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# DRB1 Gene Patterns of Two Iranian Sheep Breeds

Research Article

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## ABSTRACT

Genetic improvement programs may improve disease resistance in animal production. The best-characterized genetic control of disease resistance and immune response in animals is the one associated with the Major Histocompatibility Complex (MHC). The ovine lymphocyte antigen of DRB1 gene encodes cell surface glycoproteins that initiate immune responses by presenting processed antigenic peptides to CD4 + T helper cells. DRB1 is the most polymorphic gene in sheep and it has been extensively evaluated as a candidate marker for associations with various ovine diseases and immunological traits. Aim of this study was to analyze exon 2 Ovar-DRB1 gene polymorphism in two Iranian native sheep breeds (Afshari and Zel). PCR products were characterized by the restriction fragment length polymorphism (RFLP) technique using two restriction enzymes, *RsaI* and *HaeIII*. In the studied groups of Afshari Sheep and Zel Sheep of Iran we were able to identify 9 restriction patterns (a, b, c, d, e, f, g, h and i) for the fragments of exon 2 Ovar-DRB1 gene with *RsaI* enzymatic digestion and 5 patterns (a, b, c, d and e) with *HaeIII* enzyme, including one previously unrecognized allele (c). Our results indicate that exon 2 of the Ovar-DRB1 gene is highly polymorphic in both breeds.

KEY WORDS Afshari, MHC, Ovar-DRB1, PCR-RFLP, Zel.

## INTRODUCTION

In recent years, livestock breeders have focused on the improvement of production traits with little or no attention for improvement of disease resistance traits (Kumar *et al.* 2008). The MHC is a multi-gene family complex, controlling immunological self / non-self recognition. Amongst them are the genes that encode the cell surface glycoproteins that present peptides of foreign and self proteins to T cells, thereby controlling all specific immune responses, both cell and antibody mediated (Klein, 1986). A striking characteristic of MHC genes is their extreme polymorphism. Exon 2 of the Ovar-DRB1 gene codes for part of the MHC class II antigen binding cleft and over 80 alleles have been identified at this locus in sheep (Sayers *et al.* 2005). Several studies have reported a relationship between Class II DRB variants and resistance or susceptibility to mastitis (Dietz *et al.* 1997; Sharif *et al.* 1998), both in cattle (Xu *et al.* 1993) and in sheep (Konnai *et al.* 2003), and with protection induced by peptide vaccines against foot and mouth disease in cattle (Rupp *et al.* 2007; Garcia Briones *et al.* 2000). The polymorphism of the Ovar-DRB1 gene plays an important role in resistance to nematode infection in the Suffolk breed (Sayers *et al.* 2005).

Analysis of the Ovar-DRB1 gene in livestock is of special interest for two mains reasons: i) high functional importance of the gene (one of the key genes controlling the immune response of the organism to viral and bacterial infections and ii) high level of polymorphism (Mota *et al.* 2002; Konnai *et al.* 2003; Li *et al.* 2010). The first studies

used restriction fragment length polymorphism of amplified DNA fragments (PCR-RFLP) for Ovar-DRB1 gene assignments (Konnai *et al*. 2003; Li *et al*. 2010; Gruszczyñska *et al*. 2005).

Iran has 27 sheep breeds, which vary in their genetic productive potential (milk, meat and wool), disease resistance and fecundity, among others (Tavakolian, 2000). Iranian sheep populations can be classified according to their color and body weight (Saadat Noori and Siah Mansoor, 1982). All Iranian native sheep breeds are fat-tailed, except the Zel breed. Afshari breed is the largest sheep breed for meat production and is characterized by high reproductive performance (Saadat Noori and Siah Mansoor, 1982).

Previous studies have reported the use of *RsaI* restriction enzyme for polymorphism detection of second exon of the DRB1 gene in Sangsari (Jamshidi *et al*. 2010), Shaul, Lori-Bakhtiyari and Zandi breeds (Nikbakht *et al*. 2011) breeds. The objective of the present study was to identify genotypes and allelic frequencies of the Ovar-DRB1 in Afshari and Zel breeds. This is the first description of Ovar-DRB1 pattern in Iranian sheep breeds (Afshari and Zel) using *HaeIII* restriction enzyme.

## MATERIALS AND METHODS

### DNA extraction

Blood samples (approximately 8 to 10 mL) were collected from the jugular vein of 50 Afshari sheep and 50 Zel sheep into vacutainer tubes with 10% of 0.5 M EDTA-coated vacutainer tubes. Samples were collected from different locations in the Zanjan and Mazandaran provinces, respectively. Information on the two native sheep populations are shown in Table 1. Total DNA was isolated from 5ml blood aliquots by Miretti (2001) and was measured at 260 nm optical density according to the method described by Sambrook and Russell (2001).

### Amplification of Ovar-DRB1 Exon 2

The second exon of Ovar-DRB1 was amplified by the PCR technique in two stages. Ovar-DRB1 exon 2 was amplified by PCR with the primers of Ovar-HL030, Ovar-HL031 and Ovar-HL032. The first round of PCR was performed with primers HL030-(5′-ATC CTC TCT CTG CAG CAC ATT TCC-3′) and HL031-(5′-TTT AAA TTC GCG CTC ACC TCG CCG CT-3′) adapted from Van Eijk *et al*. 1992. One hundred ng of genomic DNA was subjected to amplification by PCR in a total volume of 25 µL, including: 1.5 m*M* $MgCL_2$ and 200 m*M* dNTPs to which 0.5 m*M* of each primer and 1 U of Taq polymerase. Reactions were performed in a thermo cycler under the following conditions: one cycle of incubation for 3 min at 94 ˚C, followed by 30 cycles of 95 ˚C for 30 s, 60 ˚C for 30 s, and 72 ˚C for 30 s, with final extension at 72 ˚C for 5 min. The constituents of the second round PCR mixtures were the same as those described above, except with only a modification of the reverse primer. We used 2.0 µL of the resulting mixture, plus primers HL030 and HL032 (5′-GTC CCG CTG CAC AGT GAA ACT CTC-3′) for the second round of PCR (adapted from Van Eijk *et al*. 1992). The conditions for the second round of PCR were one cycle for 3 min at 94 ˚C, followed by 30 cycles of 95 ˚C for 30 s, 65 ˚C for 30 s, and 72 ˚C for 30 s with a final extension at 72 ˚C for 5 min.

### Restriction endonuclease digestion

The restriction analysis of the PCR-amplified products was performed with restriction endonucleases *RsaI* and *HaeIII* according to the manufacturer's instructions. The digestion products were resolved in 12% polyacrylamide at 120 V for 60 min. A 50 bp DNA ladder (Fermentas, Germany) was used as a DNA size marker. The Ovar-DRB1 nomenclature, described by Li *et al*. 2010; Gruszczyñska *et al*. 2004; Jam shidi *et al*. 2010 was followed to identify the different allele types obtained in the present study from the different restriction enzyme patterns.

### Statistical analysis

The frequencies of alleles were estimated by PhotocaptMwver 99.03 software. Difference between the observed and expected distributions of genotypes was checked by the $\chi^2$ test. The POPGENE 1.31 program (Yeh *et al*. 1999) was used to perform statistical analysis.

## RESULTS AND DISCUSSION

### PCR amplification

Ovar-DRB1 exon 2 was amplified by PCR with the primers of Ovar-HL030, Ovar-HL031 and Ovar-HL032. One specific band of 270 bp was observed by 1% agarose gel electrophoresis.

### The result of PCR-RFLP

When the amplified products were cleaved using the restriction enzyme *RsaI*, 6 and 4 different digest patterns in the Afshari and Zel breeds were observed (Table 2), respectively. Moreover, the number of bands in the different restriction patterns of *HaeIII* ranged from 2 to 4, and the sizes of the band are illustrated in Table 3.

### Alleles and genotype frequencies

After digestion with restriction endonuclease *RsaI* and *HaeIII*, allele frequencies of Ovar-DRB1 gene in two Iranian native sheep were determined. The allele frequencies of PCR-RFLP in the second exon of the Ovar-DRB1 gene made by both restriction enzymes in Afshari and Zel sheep breeds were shown in the Table 4.

**Table 1** Phenotypic traits (color and body weight), horn existence and number of flocks of two native sheep breeds

| Breed | Colour | Weight ♂ | Weight ♀ | Horn ♂ | Horn ♀ | Flock |
|---|---|---|---|---|---|---|
| Afshari | Brown (from light to black) | 85 | 65 | × | × | 7 |
| Zel | White, brown and black (dark head and legs) | 55 | 50 | √ | × | 6 |

**Table 2** Distribution of *RsaI* restriction patterns obtained for exon 2 of the Ovar-DRB1 gene in Afshari (A) and Zel (Z) sheep breeds

| RE | Size | Breed | | | | |
|---|---|---|---|---|---|---|
| *RsaI* | bp | A | Z | Sa | Sh | Ph, Pl |
| a | 222 / 48 | * | * | * | * | * |
| b | 270 | * | - | - | * | * |
| c | 177 / 93 | * | - | * | - | * |
| d | 141 / 129 | * | - | * | - | - |
| e | 104 / 69 / 50 / 47 | * | - | * | * | * |
| f | 117 / 106 / 47 | * | - | * | - | - |
| g | 130 / 56 / 51 / 33 | - | * | * | - | * |
| h | 110 / 103 / 57 | - | * | * | - | - |
| i | 110 / 68 / 57 / 23 / 12 | - | * | * | * | * |

* Pattern identified in Afshari (A) and Zel (Z) sheep breeds corresponding with Sangsari (Sa) sheep (Jamshidi *et al*. 2010), Shaul (Sh) sheep (Nikbakht *et al*. 2009) and Polish Heath (PH) Sheep and Polish Lowland (PL) Sheep described by Gruszczyñska *et al*. (2005).

**Table 3** Distribution of *HaeIII* restriction patterns in two Iranian native sheep breeds using PCR-RFLP method

| RE | Size | Breed | | |
|---|---|---|---|---|
| *HaeIII* | bp | A | Z | K |
| a | 147 / 123 | * | * | * |
| b | 152 / 66 / 52 | * | * | * |
| c | 204 / 66 | * | * | - |
| d | 145 / 111 / 14 | - | * | * |
| e | 145 / 64 / 51 / 10 | - | * | * |

* Pattern identified in Afshari (A) and Zel (Z) sheep breeds corresponding with Kazakh (K) sheep (Li *et al*. 2010).

The restriction pattern analyses used the patterns described by Gruszczyñska *et al*. 2005; Nikbakht Boroujeni *et al*. 2009; Jamshidi *et al*. 2010 of which we detected 6 (Afshari) and 4 (Zel) of *RsaI* pattern.

**Table 4** Allelic frequencies of second exon of the MHCDRB1 gene in Afshari (A) and Zel (Z) sheep populations, using *RsaI* and *HaeII* restriction enzymes

| *RsaI* | | *HaeIII* | |
|---|---|---|---|
| A | Z | A | Z |
| a (18.78) | a (40) | a (61.2) | a (39) |
| b (25) | g (30) | b (22.2) | b (27.7) |
| c (6.25) | h (20) | c (16.6) | c (11.1) |
| d (12.5) | i (10) | - | d (11.1) |
| e (25) | - | - | e (11.1) |
| f (12.5) | - | - | - |

In addition, based on Li *et al*. (2010) patterns, 3 and 5 alleles were detected in *HaeIII* pattern of Afshari and Zel, respectively. The genotype frequencies distribution of the *RsaI* and *HaeIII* patterns in two studied breeds are shown in Table 5.

**Statistical analyses**

By Chi-Square test, ovar-DRB1 exon 2 of Afshari and Zel sheep breeds was analyzed to determine whether it fitted the Hardy-Weinberg equilibrium.

The Chi-Square values of two restriction enzymes *RsaI* and *HaeIII* patterns of both breeds were not within Hardy-Weinberg equilibrium.

***RsaI* Patterns**

When the amplified products were cleaved using the restriction enzyme *RsaI*, 6 different digest patterns were observed in the population of Afshari sheep and 4 different patterns in Zel sheep. In Afshari sheep all patterns except pattern b were similar to those previously reported by Jamshidi *et al*. 2010.

In the last mentioned study, two putative novel PCR-RFLP *RsaI* pattern (d and f) in Sangsari sheep was introduced, and such pattern was also observed in Afshari sheep. In Zel sheep, only one pattern (a) among 4 is similar to the Afshari.

All Zel patterns were detected in other Iranian sheep breeds (Sangsari, Shaul, Zel and Lori-Bakhtiyari) previously described by Jamshidi *et al*. (2010) and Nikbakht *et al*. (2011).

The study of Jamshidi *et al*. (2010) on 138 Sangsari sheep showed that the most frequent Ovar-DRB1allele, based on *RsaI* digestion, was pattern a (0.398), and interestingly, similar results were reported also in Zel sheep. In Afshari sheep, 2 alleles (b and e) accounted for 50% of the alleles.

Genotypes ee and gg were the most common in Afshari and Zel breeds, respectively. Nikbakht *et al*. (2011) studied the polymorphism of Ovar-DRB1 exon 2 of three fat-tailed Iranian sheep breeds by PCR-RFLP with the restriction enzyme *RsaI*. However, with the restriction enzyme *RsaI*, most genotype frequencies belonged to the ee, ii and ee for Lori-Bakhtiyari, Shaul and Zandi Breeds, respectively.

In the work by Nikbakht *et al*. (2011), three sheep populations of Iran (Shaul, Lori-Bakhtiyari and Zandi) were studied by digestion of the 308 bp PCR fragment with *RsaI*, and a total of 7 alleles and 12, 17 and 11 genotypes were found.

In another report on allelic variations in the DRB1 gene in Iranain Sangsari sheep breed, 8 alleles and 13 genotypes were found (Jamshidi *et al*. 2010).

**Table 5** Genotypic frequencies of allele combinations as detected after RsaI and Hae III digestions of the second exon of MHC-DRB1 gene in Iranian Afshari (A) and Zel (Z) Sheep

| *RsaI* | | *HaeIII* | |
|---|---|---|---|
| A | Z | A | Z |
| aa (0.2) | aa (0.3) | aa (0.8) | aa (0.74) |
| ab (0.02) | ag (0.06) | ac (0.1) | ab (0.02) |
| bb (0.16) | ai (0.18) | bb (0.08) | bb (0.1) |
| be (0.02) | gg (0.44) | cc (0.02) | cc (0.02) |
| cd (0.02) | hh (0.02) | - | dd (0.06) |
| de (0.02) | - | - | ee (0.06) |
| ee (0.54) | - | - | - |
| ff (0.02) | - | - | - |

Similarly, in Polish Heath (PH) Sheep and Polish Lowland (PL) Sheep described by Gruszczyñska *et al*. (2005) 8 alleles and 13 genotypes were described, whereas in our studies 6 (Afshari) and 4 (Zel) alleles and 8 (Afshari) and 5 (Zel) genotypes were reported. This difference may be due to the different numbers and species of sheep.

### *HaeIII* Patterns

When each amplified product was digested by the *HaeIII* restriction enzymes, the cleavage and allele patterns were in accordance with Li *et al*. (2010). Alleles a, b and c were found in A and Z, similarly. Also, patterns d and e were identified only in Zel sheep, and their presence in Kazakh sheep was explained earlier by Li *et al*. (2010).

With the use of *HaeIII* restriction enzyme, 3 alleles and 4 genotypes were found in Afshari population, whereas 5 alleles and 6 genotypes characterized the Zel population, and their genotypic restriction map is shown in Table 5.

A simulation of separation of the published sequences with the *HaeIII* enzyme revealed that similarly as in Afshari Sheep (0.612) and Zel Sheep (0.39), pattern a (147/123 bp) was significantly more common than other patterns in both breeds. Moreover, aa was the most frequent genotype in Afshari (0.80) and Zel (0.74) sheep breeds, detected by exon 2 *HaeIII* digestion pattern; however, in Kazakh breed, the most frequently observed pattern was b (25% in hydatidosis negative and 31% in hydatidosis positive; Li *et al*. (2010).

In vertebrates, the MHC plays a central role in foreign antigen recognition and immune response to pathogens. Some Ovar-DRB1 alleles may be better suited to display antigens to certain diseases and so generate a better immunity through an improved T-cell response repertoire. In the study of Ovar-DRB1 exon 2 of Kazakh sheep by Li *et al*. (2010) the frequencies of genotypes were compared between sheep affected by hydatidosis and controls by statistical methods. Statistical analysis, showed that the genotype frequencies of *HaeIII* dd in control sheep were higher ($P<0.01$) than in hydatidosis sheep, indicating that a strong association exists between these genotypes and hydatidosis resistance. In our study this genotype was found in Zel sheep.

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