Association between PCR-RFLP of Melatonin Receptor 1a Gene and High Prolificacy in Small Tail Han Sheep*

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ABSTRACT : Melatonin regulates circadian rhythms and reproduction changes in seasonally reproductive mammals through binding to high-affinity, G-protein-coupled receptors. Small Tail Han sheep that has significant characteristics of high prolificacy and non-seasonal ovulatory activity is an excellent local sheep breed in P. R. China. The exon 2 of the ovine melatonin receptor 1a (*MTNR1A*) gene was amplified and a uniform fragment of 824 bp was obtained in 150 ewes of Small Tail Han sheep. The 824 bp PCR product was digested with restriction endonucleases Mnl I and Rsa I , and genetic polymorphism was detected by PCR-RFLP. Polymorphic Mnl I site was detected at base position 605 of the exon 2 of the *MTNR1A* gene. There were two kinds of genotypes in Small Tail Han sheep, AB (303 bp, 236 bp/67 bp) and BB (236 bp/67 bp, 236 bp/67 bp). The results indicated that genotype AA (303 bp, 303 bp) at Mnl I -RFLP site did not exist in non-seasonal estrous Small Tail Han sheep, which suggested that there was an association between genotype AA (303 bp, 303 bp) and reproductive seasonality in sheep. Polymorphic Rsa I site was detected at base position 604 of the exon 2 of the *MTNR1A* gene. Three kinds of genotypes were found in Small Tail Han sheep, AA (290 bp, 290 bp), AB (290 bp, 267 bp/23 bp) and BB (267 bp/23 bp, 267 bp/23 bp). Least squares means of litter size in the first parity and the second parity for genotype AA (290 bp, 290 bp) at Rsa I -RFLP site were 0.43 and 1.06 more than those for genotype AB (290 bp, 267 bp/23 bp) in Small Tail Han sheep. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12 :1701-1704)***

Key Words : Sheep, Prolificacy, Reproductive Seasonality, Melatonin Receptor 1a Gene, PCR-RFLP

INTRODUCTION

Melatonin, secreted by pineal gland and retina, regulates circadian rhythms and reproduction changes in seasonally reproductive mammals through binding to high-affinity, G-protein-coupled receptors (Dubocovich et al., 1987; Vanecek, 1988; Reppert et al., 1988; Klein et al., 1991; Roca et al., 1996; Barrett et al., 1997; Kokkola et al., 1998; Ebisawa et al., 1999). The circadian effects of melatonin may be mediated by melatonin receptors in the hypothalamic suprachiasmatic nucleus, the site of a circadian clock (for reviews see Klein et al., 1991; Weaver et al., 1996), and the reproductive effects mediated by melatonin receptors in the hypophyseal pars tuberalis (Reppert et al., 1994). A high-affinity melatonin receptor that mediates these two major biological functions of melatonin in mammals was cloned by Reppert et al. (1994). Slaugenhaupt et al. (1995) mapped melatonin receptor 1a gene to human chromosome 4q35.1 and the proximal portion of mouse chromosome 8. By microsatellite markers and two-point linkage analysis, Messer et al. (1997) mapped ovine melatonin receptor 1a (*MTNR1A*) gene to ovine chromosome 26, between microsatellites CSSM43 and BM6526. Pelletier et al. (2000) discovered the association between genotype 303 bp/303 bp for site Mnl I and seasonal anovulatory activity in Merinos d'Arles ewes.

Small Tail Han sheep that has significant characteristics of high prolificacy and non-seasonal ovulatory activity is an excellent local sheep breed in P. R. China. The lambing percentage averaged 261% (Zheng, 1989) and 265.2% (Wang et al., 1990) in Small Tail Han sheep of Shandong Province, P. R. China. The objectives of the present study were firstly to analyze the polymorphism of *MTNR1A* gene, and secondly to examine the relationship between *MTNR1A* gene and high prolificacy in Small Tail Han sheep.

MATERIALS AND METHODS

Genomic DNA preparation

Blood samples of 10 ml were collected from 150 ewes of Small Tail Han sheep along with data on litter size in Jiaxiang Breeding Sheep Farm in Shandong Province, P. R. China. DNA was extracted from blood samples collected using acid citrate dextrose as an anticoagulant. Genomic sheep DNA was dissolved in TE buffer and kept at -20°C.

Primer sequences

Primers for the exon 2 of ovine *MTNR1A* gene were as follows (Messer et al., 1997):

Forward: 5'-TGTGTTTGTGGTGAGCCTGG-3'
Reverse: 5'-ATGGAGAGGGTTTGCGTTTA-3'

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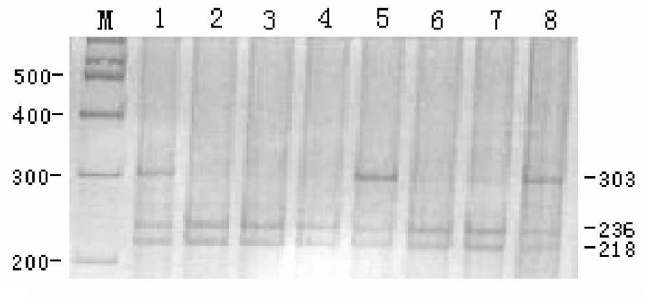

Figure 1. Band patterns of the exon 2 of *MTNR1A* gene digested with *Mnl* I in Small Tail Han sheep. M:100 bp DNA Ladder; lanes 1, 5, 8: AB genotype; lanes 2, 3, 4, 6, 7: BB genotype.

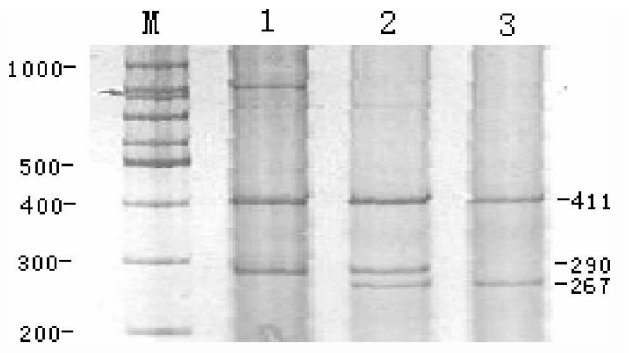

Figure 2. Band patterns of the exon 2 of *MTNR1A* gene digested with Rsa I in Small Tail Han sheep. M:100 bp DNA Ladder; lane 1: AA genotype; lane 2: AB genotype; lane 3: BB genotype.

PCR conditions

Polymerase chain reaction (PCR) was performed in 25 μl volume containing approximately 50 ng genomic sheep DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 200 μM each dNTP, 1.5 mM $MgCl_2$, 1.0 μM each primer and 1.0 unit Taq DNA polymerase (SABC, Beijing, China). PCR conditions were as follows: denaturation at 94 °C for 4 min, followed by 33 cycles of denaturation at 94 °C for 1 min, annealing at 63°C for 1 min, extension at 72°C for 1.5 min, with a final extension at 72°C for 10 min on Gene Amp PCR System 9600 (PERKIN ELMER Co., USA).

PCR products of 12 μl were digested separately with 2 U Mnl I (New England Biolabs, Beverly, MA, USA) and 2 U Rsa I (Promega, Madison, WI, USA) at 37°C overnight. The resulting fragments were separated by electrophoresis on 8% polyacrylamide gels. Gels were stained with silver nitrate (silver staining) after electrophoresis to read fragment sizes.

Statistical analysis

The following statistical model was fitted to compare difference of litter size among *MTNR1A* genotypes.

$$y_{ij}=\mu+G_i+e_{ij}$$

where y_{ij} is phenotypic value of litter size, μ is population mean, G_i is the fixed effect of the i^{th} genotype, and e_{ij} is random error effect of each observation. Calculations were achieved using Proc GLM of SAS (Ver 8.1).

RESULTS

PCR-RFLP analysis of the exon 2 of ovine *MTNR1A* gene

In the present study, the primers for the exon 2 of ovine *MTNR1A* gene were used for amplification genomic DNA of Small Tail Han sheep and a uniform fragment of 824 bp was obtained after 1.5% agarose gel electrophoresis in Small Tail Han sheep. The 824 bp PCR product was digested with two restriction endonucleases Mnl I and Rsa I and genetic polymorphisms were investigated by PCR-RFLP. According to the sequence (U14109) of ovine *MTNR1A* gene in GenBank, there were seven restriction endonuclease Mnl I sites (218 bp+36 bp+67 bp+236 bp+22 bp+28 bp+82 bp+135 bp) and four restriction endonuclease *Rsa* I sites (53 bp+267 bp+23 bp+411 bp+70 bp) in 824 bp fragment for the exon 2 of ovine *MTNR1A* gene.

A biallelic polymorphism was found with restriction endonuclease Mnl I, which cuts the amplicon to several fragments (Figure 1). Allele A, in which the polymorphic restriction site at position 605 is absent, is characterized by the presence of the largest fragment of approximate length 303 bp, while for allele B, which possesses the polymorphic restriction site, this fragment is cut to yield a fragment of about 236 bp and a short fragment approximately 67 bp barely detectable on the gel. Only two genotypes were detected in Small Tail Han sheep, AB (303 bp, 236 bp/67 bp) and BB (236 bp/67 bp, 236 bp/67 bp).

A biallelic polymorphism was found with restriction endonuclease Rsa I, which cuts the amplicon to several fragments (Figure 2). Allele A, in which the polymorphic restriction site at position 604 is absent, is characterized by the presence of the largest fragment of approximate length 290 bp, while for allele B, which possesses the polymorphic restriction site, this fragment is cut to yield a fragment of about 267 bp and a short fragment approximately 23 bp barely detectable on the gel. Three genotypes were detected in Small Tail Han sheep, AA (290 bp, 290 bp), AB (290 bp, 267 bp/23 bp) and BB (267 bp/23 bp, 267 bp/23 bp).

Gene frequency and genotype frequency of the exon 2 of *MTNR1A* gene in Small Tail Han sheep were presented in Table 1. From Table 1 it can be seen: (i) for Mnl I site, there was a big difference between frequency of alleles A and B. (ii) for Rsa I site, there was no big difference between frequency of alleles A and B.

Table 1. Gene frequency and genotype frequency of the exon 2 of *MTNR1A* gene in Small Tail Han sheep

Restriction endonuclease	No.	Gene frequency		Genotype frequency		
		A	B	AA	AB	BB
Mnl I	106	0.2545	0.7455	0	0.509	0.491
Rsa I	101	0.4755	0.5245	0.208	0.535	0.257

Table 2. Least squares means (LSM) and standard errors (SE) for litter size of different genotypes of 2 sites in *MTNR1A* gene in Small Tail Han sheep

Genotype	No.	First parity		Second parity	
		LSM	SE	LSM	SE
Mnl I					
AB	54	1.70	0.08	2.69	0.11
BB	52	1.50	0.08	2.52	0.11
Rsa I					
AA	21	1.95^{A}	0.13	3.19^{A}	0.13
AB	54	1.52^{B}	0.08	2.13^{B}	0.08
BB	26	1.73^{AB}	0.12	2.25^{B}	0.12

Means with the different superscripts within the same column differ significantly ($p<0.01$).

Effects of *MTNR1A* gene on high prolificacy in Small Tail Han sheep

Results of variance analysis indicated that site for Mnl I of *MTNR1A* gene had no significant effect on litter size in both the first parity and the second parity in Small Tail Han sheep ($p>0.05$); site for Rsa I had significant effect on litter size in the first parity ($p<0.05$) and highly significant effect on litter size in the second parity ($p<0.01$).

Least squares means (LSM) and standard error for litter size of different genotypes of 2 sites in *MTNR1A* gene in Small Tail Han sheep are shown in Table 2. It can be seen from Table 2, relationships of LSM for litter size of two genotypes for Mnl I site in both the first parity and the second parity are AB>BB, but the difference is not significant ($p>0.05$). The relationships of LSM for litter size of three genotypes for Rsa I site in the first parity are AA>BB>AB, in which the difference between AA and AB is highly significant ($p<0.01$), AA had 0.43 more lambs than AB. The relationships of litter size of three genotypes for Rsa I site in the second parity are AA>BB>AB, in which difference between BB and AB is not significant ($p>0.05$), AA has 0.94 more lambs than BB ($p<0.01$), AA has 1.06 more lambs than AB ($p<0.01$).

DISCUSSION

Site for Mnl I and reproductive seasonality

Pelletier et al. (2000) studied the exon 2 of the *MTNR1A* gene in two groups of Merinos d'Arles ewes (one group seasonal ovulatory and the other non-seasonal estrous) and found that there was an association between genotype -/- (303 bp, 303 bp) for Mnl I site at position 605 and seasonal anovulatory activity in Merinos d'Arles ewes. In the present study, genotype AA (303 bp, 303 bp) was not detected in non-seasonal estrous Small Tail Han sheep, which confirmed the conclusion of Pelletier et al. (2000) that AA was related with seasonal anovulation in sheep. However, the results of Migaud et al. (2002) on the exon 2 of the *MTNR1A* gene in two breeds of goat with different reproductive seasonality indicated that no relationship could established between the *MTNR1A* gene structure and the expression of reproductive seasonality in goats. The difference on relationship between *MTNR1A* gene and reproductive seasonality in sheep and goats should arouse enough emphasis and the deepgoing studies are expected.

Relationship between *MTNR1A* gene and high prolificacy

The relationships of litter size of three genotypes for Rsa I site in both the first parity and the second parity are AA>BB>AB. If A allele is dominant over B allele, AB genotype generally shows better phenotype. That was not this case in the present study. The reason may be less samples analyzed or more complicated interaction between A allele and B allele. So, the results obtained in this study need be confirmed by enlarging sheep breeds and samples.

The relationships between two restriction sites for *MTNR1A* gene and litter size in Small Tail Han sheep were preliminary because of less samples detected in the present study, further analyses are need by expanding sheep breeds and samples.

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