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STUDY ON GENETIC VARIABILITY IN MHC-DRB1 SECOND EXON IN MAKUIE SHEEP BREED POPULATION

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In the present study polymorphism of the exon 2 of *MHC* (Major Histocompatibility Complex) gene in Makuie sheep breed was studied. Genomic DNA from blood samples of 90 sheep was extracted and a 279 bp *MHC* exon 2 fragment was amplified using polymerase chain reaction (PCR). PCR products were subjected to enzymatic digestion using RsaI endonuclease. Digested PCR products were electrophoresed on 2% agarose gel. The results showed the existence of 10 alleles: A, B, E, F, I, M, O, P, Q and V for the exon 2 of the *MHC* gene, with the frequencies of 0.4756, 0.0976, 0.0183, 0.0366, 0.0549, 0.0122, 0.1098, 0.0915, 0.0854 and 0.0183, respectively. Eighteen genotypes: AA, AB, AE, FF, AM, BO, EO, IO, OM, AP, BP, OP, PP, AQ, OQ, PQ, QQ and AV with the frequencies of 0.317, 0.1585, 0.0121, 0.0365, 0.0121, 0.0243, 0.0243, 0.1097, 0.0121, 0.0487, 0.0121, 0.0365, 0.0365, 0.0487, 0.0121, 0.0121, 0.0487 and 0.0365, respectively were identified in the population under study. Effective number of alleles and heterozygosity for the examined region were 3.7231 and 0.7314, respectively. Chi-square test showed that the examined sheep population was not in Hardy-Weinberg equilibrium in the examined region.

Key words: genetic variation, Hardy-Weinberg equilibrium, Makuei sheep, PCR-RFLP, west Azerbaijan

INTRODUCTION

Major histocompatibility complex (*MHC*) plays an important role in the immune response of animals. Extreme polymorphism in the *MHC* gene enables the host to recognize

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enormous numbers of foreign peptides triggering an immune response (TIZARD *et al.,* 2008). Actually, cell surface proteins coded by gene families in the *MHC* loci are classified as class I or class II based on differences in the cellular distribution, molecular weight and functions. Class I genes of the *MHC* code for proteins that found in almost all nucleated cell of body and class II proteins are primarily restricted to the surface of immune cells and are responsible for immune regulation (DUKKIPATI *et al.,* 2006a; AHMED *et al.,* 2006).

Sheep *MHC* is known as Ovar and it located on chromosome 20 (BOZKAYA *et al.,* 2007). Exon 2 of the *MHC* gene encode the outer domain of the *MHC* molecule that composed none covalently linked subunits (α and β), which is the binding area for antigens (GRUSZCZYNSKA *et al.,* 2005). *MHC* gene at *DRB1* region consists of one exon and one intron in all studied species (GELDERMANN *et al.,* 2006). Among Ovar class II genes, the expressed DR beta1 gene has been found to be high polymorphic.

To date at least 160 *Ovar-DRB1* alleles have been identified by DNA sequencing of the exon 2 region in various sheep breeds. Several reports are available which describe associations between special alleles and disease resistance (KONNAI *et al.,* 2003b; DUKKIPATI *et al.,* 2006b; LI *et al.,* 2011). In a study microsatellite markers were used to investigate any genetic variation in the exon 2 of the *DRB1* locus (KENNEDY *et al.,* 2011). A positive relation was found between microsatellite polymorphism in this region and fertility and grows traits in sheep. The relation between polymorphism in the *MHC-DRB1* region with reproduction, milk production and grows traits have been identified by many authors (MIR *et al.,* 2008; GELDERMANN *et al.,* 2006).

Makuie sheep breed is fat-tailed sheep with medium body size and black spots on face and feet. They are housed in east and west Azerbaijan provinces of Iran and their main products are meat and wool (KHALDARI, 2003). The present research focused for the first time on the analysis of the genetic variation in the *MHC* class II *DRB1* gene in Makuie sheep breed using PCR-RFLP method.

MATERIALS AND METHODS

Sampling and DNA extraction

A number of 90 blood samples comprising both sexes of Makuie sheep breed were randomly collected in a research station of Maku city in west-Azerbaijan. The whole blood was preserved in ethylene diamine tetra acetic acid (EDTA)-coated tubes and stored at –20 °C. Genomic DNA was extracted from 0.3 mL blood using the genomic DNA purification kit (Ferementas Cat. No 0512) according to manufactori's instructions. Quality and quantity of extracted DNA was measured on 0.8% agarose gel prepared in 0.5 X TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA, PH 8.0) and visualized with ethidum bromide (1.0 mg mL^{-1}) and photographed under UV light using a Gel-Doc image analysis system (Gel Logic 212 PRO, USA).

Amplification of the exon 2 of the *MHC-DRB1* gene

The amplification of the exon 2 region of the *MHC-DRB1* gene was achieved using primers MHC-Forward (5′-TCTCTGCAGCACATTTCCTGG-3′) and MHC-Reverse (5′-CTCGCCGCTGCACAGTGAAAC-3′) amplifying a fragment of 279 bp as described by Tkacikova *et al,* (2005). The PCR were performed in a final volume of 25 µL containing 100 ng template DNA, 0.5 µM of each primer, 2.5 µL of 10 × PCR buffer, 25 µM each of dATP, dCTP,

dGTP and dTTP, 2 mM $MgCl_2$, 2.5 U Taq DNA polymerase (CinnaGen, Tehran, Iran) using a 96-well Eppendorf Mastercycler Gradient (Type 5331, Eppendorf AG, Hamburg, Germany). The solution was initially denatured at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 90 s, annealing at 60 °C for 30 s and extension at 72 °C for 90 s and a final extension at 72 °C for 10 min. Five µL of PCR-products were electrophoresed on 2% agarose gels in order to check the quality and specificity of amplification.

Restriction Fragments Length Polymorphism

The restriction enzyme RsaI was used to examine the nucleotide sequence variation at the exon 2 region of the *MHC-DRB1* gene. Seven µL of PCR product was mixed and treated with 0.5 unit of RsaI, 1 µL green buffer, 6.5 µL distilled water in a final reaction volume 15 µL according to the manufacturer's instruction (Fermentas). The restricted fragment was analyzed by electrophoresing product on 2% agarose gel in 0.5 X TBE buffer, stained with ethidium bromide and photographed under UV light using a Gel-Doc image analysis system (Gel Logic 212 PRO, USA).

Population genetic analysis

The allelic and genotypic frequencies and observed and expected Nei's heterozygosities (He = 1- $\sum P_i^2$, where P_i is the frequency of allele i) were estimated by using PopGene32 version 1.31 (YEH *et al.,* 1999). Hardy-Weinberg equilibrium test was performed in the PopGene32.

RESULTS

Amplification of *MHC-DRB1* second exon

The amplification of a 279 bp fragment of the exon 2 of the *MHC-DRB1* gene was successful in our first attempt. All DNA extract from blood samples yielded a specific single band. Therefore, the PCR products were directly used for RFLP analysis.

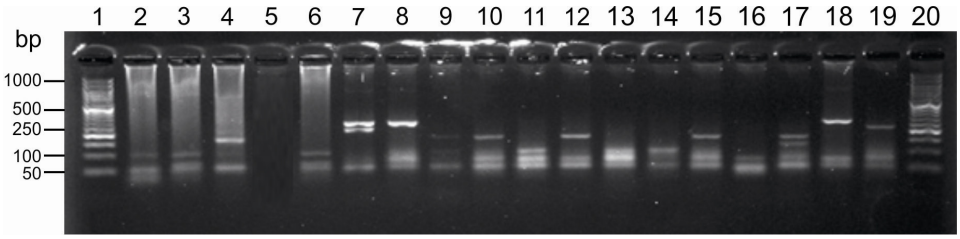

Figure1. PCR-RFLP analysis of the second exon of *Ovar-DRB1* gene (279bp) from Iranian Makoei sheep. Lane 1 and 20: 50 bp ladder marker, lane 2: genotype AE, lane 3 and 14: genotype AA, lane 4 and 9: genotype AB, lane 6,13 and 15: genotype IO, lane 7: genotype PQ, lane 8 and 18: genotype OP, lane 10 and 12: genotype BO, lane 11 and 17: genotype OM, lane 16: genotype EO, lane 18: genotype OP, lane 19: genotype QQ.

RFLP analysis of amplified fragments

Amplified products of *MHC-DRB1* second exon were digested using RsaI restriction enzyme. Within ninety digested samples a number of 25 different patterns were observed. The banding patterns of some individuals have been shown in Figure 1. By analyzing restriction map, polymorphic sites were detected at positions 240, 228, 105, 72, 54 and 51 (Table 1). Ten alleles and 18 genotypes were identified in the tested population (Table 1). Allele A and genotype AA with the frequencies of 0.4756 and 0.317, respectively, were the most frequent allele and genotype in the studied Makuie sheep breed population. Observed homozygosity and heterozygosity were 0.439 and 0.561, respectively. Expected homozygosity and heterozygosity were 0.2641 and 0.7359, respectively (Table 1). Shannon's information index (I) was 1.7323 (Table 1). Chi-square test showed that the examined sheep population was not in Hardy-Weinberg equilibrium ($P \geq 0.05$) for the examined region.

Table 1. Allele and genotypic frequencies for the exon 2 of the MHC-DRB1 gene in Iranian Makuie sheep breed.

Genetic diversity statistics	Value	Allele	Frequency	Genotypic frequencies	Number of sheep	Frequency
N_A	10	A (105bp/69bp/54bp/51bp)	0.4756	AA (105bp/69bp/54bp/51bp)	25	0.317
N_E	3/7231	B (123bp/105bp/51bp)	0.0976	AB (123bp/105bp/69bp/54bp/51bp)	13	0.1585
I	1/7323	E (72bp/58bp/54bp/51bp/44bp)	0.0183	AE (105bp/72bp/69bp/58bp/54bp/51bp/44bp)	1	0.0121
Observed homozygosity	0/439	F (135bp/54bp/51bp/39bp)	0.0366	FF (135bp/54bp/51bp/39bp)	3	0.0365
Observed heterozygosity	0/561	I (103bp/72bp/54bp/50bp)	0.0549	AM (135/105bp/72bp/69bp/54bp/51bp/42bp/30bp)	1	0.0121
Expected homozygosity	0/2641	M (135bp/72bp/42bp/30bp)	0.0122	BO (240bp/123bp/105bp/51bp/39bp)	2	0.0243
Expected heterozygosity	0/7359	O (240bp/39bp)	0.1098	EO (240bp/72bp/58bp/54bp/51bp/44bp/39bp)	2	0.0243
Average heterozygosity	0/7314	P (279bp)	0.0915	IO (240bp/103bp/72bp/54bp/50bp/39bp)	9	0.1097
		Q (228bp/51bp)	0.0854	OM (240bp/135bp/72bp/42bp/39bp/30bp)	1	0.0121
		V (156bp/72bp/51bp)	0.0183	AP (279bp/105bp/69bp/54bp/51bp)	4	0.0487
				BP (279bp/123bp/105bp/51bp)	1	0.0121
				OP (279bp/240bp/39bp)	3	0.0365
				PP (279bp)	2	0.0365
				AQ (228bp/105bp/69bp/54bp/51bp)	4	0.0487
				OQ (228bp/240bp/51bp/39bp)	1	0.0121
				PQ (279bp/228bp/51bp)	1	0.0121
				QQ (228bp/51bp)	3	0.0487
				AV (156bp/105bp/72bp/69bp /54bp/51bp)	2	0.0365

N_A = Number of alleles; N_E = effective number of alleles; I = Shannon's information index.

DISCUSSION

Polymorphism in the exon 2 region of the *DRB1* gene in Iranian Makuie sheep breed (fat-tailed sheep breed) was studied by PCR-RFLP. Compared to cattle and other animals, *Ovar-DRB1* locus is poorly studied in Iranian sheep breeds. Different methods have been used to study genetic variation in the *Ovar-DRB1* gene in various sheep breeds. Among different methods, PCR-RFLP analysis has been found a valuable technique in identifying genetic variation of the *DRB1* gene in farm animals (KONNAI *et al.,* 2003a, b; GRUSZYNSKA *et al.,* 2005).

Ten alleles and eighteen genotypes were identified in the exon 2 region of the *MHC-DRB1* gene in Makuie sheep breed population. The most frequent allele and genotype were A allele and AA genotype in the frequencies of 0.4756 and 0.317, respectively. Our results partly were in agreement with the results of KONNAI *et al*. (2003b) and GRUSZCZYNSKA *et al*. (2005). KONNAI *et al*. (2003a) reported alleles Q and P in Polish Heath and Polish Lowland sheep breeds with 6 and 13 different patterns respectively. GRUSZCZYNSKA *et al*. (2005) reported alleles B, E, D and F in Suffolk sheep breed. Other alleles (A, I, M, O and V) were not reported in sheep breeds until now. CHARON *et al.* (2000) have shown positive association between *MHC-DRB1* alleles with reduced faecal egg counts in parasitic infestations. Ovine *MHC* class II *DRB1* alleles reported to be associated with susceptibility to development of bovine leukemia virus-induced ovine lymphoma (NAGAOKA *et al.,* 1999). In recent researches we couldn't find any creature without *MHC* molecules. This truth can indicate that creatures without *MHC* couldn't survive longtime after their birth because any slight defect or mutation in *MHC* molecules would cause intense immunological disorder (DUKKIPATI *et al.,* 2006b).

Shannon's information index revealed high genetic diversity within studied population. The chi-square test showed significant ($P \geq 0.05$) deviation from Hardy-Weinberg equilibrium at the *MHC-DRB1* gene in the studied population. This was the first report on studying the polymorphism in the *MHC-DRB1* locus in Makuie sheep breed. The previous breeding programs in the most research centers of Iran were based on only phenotypic characters. This study may be considered as an introductory to understanding the genetic variability on native sheep breeds in the Azerbaijan regions by using molecular techniques that do not affect by environmental effects.

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ISPITIVANJE GENETČKE VARIJABILNOSTI U MHC-DRB1 DRUGOM EGZONU U SELEKCIONOJ POPULACIJI MAKUIE OVCE

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Izvod

Vršena su ispitivanja polimorfizma egzona 2 MHC (Major Histocompatibility Complex) gena kod Makuie ovce. Ekstrahovana genomska DNK iz uzoraka krvi 90 ovaca I *MHC* exon 2 fragment veličine 279 bp je amplifikovan koristeći PCR. PCR produkti su digestovani endonukleazom RsaI I razdvajani na 2% agaroznom gelu. Dobijeni rezultati su pokazali da postoji 10 alela,: A, B, E, F, I, M, O, P, Q i V za egzon 2 ispitivanog gena, različite učestalosti (0.4756, 0.0976, 0.0183, 0.0366, 0.0549, 0.0122, 0.1098, 0.0915, 0.0854 i 0.0183). Identifikovano je 18 genotipova , AA, AB, AE, FF, AM, BO, EO, IO, OM, AP, BP, OP, PP, AQ, OQ, PQ, QQ i AV učestalosti 0.317, 0.1585, 0.0121, 0.0365, 0.0121, 0.0243, 0.0243, 0.1097, 0.0121, 0.0487, 0.0121, 0.0365, 0.0365, 0.0487, 0.0121, 0.0121, 0.0487 i 0.0365. Efektivan broj alela i heterozigotnosti u ispitivanom regionu je utvrđen, 3.7231 i 0.731. Hi- kvadrat test je pokazao da ispitivana populacija ovaca nije bila u Hardy-Weinberg ekvilibriumu u ispitivanom region.

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