romanov sheep. 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 (0.6). In a seasonal 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, 0.205 in prolific Olkuska sheep, Polish Mountain sheep, Suffolk and F1 (Merino-Romanov) crosses, respectively.

Table 2. 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, BW1,3,6,9,12: bosy weight at 1, 3, 6, 9 and 12 months of age. * $P < 0.05$.

Results of variance analysis indicated that there was no association between the *MTNR1A/MnlI* locus and litter size in Zandi sheep flock (table 2). Chu et al. (2003) showed that the marker site for *MnlI* of *MTNR1A* gene had no significant effect on litter size in both the first and the second parity in Small Tail Han sheep. Reports on the effect of genotype at the *MTNR1A* loci on the sheep litter size indicated that there was no significant effect on this

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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).
In the present study, the *MTNR1A/Mnl*I genotypes were significantly associated with BW1 ($p<0.05$). Least square analysis indicated that Mm genotypes had higher BW1 than MM genotype.

Conclusion

Two genotypes of MM and Mm were found in *MTNR1A/Mnl*I marker site in Zandi sheep. Marker-trait association analysis showed a significant effect of the *MTNR1A/Mnl*I polymorphism on BW1. No any associations were found between observed genotypes and litter size in Zandi sheep flock.

References

1. Barrett, P., S. Conaway, R. Jockers, A.D. Strosberg, B. Guardiola-Lemaitre, P. Delagrange, P.J. Morgan. 1997. Cloning and functional analysis of a polymorphic variant of the ovine Mel 1a melatonin receptor. *Biochim. Biophys. Acta.,* 1356: 299–307.
2. Bartness, T., E. L. Bittman, M. H. Hastings, J. B. Powers, B. Goldman. 1993. Timed melatonin infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J. Pineal Res.,* 15:161-190
3. Chu, M.X., C. L. Ji, G. H. Chen. 2003. Association between PCR-RFLP of melatonin receptor 1a gene and high prolificacy in Small Tail Han sheep. *Asian-Aust. J. Anim. Sci.,* 16, 1701–1704.
4. Chu, M. X., Y.Q. He, D.X. Cheng, S.C. Ye, L. Fang, J.Y. Wang. 2007. Association between expression of reproductive seasonality and alleles of melatonin receptor 1A in goats. *Anim. Repro. Sci.,* 101: 276–284.
5. Gall, C. V., G. H. Stehle, D. R. weaver. 2002. Mammalian melatonin receptors: molecular biology and signal transduction. *Cel. Tiss. Res.,* 309:151–162
6. GhiasI, H., M. R. Nasiry, A. R. H. Mousavi, A. A. Mousavizadeh, A. Javad Manesh. 2006. Genetic polymorphism of the melatonin receptor 1A locus in Iranian Shall and Karakul sheep. *Iran. J. Biotech.,* 4 (3): 201-203.
7. Hernandez, X., L. Bodin, D. Chesneau, D. Guillaumw, P. Chemineau, B. Malpaux, M. Migaud. 2005. Relationship between MT1 melatonin receptor gene polymorphism and seasonal physiological responses in Île-de-France ewes. *Reprod. Nutr. Dev.,* 45: 151-162.
8. Kaczor, U., M. kamiecik, E. Molik, T. Rycheusz. 2006. Polymorphism in the melatonin receptor gene MT1 (locus *MTNR1A*) in sheep. *Arch. Tierz.,* 49: 257-262
9. Malpaux, B., M. Migaud, H. Tricoire, P. Chemineau. 2001. Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. *J Biol. Rhythms.,* 16: 336-347
10. Messer, A.L., L. Wang, C. K. Tuggle, M.F. Rothschild. 1997. Mapping of the melatonin receptor 1a (MTNR1A) gene in pigs, sheep, and cattle. *Mammal. Genome.,* 8: 368-370.
11. Mura, M.C., S. Luridiana, G. M. Vacca, P. P. Bini, V. Carcangiu. 2010. Effect of genotype at the MTNR1A locus and melatonin treatment on first conception in Sarda ewe lambs. *Thriogen.,* 74: 1579-1586.
12. Notter, D. R., N.E. Cockett, T.S. Hadfield. 2003. Evaluation of melatonin receptor 1a as a candidate gene influencing reproduction in an autumn-lambing sheep flock. *J. Anim. Sci.,* 81: 912-917.

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13. Pelletier, J., L. Bodin, E. Hanocq, B. Malpaux, J. Teyssier, J. Thimonier, P. Chemineau. 2000. Association between expression of reproductive seasonality and alleles of the gene for Mel1a receptor in the ewe. *Biol. Reprod.*, 62:1096-1101.
14. Reppert, S.T., D.R. Weaver, T. Ebisawa. 1994. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. *Neuron,*13 (5): 1177-85.
15. SAS Institute Inc. 2000. SAS/STAT User's Guide: Version 8.1st edn. SAS Institute Inc., Cary, North Carolina, USA.
16. Teyssier, J., M. Migaud, N. Debus, C. Maton, E. Tillard, B. Malpaux, P. Chemineau, L. Bodin. 2010. Expression of seasonality in Merinos d'Arles ewes of different genotypes at the MT1 melatonin receptor gene. *Anim.,* 5(3): 329-336.
17. Trecherel, E., M. Batailler, D. Chesneau, P. Delagrange, B. Malpaux, P. Chemineau, M. Migaud. 2010. Functional characterization of polymorphic variants for ovine MT1 melatonin receptors: Possible implication for seasonal reproduction in sheep. *Anim. Reprod. Sci.,* 122: 328-334.

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