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MTNR-1A Gene Polymorphism in Two Tropical Sheep breeds of South India

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Abstract

The present study was carried out to evaluate the genetic polymorphism in Ovine *MTNR1A* of two tropical breeds ie., highly prolific NARI Suwarna sheep and Mandya sheep from southern peninsular region of India with respect to differential fertility. Fifty animals from each breed were randomly selected and subjected to *RsaI*-RFLP analysis of exon-2 of Ovine *MTNR1A*. The study revealed monomorphic patterns in all the animals for both the breeds. PCR amplicon of 824 bp fragments with four cut sites at 53 bp, 320 bp, 343 bp and 754 bp, resulted in 411 bp, 267 bp, 70 bp, 53 bp and 23 bp. Only presence of CC genotypes at rs406779174 SNP of Ovine *MTNR1A* in both the breeds supports non-seasonality in breeding of these animals and no association of SNP with increased fecundity of NARI Suwarna sheep over Mandya sheep.

Introduction

Reproductive efficiency in sheep often varies among the breeds, which is largely influenced by genetic and environmental factors. Seasonal variation in reproductive activity is controlled by photoperiod in sheep (Thiery *et al*., 2002). The non-seasonal sheep breeds exhibit reproductive responses to improved nutrition irrespective of photoperiod, whereas the seasonal breeds respond to nutrition only during breeding season (Hotzel *et al.,* 2003). The endocrine signal, circadian rhythm of melatonin secretion is responsible for mediating the effects of photoperiod on the hypothalamo pituitary axis and plays an important role in regulation of seasonal reproduction (Gall *et al.,* 2002). Reproductive effects of melatonin are mediated via ligand specific guanine nucleotide-binding protein (G-protein) coupled receptors located in the hypophyseal pars tuberalis (Ebisawa *et al.,* 1994).

Two melatonin receptor subtypes: *Mel1a* (or *MTNR1A*) and *Mel1b* (or *MTNR1B*) have been characterized in mammals (Reppert *et al*., 1994). Melatonin receptor 1A gene (*MTNR1A)* is located on chromosome 26 of the sheep genome. It consists of two exons and an intronic sequence of about 8 kb in length (Reppert *et al.,* 1994). Exon 1 codes for the first transmembrane domain and the first intracellular loop while exon 2 codes

for the remaining part of the receptor, which has been extensively reported in the literature (Barrett *et al.,* 1997). The *MTNR1A* has been identified as a candidate gene which plays a key role in the photoperiodic control of seasonality mediated by the circadian changes in melatonin concentrations (Carcangiu *et al.,* 2011 and Pelletier *et al.,* 2000). *Mel1a* has been reported to be present in small ruminants (Migaud *et al*., 2002). Several studies have reported relationships of *MTNR1A* with seasonal reproduction activity (Pelletier *et al.,* 2000 and Carcangiu *et al.,* 2011). Since Mandya and NARI Suwarna are two tropical sheep breeds from southern peninsular region of India with varied reproductive efficiency producing single and multiple lambs per lambing, respectively, the present study was carried out to elucidate the polymorphism of *MTNR1A* in Mandya and NARI Suwarna sheep and its role in differential fertility.

Materials and methods

Fifty unrelated Mandya sheep were randomly selected from the flock maintained at Livestock Information and Research Center (Mandya sheep), Nagamangala and farmer's flock from the breeding tract and fifty unrelated NARI Suwarna sheep were randomly selected from the flock maintained at regional campus, KVAFSU, Bengaluru. In the beginning about 9 ml of venous blood was collected through jugular vein puncture in vacutainer tubes containing EDTA (1.5 mg/ml). Blood samples were stored at 4 °C till further processing. Genomic DNA was isolated by following Miller's high salt method (Miller *et al.,* 1988). The quality and quantification of the DNA was ascertained by electrophoretic analysis using 0.8% agarose gel.

A standard procedure of PCR was adopted to amplify the exon-2 segment of the ovine *MTNR1A* gene from the genomic DNA template. The primer sequences previously published by Reppert *et al*. (1994) were utilized. The sequence of the forward primer was 5' TGT GTT TGT GGT GAG CCT GG 3' and that of the reverse primer was 5' ATG GAG AGG GTT TGC GTT TA 3'. The PCR was carried out in 25 µl reaction mixture containing 1 µl each of forward and reverse primers of 10 pM/ µl, 1 µl of genomic DNA of 100 ng/ µl, 12.5 µl of 2X Red PCR Master Mix and 9.5 µl of nuclease free water. The PCR cyclic conditions were: initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation (94 °C for 1 min), annealing (61 °C for 45 sec) and extension (72 °C for 2 min) and final extension at 72 °C for 8 min. The PCR amplification was confirmed on 1.5% agarose gel. The restriction enzyme digestion was carried out in 15 µl reaction mixture containing autoclaved triple distilled water 8.5 µl, 10 x Assay buffer for RE 1.0 µl, *Rsa*I (10 U/ µl) 0.5 µl and PCR product 5.0 µl. The reaction mixture was incubated at 37 °C for 10 minutes. The restriction enzyme digested PCR products were electrophoresed on 3% percent agarose gel. The restriction pattern resolved by agarose gel electrophoresis was photographed and analyzed using Gel Documentation System (Bio Rad Molecular imager Gel Doc XR+, USA).

Results and discussion

Melatonin exerts circadian rhythms and reproduction changes in seasonally reproducing mammals through binding to high-affinity, G-protein coupled receptors. In the present study PCR–RFLP technique was employed to verify the polymorphism in exon-2 of Ovine *MTNR1A* gene in two tropical sheep breeds with varied fertility. PCR-RFLP analysis of the *MTNR1A* exon-2 resulted in a similar banding pattern in all the samples studied from both Mandya and NARI Suwarna sheep breeds indicating absence of polymorphism at rs406779174 SNP in the exon-2 of *MTNR1A* gene sequence (Fig.1).

Polymorphism at rs406779174 SNP is attributable to the presence or absence of second *Rsa*I cleavage site in the 824 bp PCR amplicon. The presence of nucleotide C at 322 bp position of the amplified product creates restriction site, resulting in five DNA fragments of 411bp, 267 bp, 70 bp 56 bp and 23 bp and is considered as allele 'R', whereas the presence of nucleotide T at 322 bp position of the amplified product resulting in absence of RE cut site resulting in 411 bp, 290 bp, 70 bp and 56 bp DNA fragments and is considered as allele 'r' (Meena *et al.,* 2013). In the present study electrophoretic analysis revealed clear bands of 411 bp and 267 bp and faint bands of 70 bp and 56 bp indicating presence of only one allele in homozygous state in all the animals (Fig.2).

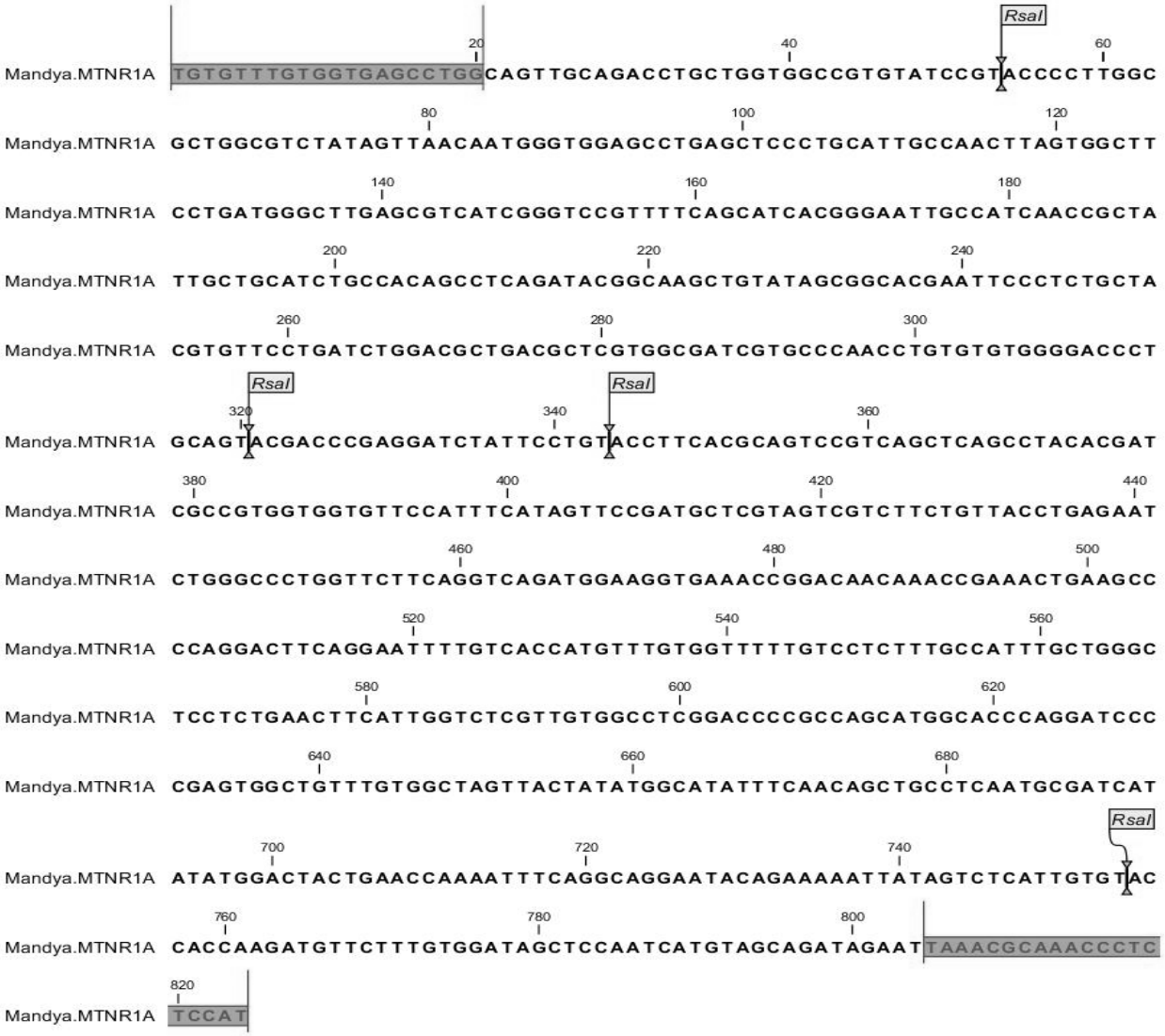

Figure 1: RE mapping and Nucleotide sequence of PCR amplified exon-2sequence of *MTNR1A* gene in Mandya and NARI Suwarna sheep

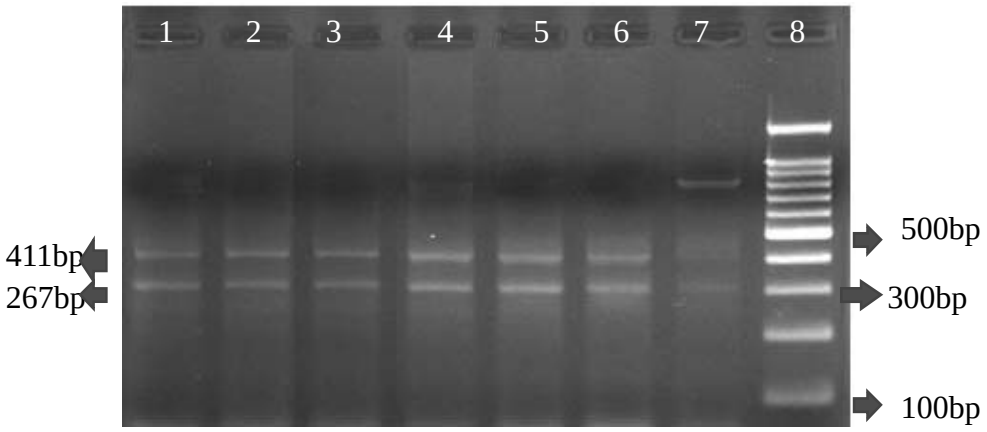

Figure 2: *Rsa*I RFLP of exon-2 of *MTNR1A* gene in NARI Suwarna and Mandy sheep exhibiting monomorphic pattern.

It is evident from the literature that rs406779174 SNP of Ovine *MTNR1A* exon-2 resulted in all the possible genotypes viz., RR (CC), Rr (CT) and rr (TT) in several sheep breeds. In the sheep breeds of temperate regions the frequency of allele R was low to moderate and ranged from 0.04 in Suffolk breed (Chu *et al.,* 2006; Ioannis *et al*., 2016)) to 0.58 in Sarda sheep breed (Mura *et al.,* 2014). India has extraordinary variety of climatic regions, ranging from tropical in the south to temperate and alpine in the Himalayan north. The frequency of allele R for hilly temperate breeds Sandya and Nilgiri was 0.46 and 0.56 respectively and for Malpura and Patanwadi breeds from dry aired regions of Rajasthan was 0.93 and 0.72 respectively (Saxena *et al*., 2015). In the present study lack of polymorphism *at Rsa*I/*MTNR1A* gene and presence of only R type of allele in Mandya sheep may be attributable to its higher acclimatization to tropical climate. Similarly, no evidence for polymorphism of *Rsa*I/*MTNR1A* gene observed in NARI Suwarna sheep in spite of it being developed from Garole sheep with moderate frequency of both the alleles. The probable reason for absence of polymorphism in NARI Suwarna sheep may be attributable to the fact the breed is Deccani sheep with only introgression of Fec-B gene from Garole. (Nimbkar *et al.,* 2009). Further, the absence of polymorphism in both highly prolific NARI Suwarna sheep as well as Mandya sheep indicates no association of SNP with increased fecundity of NARI Suwarna sheep.

In conclusion the present study revealed the absence *Rsa*I/*MTNR1A* polymorphism of exon-2 sequence in both Mandya and NARI Suwarna sheep. Only one genotype was observed in all the animals studied. However, the present observation needs to be revalidated involving a larger sample size and more number of south Indian tropical sheep breeds.

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