LINKAGE DISEQUILIBRIUM BETWEEN MHC-LINKED MICROSATELLITE LOCI IN WHITE KARAMAN, AWASSI AND MERINOLANDSCHAF SHEEP BREEDS

Faruk BOZKAYA[1] Ercan KURAR[2]

[1]Harran Üniversitesi, Veteriner Fakültesi, Zootekni Anabilim Dalı, Şanlıurfa –TÜRKİYE

[1]Selcuk Üniversitesi, Veteriner Fakültesi, Zootekni Anabilim Dalı, Konya –TÜRKİYE

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ABSTRACT

The purpose of this study was to examine the presence of linkage disequilibrium between nine microsatellite loci in and out of Major Histocompatibility Complex (MHC) as a description of population structure in White Karaman, Awassi and Merinolandschaf sheep populations. Of the 108 pairwise comparisons a significant linkage disequilibrium was observed between six and seven loci pair after exact test and pooling, respectively. Only two and three of the significant deviations were present between MHC-linked loci. The results indicated that the populations analyzed in this study were mostly in equilibrium and that selection did not play a major role to *maintain the linkage disequilibrium between MHC-linked loci in the populations studied.*

Key Words: *Microsatellite, MHC, Sheep*

ÖZET

Akkaraman, İvesi ve Merinolandschaf Koyun Irklarında MHC ile Bileşik Mikrosatellit Lokusları Arasında Bileşiklik Dengesizliği

Sunulan çalışmada, Akkaraman, İvesi ve Merinolandschaf koyun ırklarında Büyük Doku Uyuşum Komplexi (MHC) ile bileşik olan ve olmayan mikrosatellit lokusları açısından bileşiklik dengesizliği olup olmadığı araştırıldı. Toplam 108 karşılaştırmada normal ve toplulaştırılmış analiz sonucunda sırasıyla altı ve yedi tane lokus çifti arasında önemli bileşiklik dengesizliği tespit edildi ($p < 0,05$). Normal ve toplulaştırılmış analiz sonucunda önemli sapmalardan sırasıyla yalnız ikisi ve üçünün MHC ile birleşik lokuslar arasında olduğu bulundu. Sonuç olarak araştırmaya konu olan populasyonların incelenen lokuslar açısından büyük oranda denge durumunda bulunduğu ve MHC ile birleşik lokuslar arasındaki bileşiklik dengesizliğinin seleksiyon yoluyla sürdürülmediği kanısına varıldı.

Anahtar Kelimeler: *Mikrosatellit, MHC, Koyun*

INTRODUCTION

Linkage disequilibrium (LD) or genotypic disequilibrium can give information about population structure and history. LD is also widely used to detect genes associated inherited diseases (1). It is also important to have information about the presence of LD in a population for forensic purposes such as identity or parentage testing (2).

Major Histocompatibility Complex (MHC) plays a key role in the immune response and is thought to be under some selection pressures due to some pathogenic agents (3, 4). Therefore, several studies indicated close associations between polymorphisms of MHC-genes and resistance to some animal diseases (4-6). It was found in different studies that microsatellite loci located within MHC showed high levels of LD but not between MHC and flanking markers in some free living sheep populations (7, 8).

Microsatellites are noncoding DNA fragments with short tandem repeat sequences (STR). The motives of one to four nucleotides can repeat tandemly up to thirty times. Repeat regions are flanked with nonrepeat sequences. Due to their high mutation rates, microsatellites show a great deal of polymorphism (9). Microsatellites are widely used for examining of population structure, gene and Quantitative Trait Loci (QTL) mapping and forensic purposes due to their high polymorphisms and the simplicity of their analysis (2, 10-13). Primer binding regions of some microsatellite loci are highly conserved, which thereby allows the use of same microsatellite markers in closely related species such as cattle, sheep and goat. For example, about 70% of the bovine derived microsatellites reciprocally amplify ovine, and over 60% of these are informative in sheep (14, 15).

The purpose of this study was to examine presence of LDs between six MHC linked microsatellite loci and to compare with that between MHC unlinked loci in White Kraman, Awassi and Merinolandschaf sheep populations.

MATERIALS and METHODS

Animal material : Blood samples were collected randomly from three local sheep breeds. Two of them were from Turkey (White Karaman sheep (n=60) from Central Anatolia and Awassi sheep (n=63) from South-East Anatolia) and the other one was from Germany (Merinolandschaf (n=70) from Baden-Würtemberg). The blood samples collected from Turkey were conserved with NaCl until DNA extraction. However, the blood samples from Germany were deep-frozen. DNA was extracted by using a Phenol-Chloroform extraction following Proteinase-K digestion.

Microsatellite loci : Nine microsatellite loci were included in the analysis. Six of them were linked with MHC located on sheep chromosome (OAR) 20 (16). The other microsatellite loci were located on different sheep chromosomes and were not linked with MHC. These unlinked loci were used for comparing of the results with those linked with MHC. Table 1 gives some information about the loci studied.

Table 1: Information about the microsatellite loci

Locus	Chromosom	Repeat motive	References
MHC-linked			
MSDRB	20 (Class IIa)	$(GT)_n(GA)_m$	Schweiger et al. (17)
DYMS1[1)]	20 (Class IIb)	$(CA)_{12}$	Buitkamp et al. (18)
SMHCC1[1)]	20 (Class I)	$(CA)_{20}$	Groth and Wetheral (19)
BF[1)]	20 (Class III)	$(CA)_{24}$	Groth and Wetheral (20)
BM1258[2)]	20 (Adjacent to Class IIa)	$(GT)_{16}$	Bishop et al. (10)
BM1818[2)]	20 (Adjacent to Class I)	$(GT)_{13}$	Bishop et al. (10)
MHC-unlinked			
ILSTS005[2)]	7	$(AT)_6(GT)_9(AT)_7$	Brezinsky et al. (11)
ILSTS011[2)]	9	$(CA)_{11}$	Brezinsky et al. (12)
ILSTS059[2)]	13	$(CA)_4(GT)_{21}$	Kemp et al. (13)

[1)]Locus was defined in sheep, [2)]Locus was defined in cattle

Table 2: PCR conditions used for amplification of different loci

		Conditions (C°/sec)		
Locus	$MgCl_2$(mM)	Denaturation	Annealing	Extention
MSDRB, BF, SMHCC1	1,6	94/30	60/60	72/60
DYMS1	1,6	94/30	52/60	72/60
BM1258, BM1818	1,4	94/15	58/30	72/20
ILSTS11, ILSTS059	1,6	94/30	60/30	72/30
ILSTS005	1,6	94/30	54/30	72/30

Marker genotyping : The microsatellite loci were amplified by Polymerase Chain Reaction (PCR) using fluorescein labeled primers, and the fragment lengths of the amplification products were analyzed by the use of an A.L.F. Sequencer (Pharmacia) on %5 HydroLynk gels. The lengths of the amplified fragments were measured by using two internal standard markers, which were the amplification products of a λ-Phage-DNA (Mutant CI857SAM7).

Electrophoresis was performed in 0.6 % TBE buffer at running condition of 1500 V, 45 mA and 50 °C for 3 hrs. Analysis of data was carried out with AlleleLinks software (Pharmacia, Freiburg, Germany).

PCR conditions used were as follows: Initial denaturation for 3 min at 94 °C followed by 30 PCR cycles each with 30-60 sec. at 52-60 °C, 30-60 sec. at 72 °C and 30-60 sec at 94 °C, and a final extension for 5 min at 72 °C (Table 2). PCR reactions were carried out for volumes of 12,5 µl with 0.4-0,8 µM primer, 200 µM each dNTP, 100 ng genomic DNA, 1,4-1,6 mM MgCl2 (Table 2) and 0.5 units of Taq polymerase (Roth, Karlsruhe).

Statistical analysis : To estimate the genotypic disequilibrium between loci GENEPOP Version 3.3 (21), was used. GENEPOP creates contingency tables for all pairs of loci in each sample, then performs a probability test (Fisher's exact test) for

each table using a Markov-Chain-Method. As no family information was available, the haplotype frequencies could not be estimated. But, the observed genotypic frequencies were compared with the expected genotypic frequencies under equilibrium conditions. The null hypothesis (Ho) was that genotypes for one locus are independent from the genotypes for another locus.

RESULTS

Table 3 shows the pairs of loci and p-values for the populations in which significant LDs were observed after the exact test and pooling. Of 108 comparisons after exact test, six significant LDs were observed. In the White Karaman population, significant LDs were observed for four pairs of loci while, significant deviations were found for only two pairs of loci in Merinolandschaf. In Awassi population, no significant deviation from equilibrium was observed. Significant deviations observed in White Karaman were between loci which were known to be unlinked. In Merinolandschaf, however two significant deviations were observed between loci which were known to be linked with MHC on OAR20 (16, 22). The highly significant deviations observed from the expectations could have been due to some rare alleles. To test this, the most frequent allele of a locus was considered as an allele and remaining all other alleles pooled to a second allele; and the analysis was run again. Also after pooling, significant LDs ($p < 0,05$) could be observed for seven pairs of loci in different populations. After pooling four significant deviations were observed between loci which were unlinked.

Table 3: Significant p-values for the loci pair examined before and after pooling

	unpooled		pooled	
Population	Loci pair	p	Loci pair	p
WK	SMHCC1-ILSTS059	**	SMHCC1-DYMS1	*
	BM1258-ILSTS059	*		
	BF-ILSTS011	***		
	BM1258-ILSTS011	**		
AW			MSDRB-ILSTS059	*
			SMHCC1-ILSTS011	*
ML	SMHCC1-BF	**	DYMS1-BM1818	*
	SMHCC1-BM1818	***	MSDRB-BM1258	*
			ILSTS005-BM1258	*
			ILSTS011-BM1258	*

WK: White Karaman; AW: Awassi; ML: Merinolandschaf
*: $p < 0,05$ **: $p < 0,01$ ***: $p < 0,001$

DISCUSSION

The purpose of this study was to test the presence of LD between some microsatellite loci in and out of MHC in White Karaman, Awassi and Merinolandschaf sheep populations. It was not intended to estimate the degree of LD for the population and loci. Nor was aimed to estimate the gametic frequencies or recombination rate, as no family information was available.

LD can arise via linkage, selection, population admixture with different allele frequencies, random drift in small populations, different allele frequencies in males and females (23). Investigations of different populations showed that LD decreased with the marker distance only for closely linked loci (24, 25). However, if there were not other factors, LD due to linkage would be lost after some generations depended on the recombination frequencies between loci (23). Since the samples used in this study were collected randomly from the populations, the animals were as unrelated as possible, and the breeds were old, significant LD due to linkage was not thereby expected. In this study, significant LDs have been found even between unlinked loci or loci mapped with great distance on the same chromosome and these findings agree with this suggestion. This was the case for both before and after pooling. Therefore, it seems that there is no tendency in the sheep populations studied to maintain LD even between closely linked loci in MHC, i.e. due to selection. These results contrast with those of Paterson (8), who found significant LD between MHC linked loci in a free living sheep population. Paterson (8) concluded that LD between the MHC linked loci were maintained due to selection. The populations studied

here were kept under the control of human. Thus the selection pressure of diseases on the populations here might have been less than that on the sheep population studied by Paterson (8). The investigations of Ohta (26) and Farnir et al. (24) showed that selection played a less important role than other factors, i.e. inbreeding or population subdividing.

The significant LDs found between unlinked loci in this study is in accordance with the results of Farnir et al. (24) who found significant LD (at $p < 0,05$) between unlinked loci with a frequency of 12% (out of 281 microsatellite markers). Nevertheless, Tenesa et al. (25) could not observe any significant LDs between unlinked loci (13 loci on two chromosomes, n=50), while they found significant LDs between loci closer than 10 cM. However it is difficult to compare the results of this study with those of Farnir et al. (24) and Tenesa et al. (25) because, the frequencies of gametic genotypes were not estimated here.

After pooling significant deviations were observed between loci and the p-values decreased as low as $< 0,05$. These results indicate that the significant deviations observed from HWE before pooling could have been due to some rare alleles. A total of 108 tests were performed for three populations [9x(9-1)/2)x3] and seven significant deviations (6,48%) were observed in all populations. This kind of significance can be observed just by chance, if the sample size is small. Significant deviations after pooling were observed only for one or two pairs of loci in the Turkish sheep populations. Significant LDs after pooling between the locus BM1258 and others in Merinolandschaf, seem to be specific for this locus, and could be because of some null alleles. Null alleles arise due to mutations in the nucleotide sequence of the primer binding regions, so that some alleles can not be amplified by using PCR (27).

These results suggest that a population admixture did not occur between the sheep populations studied to cause significant disequilibrium between loci tested. Furthermore, the results suggest that the lack of significant LDs did not indicate a localisation of loci on different chromosomes. As a conclusion the results indicated that the populations in this study were mostly in equilibrium and that selection did not play a major role to maintain the linkage disequilibrium between MHC-linked loci in the populations studied.

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Yazışma Adresi: Faruk BOZKAYA Harran Üniversitesi, Veteriner Fakültesi, Zootekni Anabilim Dalı, 63300 Şanlıurfa –TÜRKİYE
Tel: 0 414 312 84 56 / 2439 e-posta: farukbozkaya@harran.edu.tr