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Novel Polymorphism at Exon 2 of Caprine MHC Class II DRB3 Gene in Marwari Goats

Om Prakash[1], Pushpendra Kumar[1]*, Kush Shrivastava[1], Thirunavukkarasu, S.B.[1], Nihar Ranjan Sahoo[1], Amit Kumar[1], Anuj Chauhan[1], Bharat Bhushan[1], Ruchi Pachaury[2], Rohit Charan[2], Rajni Chaudhary[1], Vaishali Sah[1], Arvind Prasad[3], P.K. Bharti[4] and B.H.M. Patel[5]

[1]Molecular Genetics Lab, Division of Animal Genetics, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly (UP), INDIA

[2]District Diagnostic Laboratory, Animal Husbandry Department, Barmer (Raj), INDIA

[3]Division of Veterinary Parasitology, ICAR-IVRI, Izatnagar, Bareilly (UP), INDIA

[4]Livectock Production and Management Section, ICAR-IVRI, Izatnagar, Bareilly (UP), INDIA

[5]Principal Scientist, ICAR-IVRI, Hebbal, Bangalore (Karnataka), INDIA

**Corresponding author: P Kumar; Email: pushpendra64@gmail.com*

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ABSTRACT

The highly polymorphic genes of the MHC play a major role in the immune recognition of pathogens and parasites. The purpose of this study was to study the polymorphism of CLA-DRB3 (Caprine Leukocyte Antigen- antigen D Related β3-chain) gene in Marwari goat using PCR-RFLP technique. A region of exon 2 encompassing 285 bp fragment of DRB 3.2 gene in Marwari goats was amplified by polymerase chain reaction (PCR). The restriction digestion by *Hin1I* revealed two alleles, A and G with frequencies 0.452 and 0.548, respectively and three genotypes, AA (285 bp), AG (285/174/111 bp) and GG (174/111 bp) with frequencies 0.242, 0.420 and 0.338, respectively. The polymorphic information content (PIC) value and expected heterozygosity were 0.373 and 0.495, respectively which were high in both cases. The present study shows polymorphic nature of MHC Class II DRB3 gene at this locus in Marwari goats.

Keywords: CLA-DRB3 gene, PCR-RFLP, polymorphism, Marwari goat, *Hin1I*

Goat major histocompatibility complex (MHC), also known as caprine leukocyte antigen (CLA) or goat leucocyte antigen (GoLA) system has been shown to be similar to that of cattle which have two expressed class II antigens namely, DQ and DR (Takada *et al.*, 1998). The highly polymorphic genes of the MHC play a key role in the immune recognition of pathogens and parasites (Klein *et al*., 1993). Products of Class I and II genes, the histocompatibility molecules, are of paramount importance as these present antigens to T-lymphocytes, thereby eliciting immune responses (Dukkipati *et al*., 2006). Among the domesticated species, the MHC of sheep and goat is poorly characterized, but the general structure of the MHC is conserved among mammalian species (Amills *et al*., 1998).

Among the three classes of MHC, genes of class I and II exhibits most of the polymorphism (Trowsdale, 1996). Amills *et al*. (1995) for the first time reported polymorphism at caprine MHC locus. The polymorphic pattern in exon 2 of MHC class II DRB3.2 gene observed in Raeini Cashmere goats (Baghizadeh *et al*., 2009), Jamunapari goats (Khobra *et al*., 2012) and Rohilkhandi goats (Shrivastava *et al*., 2015; Shrivastava., 2015) at different loci. Marwari goats are more adapted to harsh climatic conditions of the desert with the scarcity of both feed and water. Polymorphism of MHC class II DRB3

gene has not been reported in the Marwari breed of goats so far. The present study was undertaken with the aim to find out the novel polymorphism at exon 2 of caprine MHC Class-II DRB3 gene in Marwari goats.

MATERIALS AND METHODS

Location

The study was undertaken at Molecular Genetics Laboratory, Division of Animal Genetics, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly (UP).

Animals samples

A total of 210 goats of Marwari breed were randomly selected from the farmers' herd located three kilometers away from Barmer city (Thar Desert region) of Rajasthan.

Sampling and analytical methods

About 5 ml of blood was collected under sterile conditions from the jugular vein of goats by using 2.7% EDTA as an anticoagulant in a 15 ml polypropylene centrifuge tubes to prevent clotting. All the blood samples were kept in -20°C till further processing for DNA extraction.

Genomic DNA extraction

The genomic DNA was isolated from whole blood by phenol-chloroform extraction and ethanol precipitation method as per the standard protocol (Sambrook and Russell, 2001).

Locus under investigation

The locus under investigation was selected from NCBI GenBank database. Two sequences with accession numbers KP888556 and KP888557 (Shrivastava *et al*., 2015) were utilized for this study which was conducted in order to find out the polymorphism at MHC Class II DRB gene in Rohilkhandi breed of goats. The sequences were aligned by using MEGA 6.0 software to get variations among the full length of sequences. The SNP (A/G) at 172 bp position was chosen from 285 bp fragment. Then, single cutter restriction enzyme (*Hin1I*) was selected by using NEBcutter V2.0 online available software. The *Hin1I* enzyme digests the 285 bp fragment and creates sticky ends at 173/175 bp position.

Polymerase chain reaction (PCR)

To get the desired 285 bp fragment, PCR was performed using primers with the sequence of the forward and reverse primers were 5′-TAT CCC GTC TCT GCA GCA CAT TTC-3′ and 5′-TCG CCG CTG CAC ACT GAA ACT CTC-3′, respectively (Amills *et al*., 1998). The PCR reaction was performed in 25 µl reaction mixture that included 1 µl (10 pmol) of each primer, 12.5 µl of 2X PCR master mix (Thermo Scientific) and 1 µl of 50 to 100 ng/µl of goat genomic DNA as a template and finally, 9.5 µl of nuclease free water was added to reaction mixture. The PCR conditions included initial denaturation at 95°C for 5 minutes followed by 40 cycles each of denaturation at 95°C for 1 minute, annealing at 59.5°C for 45 seconds, extension at 72°C for 1 minute and then a final extension at 72°C for 5 minutes. The 5 µl PCR products were checked by 1.5% agarose gel electrophoresis in order to check the quality and specificity of PCR product using ethidium bromide staining. Finally, the gels were photographed under UV light with a gel documentation system (Syngene).

Restriction enzyme digestion and electrophoresis

About 10 µl of PCR products were digested with *Hin1I* (Thermo Scientific) restriction endonuclease (2U) with the appropriate buffer supplied with the enzyme at 37ºC for 16 hours in a water bath. The enzyme *Hin1I* was inactivated by incubation at 65°C for 20 minutes. Digested PCR products were revealed by gel electrophoresis by using 2% agarose gel electrophoresis. Finally, the gels pictures were saved with a gel documentation system.

Statistical analysis

After enzymatic digestion, the allelic and genotypic frequencies of the locus at CLA-DRB3 gene fragment were estimated by standard procedure as given below (Falconer and Mackay, 1996):

$$\text{Genotype frequency} = \frac{\text{Number of individuals of a particular genotype}}{\text{Total Number of individuals of all genotypes}}$$

Table 1: Allele-wise gene and genotype frequency

Locus	Allele	Count	Frequency	SE*	Genotype	Count	Frequency
Hin1I	A	187	0.4517	0.0269	AA	50	0.242
	G	227	0.5483	0.0269	AG	87	0.420
	Total	**414**	**1**		GG	70	0.338
					Total	**207**	**1**

*Standard error

Table 2: Chi-square test values for HWE at Hin1I locus

Locus	Number of individuals	Number of alleles	PIC	Test for HWE			
				Chi-square	Df	Pr>Chi-square*	p** exact
Hin1I	207	2	0.373	4.905	1	0.0267	0.0266

*p-value for the Chi-square test; **an estimate of the exact p-value for the HWE test; HWE=Hardy–Weinberg equilibrium; PIC=Polymorphic information content

Table 3: Summary of heterozygosity statistics of Hin1I locus

Locus	Sample Size	Obs_Hom*	Obs_Het*	Exp_Hom[1]	Exp_Het[1]	Nei[2]	Na[3]	Ne[4]	I[5]
Hin1I	414	0.580	0.420	0.504	0.497	0.495	2.000	1.982	0.689

*Observed homozygosity and heterozygosity; [1]Expected homozygosity and heterozygosity; [2]Nei's expected heterozygosity; [3]na=Observed number of alleles; [4]ne=Effective number of alleles; [5]I=Shannon information index

$$\text{Gene frequency} = (2D+H)/2N$$

Where,

D = Number of homozygote animals of a particular genotype

H = Number of heterozygote animals having both alleles

N = Total number of individuals

The observed heterozygosity and unbiased estimate of gene diversity, neutrality ratios and test for deviations of the genotypic frequencies from Hardy-Weinberg equilibrium were done by using POPGENE V 1.32 software.

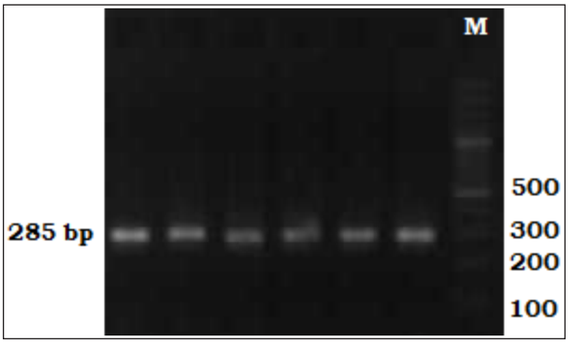
RESULTS AND DISCUSSION

A region of 285 bp CLA-DRB3 gene in Marwari goats was amplified (Fig. 1). The digestion of PCR product by *Hin1I* restriction enzyme revealed AA, AG and GG genotypes, respectively (Fig. 2). The genotypic frequency of homozygote AA, GG and heterozygote AG were 0.242, 0.338 and 0.420, respectively. The frequency of G allele (0.548) was higher was higher for A allele (0.452) (Table 1).

The locus showed a polymorphism information content (PIC) value 0.373 and heterozygosity value 0.495, respectively. The summary of markers in relation to PIC, and test for HWE are shown in Table 2. Further analysis of marker data was done by POPGENE V 1.32 to obtain other heterozygosity statistics. The observed numbers of alleles at this locus were two and the mean effective numbers of alleles were 1.982.

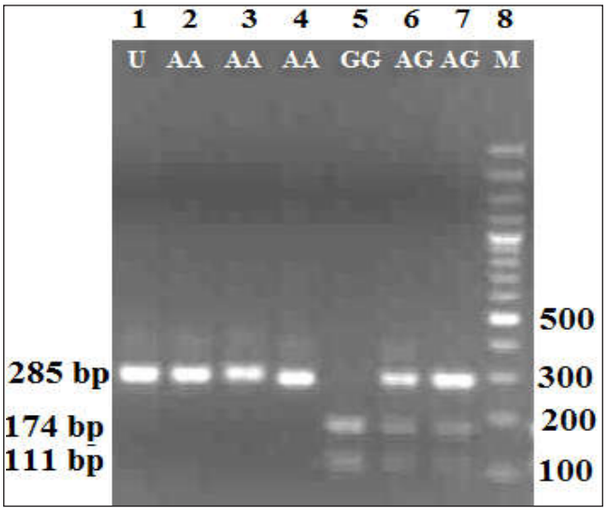
The Shannon's information index value was 0.689 (Table 3). The heterozygosity statistics at both loci are given in detail in Table 3. The F_{IS} value (Wright, 1978) for both alleles (A and G) at Hin1I locus was 0.1515. The positive value denotes an excess of homozygotes, Test for HWE

showed that population was significantly (P=0.0267) deviate from HW equilibrium at this locus, this may be due to the use of less number of males for breeding purpose.



Lane M: Marker (100 bp ladder)

Fig. 1: Amplified PCR product of 285 bp of MHC Class II DRB3 gene in Marwari goat



Lane 1: Undigested PCR product;
Lane 2-7: RE digested PCR product;
Lane 8: Marker (100 bp ladder)

Fig. 2: *HIn1I* digestion of MHC Class II DRB3 gene in Marwari goat

MHC Class II genes are having a key in conferring resistance/susceptibility to parasitic infestation (Karrow *et al*., 2014). The polymorphism at MHC loci is one of the major drivers of species survival. The polymorphism was reported in MHC Class II DRB3 gene of Marwari goats by PCR-RFLP technique. The polymorphism of this gene locus has been extensively studied by PCR-RFLP and is advocated to be used as a genetic marker for nematode resistance/susceptibility. The population genetic analysis of the genotypic data showed *Hin1I* locus has significantly deviated from HWE with comparatively less heterozygosity. However, earlier PCR-RFLP studies on MHC DRB gene have reported heterozygote excess and significant deviations from HWE using multiple restriction enzymes (Gruszczynska *et al*., 2004; Jamshidi *et al*., 2011).

In the current study, the MHC Class II DRB3 gene was found polymorphic in Marwari goats for *Hin1I* locus. The importance of this fragment is that it forms the part of antigen binding groove of the MHC gene and hence it has achieved much importance for disease resistance research. Polymorphism in this region can be studied for association study between indicator traits of gastrointestinal nematodiasis and different genotypes in Marwari goat and other Indian goat breeds.

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