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Genetic polymorphism of melatonin receptor 1A (*MTNR1A)* gene in Indian sheep breeds

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ABSTRACT

Polymorphism in the melatonin receptor 1A (MTNR1A) is associated with seasonal reproduction of sheep. Genetic polymorphism in MTNR1A gene was investigated in 4 Indian sheep breeds reared in semi-arid and arid region of the Rajasthan. In this study, blood samples were collected from 171 animals. DNA was extracted from white blood cells. The PCR product (824bp) of exon II fragment of sheep MTNR1A gene was amplified. PCR products were digested separately with 2 restriction endonucleases, viz. *MnII* and *RsaI*. Polymorphism was detected at both loci of MTNR1A gene. At MnII locus, allele frequency for Magra, Garole, Malpura and Avikalin breed was found as 0.74, 0.74, 0.78, and 0.84 for M allele and 0.26, 0.26, 0.22, 0.16 for N allele, respectively. At RsaI locus, allele frequency was found as 0.90, 0.83, 0.69, and 0.59, for R allele and 0.10, 0.17, 0.31, 0.41 for r allele, respectively. It was concluded that the frequencies of 'M' and 'R' alleles of MTNR1A were higher in Indian sheep breeds.

Key words: DNA polymorphism, Melatonin receptor 1A, Reproduction, Sheep

Melatonin, a pineal gland hormone, functions through specific receptors located in various regions of the central nervous system (CNS) including nuclei which regulate reproduction (Carcangui *et al.* 2005). Mateescu *et al.* (2009) found as association of conception rate and short lambing interval of sheep with the 'M' allele of MTNR1A gene. Considering the importance of the MTNR1A gene in reproductive seasonality, the genetic polymorphism of melatonin receptor 1A (MTNR1A) gene was studied in Magra, Garole, Malpura and Avikalin sheep breeds of India.

MATERIALS AND METHODS

Animals: Blood samples were collected randomly from 171 animals of Magra (43), Garole (44), Malpura (39) and Avikalin (45) breeds reared in the semi-arid and arid conditions of Rajasthan. Magra sheep are well known for carpet quality wool production. The wool of this breed has more lustre. The Garole is a prolific sheep breed found in the hot, humid coastal region of the southern part of the West Bengal, India. Malpura is non-prolific mutton type sheep breed, which usually produces a single lamb per ewe. Avikalin, a crossbred between native Malpura and exotic Rambouillet breeds developed at CSWRI for carpet wool.

Genomic DNA extraction: Approximately 5 ml of blood samples were collected from all animals aseptically from jugular vein in ACD (citric acid, sodium citrate, D-glucose) solution in 15 ml centrifuge tubes. DNA was extracted from white blood cells using standard phenol-chloroform extraction method. DNA samples were dissolved in 0.1X TE buffer (pH 8.0).

PCR amplification and genotyping: PCR amplification of exon II fragment of sheep MTNR1A gene was done using forward primer: 5' TGT GTT TGT GGT GAG CCT GG 3' and reverse primer: 5' ATG GAG AGG GTT TGC GTT TA 3') (Reppert *et al.* 1994). PCR reaction was carried out in 25µl volume containing 10X PCR buffer (50mM/l KCl, 10 mM/l Tris–HCl, pH 8.0, 0.1% Triton X-100), 2mM $MgCl_2$, 0.2mM of each dNTP, 5 picomole of each primer, 100 ng sheep genomic DNA and 1U Taq DNA polymerase. PCR reaction condition was as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 1 min, extension at 72°C for 1 min 20 sec and final extension at 72°C for 10 min. All PCR products were digested separately with 7U of *Mn II* restriction enzyme and 6U of *RsaI* enzyme at 37°C overnight. PCR products were resolved by electrophoresis on a 4% agarose gel.

Statistical analysis: Allelic and genotypic frequencies were calculated for the genetic variants of MTNR1A gene

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by direct counting method. The chi-square test (χ^2) was used to evaluate whether the population were in Hardy-Weinberg equilibrium. The genetic distance (Nei's 1978) was estimated between breeds by POPGENE software (v.1.32).

RESULTS AND DISCUSSION

Melatonin is involved in circadian rhythms and reproduction changes in seasonally reproducing mammals through binding to high-affinity, G-protein coupled receptors. MTNR1A gene, which is mapped on sheep chromosome 26, consists of 2 exons intervened by an intron of nearly 8kb in size (Reppert *et al*. 1994). Exon I codes for first transmembrane domain and the first intracellular loop. The exon II codes for the remaining part of the receptor, which has been widely studied for polymorphism (Barrett *et al.* 1997). Exon II shows 2 polymorphic sites reported by PCR-RFLP method (Messer *et al*. 1997). In this study, polymorphism was detected in sheep MTNR1A gene in Indian sheep breeds. The Exon II fragment of 824 bp size was amplified with consistent band intensity, analyzed by PCR-RFLP. The results indicated the presence of 7 *MnII* cleavage sites (221, 254, 324, 560, 582, 610 and 693bp) within the exon II sequence, but only 1 (324bp) was shown to be polymorphic. A biallelic polymorphism was found with restriction endonuclease *MnII*. Two alleles and 3 genotypes were detected— *MN* (303bp, 236bp and 67bp), NN (303bp) and *MM* (236bp and 67bp) (Fig.1). The most common genotype was MM, whereas the frequency of NN genotype was very low. Allelic and genotypic frequencies of MTNR1A gene for *MnII* site in 4 Indian sheep breeds are presented in Table 1.

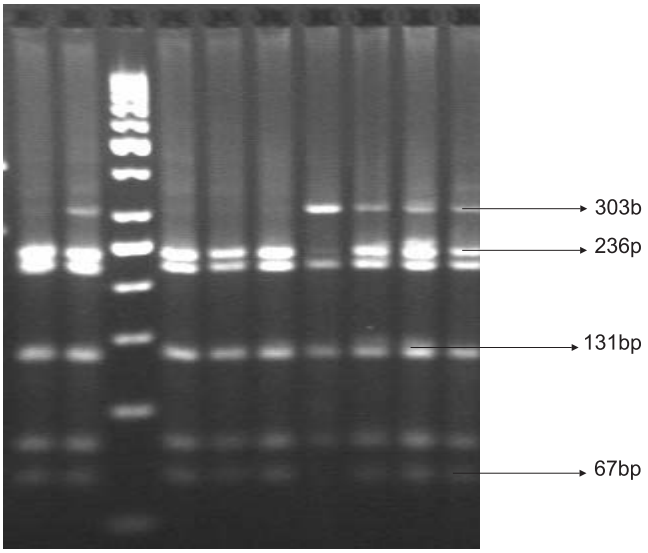

Fig. 1. Electrophoresis of PCR product digested with *MnII* restriction enzyme (4.0% agarose gel) of Magra sheep. Lane M: 50 bp DNA marker; lanes 1, 3, 4, 5: MM genotype; lanes 2, 7, 8, 9: MN genotype and lane 6: NN genotype.

The results showed a significant difference between the frequency of allele *M* and *N*. Result was in accordance with the findings of Chu *et al.* (2006) in Small Tail Han and Hu sheep breeds. However, the allelic and genotypic frequency of Suffolk, Dorset and German Mutton Merino sheep breeds was found almost equal to each other (Chu *et al.* 2006). The reason may be the sample size, which was very small in other breeds. The genetic variants of MTNR1A gene in this study were similar to the other sheep breeds studied, but there was

Table 1. Gene and genotype frequency of the exon II of MTNR1A gene for MnII site in 4 Indian sheep breeds

Breed	n	Genotype frequency			Chi-square	P Value	Allele frequency	
		MM	MN	NN			M	N
Magra	43	0.58	0.33	0.09	1.07	0.30	0.74	0.26
Garole	44	0.55	0.39	0.07	0.01	0.94	0.74	0.26
Malpura	39	0.69	0.18	0.13	9.46	0.002	0.78	0.22
Avikalin	45	0.78	0.13	0.09	11.91	0.001	0.84	0.16
Total	171	0.65	0.26	0.09	11.49	0.006	0.78	0.22

Table 2. Gene and genotype frequency of the exon II of MTNR1A gene for Rsa I site in 4 Indian sheep breeds

Breed	N	Genotype frequency			Chi-square	P value	Allele frequency	
		RR	Rr	rr			R	r
Magra	43	0.79	0.21	0.00	0.52	0.47	0.90	0.10
Garole	44	0.73	0.20	0.07	3.79	0.05	0.83	0.17
Malpura	39	0.44	0.51	0.05	1.43	0.23	0.69	0.31
Avikalin	45	0.33	0.51	0.16	0.09	0.77	0.59	0.41
Total	171	0.57	0.36	0.07	0.39	0.53	0.75	0.25

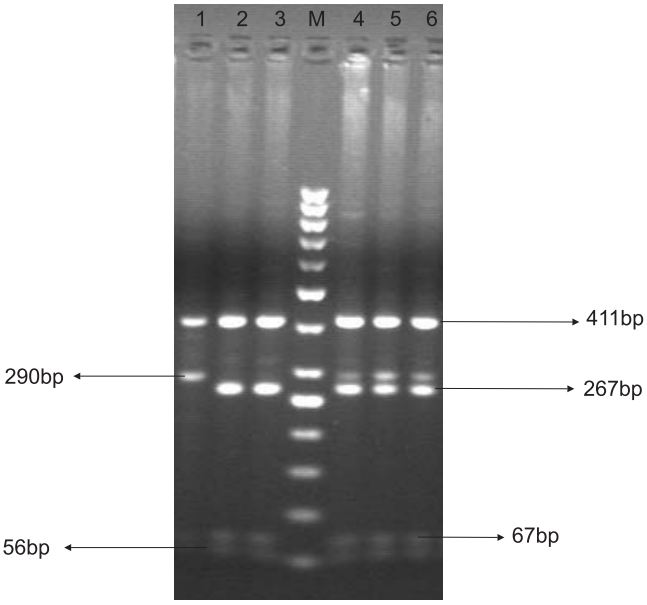

Fig. 2. Electrophoresis of PCR product digested with *RsaI* restriction enzyme (4.0% agarose gel) of Avikalin sheep. Lane M: 50bp DNA marker; lane 1: rr genotype; lines 2, 3: RR genotype and lane 4, 5, 6: Rr genotype.

a difference with respect to gene frequencies as studied in other breeds (Carcangui *et al*. 2009). The present study indicated the presence of 4 *RsaI* cleavage sites (56, 323, 346 and 757) within exon II sequence, and which generate the 5 bands of 411, 267, 67, 56 and 23 bp sizes (Fig. 2).

In the present study, the cleavage site at 323bp position was found polymorphic for *RsaI* enzyme. The polymorphism was also caused by the presence of cytosine (C) at 606bp position as confirmed by sequencing (Carcangui *et al.* 2009). Substitution of cytosine-thymine (C606T) caused the absence of this cleavage site and produces a single 290bp band, known as 'r' the allele. As a result, a biallelic polymorphism was found with *RsaI* digestion. Hence, 2 alleles (R and r) and 3 genotypes, viz. rr (290bp), Rr (290bp, 267bp and 23bp) and RR (267bp and 23bp) were detected. The present results are similar to the findings of Hristova *et al.* (2012) who found that the average genetic diversity at MTNR1A gene was highest in Bulgarian Breznishka sheep. Similarly the P-values were in correspondence with Hardy-Weinburg Equilibrium in all Bulgarian local sheep breed studied. Allelic and genotypic frequencies of the MTNR1A gene for *RsaI* site in 4 Indian sheep breeds are given in Table 2.

Contrary to the present finding, Seker *et al*. (2011) did not find any polymorphism at this site in Chios, White Karaman and Awassi sheep breeds. The frequency of 'R' allele was higher as compared to 'r' allele. In Magra and Malpura sheep breeds, rr genotype was absent it may because of the small sample size used in the study. In Malpura and Avikalin sheep breeds, Rr genotype was more frequent than the other genotype.

Table 3. Comparative genotypic frequencies for MnII and RsaI polymorphisms

	RsaI genotype		
MnII genotype	RR	Rr	rr
MM	32.16 (55)	26.32 (45)	6.43 (11)
MN	21.64 (37)	4.09 (7)	0 (0)
NN	3.51 (6)	5.26 (9)	0.58 (1)

Number of individuals given in parenthesis.

Table 4. Nei's Genetic identity among four Indian sheep breeds

Breed	Magra	Garole	Malpura	Avikalin
Magra	**	0.9975	0.9707	0.9246
Garole	0.0025	**	0.9446	0.9469
Malpura	0.0297	0.0156	**	0.9883
Avikalin	0.0784	0.0545	0.0118	**

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

In the population under study, individuals of genotypes MMRR, MMRr and MNRR had greater frequencies; where the other genotypes were at reduced frequencies. Genotype MNrr was absent. Absence of MMRR and NNrr was also reported in Dorset and its crosses by Mateescu *et al.* (2009). The genotypic frequencies for *MnII* and *RsaI* are presented in Table 3.

The chi-square test (χ^2) of genotype frequency of *RsaI* and *MnII* locus revealed that the populations were in Hardy-Weinberg equilibrium. It was observed that genotypic frequency of *MnII* locus of Avikalin and Malpura was not as per the Hardy-Weinberg equilibrium. The genetic distances and similarities among breeds are given in Table 4.

The genetic distance was found lowest between Garole and Magra (D = 0.0025), whereas higher genetic distance (D = 0.0784) between Avikalin and Magra. Avikalin had closeness with Malpura (D = 0.0118) compared with other breeds under the study and this may be due to the fact that Avikalin evolved by crossing of Rambouillet and Malpura. Mateescu *et al.* (2009) demonstrated an association of the MTNR1A gene and lambing frequency and confirmed the importance of MTNR1A gene as a potential DNA marker for out-of season breeding. Carcangui *et al.* (2011) concluded that MTNR1A gene polymorphism affects the fertility of sheep after synchronization and artificial insemination in the spring season. Genetic polymorphism at MTNR1A gene was studied for the first time in Magra, Garole, Malpura and Avikalin sheep breeds. However, further investigation is required to confirm the link of this polymorphism with seasonal reproductive activity of different sheep breeds of India reared in various climatic conditions.

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