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Microsatellite Diversity and Restriction Enzyme-based Polymorphisms of MHC Loci in Some Native Turkish Goats

Mehmet ASLAN[a], Eymen DEMİR[a], Taki KARSLI[b*]

[a]*Department of Animal Science, Faculty of Agriculture, Akdeniz University, Antalya, 07058, TURKEY*
[b]*Department of Animal Science, Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir 26160, TURKEY*

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Corresponding Author: Taki KARSLI, E-mail: takikarsli@ogu.edu.tr
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ABSTRACT

Playing a key role in immunity and autoimmunity, Major Histocompatibility Complex (MHC) contains microsatellite regions and polymorphisms associated with resistance to several diseases and thermo-physiologic characteristics in farm animals. This study aims to reveal genetic diversity in four native Turkish goat populations via MHC related gene regions including MHC-linked microsatellite markers (BF1, BM1818, BM1258, SMHCC1 and DYMS1) and MHC Class II-DRB gene. A total of 120 unrelated animals belonging to Hair (HAI), Honamlı (HNM), Kabakulak (KBK) and Norduz (NRD) from different representative populations in Antalya and Van provinces were sampled and genotyped for molecular analysis. Based on MHC-linked microsatellite markers, number of alleles ranged from 8.20 (NRD) to 8.80 (HAI and KBK) across studied goat populations. Observed heterozygosity was between 0.68 (NRD) and 0.80 (KBK), whereas expected heterozygosity ranged from 0.74 (NRD) to 0.82 (KBK) in native Turkish goats. Inbreeding coefficients were 0.04, 0.13, -0.01 and 0.09 for HAI, HNM, KBK and NRD populations, respectively. A 284 bp length PCR products belonging to MHC Class II-DRB gene region were digested separately with *Pst*I, *Taq*I, *BsaH*I and *Alu*I restriction endonucleases to assess polymorphism status together with Hardy-Weinberg equilibrium in studied goat populations. P allele frequency ranged from 0.73 (KBK) to 0.95 (NRD), while p allele frequency was between 0.05 (NRD) and 0.27 (KBK) in *Pst*I polymorphism. The highest and lowest frequency of T allele were detected in HNM (0.80) and KBK (0.49), respectively, whereas frequency of t allele was between 0.20 (HNM) and 0.51 (KBK) in *Taq*I polymorphism. G and A allele frequency were between 0.16 (HNM) - 0.39 (HAI) and 0.84 (HNM) – 0.61 (HAI), respectively in *BsaH*I polymorphism, while three different genotypes and two alleles, which were different from results reported in the literature, were observed in *Alu*I polymorphism. In this study, high genetic diversity and low inbreeding were detected in native Turkish goats according to MHC-linked microsatellite markers. Similarly, native Turkish goats hold enough polymorphisms in MHC Class II-DRB gene which gives opportunity to support selection strategies against tuberculosis and heat stress in the future.

Keywords: Genetic diversity, Heat tolerance, PCR-RFLP, SSR, Tuberculosis resistance

1. Introduction

In Turkey, goat raising is practised by smallholder farmers across the country in order to obtain specific products such as milk, meat, cashmere and angora. Although, large part of goat population (more than 90%) is Hair (HAI) goat, it possesses different varieties [Kabakulak (KBK), Pavga and Çandır] adapted to different environmental conditions (Karsli et al. 2020). There are differences between HAI and its varieties in terms of body size, fertility and milk yield (Erduran & Kırbas 2010). Moreover, a recent study based on 20 microsatellite loci demonstrated that KBK is genetically different from HAI (Karsli et al. 2020). Lower jaw and convex nose are distinctive characteristics of HNM which is raised in Muğla and Antalya provinces. Norduz (NRD) possess the lowest body size and live weight among native Turkish goats and is raised in limited region of Van province and villages close to Iran border (Kirk et al. 2004).

Today, there are many factors such as climate change and infectious diseases which negatively affect sustainability of livestock sector (Radostits et al. 2007; Rovelli et al. 2020). Genetic diversity across genome and polymorphisms in related gene regions are required to face these challenges and to maintain livestock sector in the future. Indeed, today several studies have been aimed to investigate genes related to resistance to higher temperature (Basiricò et al. 2011; Liu et al. 2011) and diseases (Vaccari et al. 2006; Pisoni et al. 2010; Cecchi et al. 2017; Gowane et al. 2018; Eren et al. 2019) in livestock species. Of these genes, Major Histocompatibility Complex (MHC) consisting of MHC Class I and MHC Class II, has a key role in immunity and autoimmunity. MHC Class I molecules transporting proteins to T cells are present in almost all cells, while MHC Class II molecules known as Antigen Presenting Cells (APC) are present in certain cells such as macrophage and B cells. Previous studies

show that there are association between polymorphisms of MHC gene regions and resistance/susceptibility to several diseases (Singh et al. 2012; Shen et al. 2014; Kim et al. 2015; Kannaki et al. 2017) and thermo-physiologic traits in livestock species. Additionally, MHC-linked microsatellite markers are commonly used to reveal genetic diversity and phylogenetic relationships among different goat breeds (Salles et al. 2011; Guang-Xin et al. 2015).

Due to developing molecular techniques, animals with higher ability of heat tolerance and resistant to specific diseases can be detected easily. Moreover, by applying Marker Assisted Selection (MAS), the frequency of desired genotypes in terms of heat tolerance and resistance to disease can be increased in populations. In this regard, this paper aims i) to reveal current genetic diversity by MHC-linked microsatellite markers and ii) to obtain preliminary results of enzyme-based polymorphisms of MHC Class II-DRB gene which may be further analysed for association studies for heat tolerance and tuberculosis resistance in native Turkish goats.

2. Material and Methods

2.1 Blood collection and DNA isolation

120 unrelated blood samples were randomly chosen from HAI (n=40), HNM (n=30), KBK (n=30) and NRD (n=20) goat populations. Blood samples of NRD were obtained from Van, while the other samples were collected from representative herds raised in different districts of Antalya, Turkey. Genomic DNA was isolated from blood samples by using a salting-out method with minor modifications (Miller et al. 1988).

2.2 PCR-RFLP analysis and microsatellite genotyping

A 284 bp length of MHC Class II-DRB gene region and five MHC Class II-linked microsatellite markers were amplified with specific primer sets (Table 1).

Table 1- Characteristics of used loci in the study

	Locus	***Method***	***Primer Sequence (5'-3')***	***PCR Products (bp)***	***RE Enzyme***	***Reference***
Gene Polymorphism	MHC Class II-DRB	PCR-RFLP	F: TATCCCGTCTCTGCAGCACATTTC R: TCGCCGCTGCACACTGAAACTCTC	284	*Pst*I, *Taq*I	Singh et al. (2012)
					*Bsa*HI, *Alu*I	Yakubu et al. (2017)
Genetic Diversity	BF1	Microsatellite	F: CAACGGTCTGCAACCGAATTACC R: CAATCCGTGGGTTGGAACACAA	159-165	-	Guang-Xin et al. (2015); Salles et al. (2011)
	BM1818		F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	244-266		
	BM1258		F: GTATGTATTTTTCCCACCCTGC R: GTCAGACATGACTGAGCCTG	98-126		
	DYMS1		F: TCCTGGGGATTCCCAATACC R: CATAGAAGTCTTCACTGGTG	171-199		
	SMHCC1		F: ATCTGGTGGGCTACAGTCCATG R: GCAATGCTTTCTAAATTCTGA	175-199		

PCR was performed in total of 50 µL volume containing 3.2 µL HQ buffer (GeneAll), 2.5 mM dNTPs, 10 pM of each primer, 2.5 U Taq DNA Polymerase (GeneAll), 50 ng template DNA and 30.9 µL ddH_2O. PCR amplifications were applied in initial denaturation at 94 °C for 5 mins, followed by 30 cycles at 94 °C for 45 s, at 55-58 °C (depending on locus) for 45 s and at 72 °C for 50 s. The final extension was carried out at 72 °C for 5 mins. Amplificated 284 bp length PCR products were separately digested with restriction enzymes of *Pst*I, *Taq*I, *BsaH*I and *Alu*I to assess polymorphism status of native Turkish goats. Restriction enzyme mixture containing 10 µL of amplified PCR products, 5 U restriction enzymes (Table 1), 4.5 µL enzyme buffer and 5 µL nuclease free water, were incubated according to manufacturer instructions (Thermo Scientific Inc.). PCR and RFLP products were separated on 1% and 3% agarose gel electrophoresis, respectively. On the other hand, a 96-well automatic fragment analyzer (Agilent 5200 Fragment Analyzer System, USA) was used to genotype individuals for five microsatellite markers.

2.3 Statistical analysis

Allele and genotype frequencies of PCR-RFLP data were calculated by using Popgene (Yeh et al. 1997) package software. Chi-Square ($\chi 2$) test was applied to determine deviation from Hardy-Weinberg Equilibrium (HWE). Allele size range, private allele and allele frequencies of microsatellite loci were calculated via Convert (Glaubitz 2004) program. Number of alleles (Na), number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity were calculated by Popgene (Yeh et al. 1997) software. Inbreeding coefficient (F_{IS}), the diversity between breeds (D_{ST}) and the coefficient of gene differentiation (G_{ST}) were detected by Fstat v.1.2. (Goudet 1995), whereas null allele frequency was calculated by using Ml-NullFreq (Kalinowski & Taper

2006) software, respectively. UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) dendrogram was constructed by Popgene (Yeh et al. 1997), while FCA (Factorial Corresponding Analysis) and Structure analysis were done by Genetix v.4.05 (Belkhir et al. 2004) and Structure v.2.2. (Pritchard et al. 2000) programs, respectively. In order to determine the best K value, Structure Harvester (Earl & vonHoldt 2012) was utilized, while Clumpak (Kopelman et al. 2015) was used to visualise the result of structure analysis.

3. Results and Discussion

3.1 Genetic diversity among native Turkish goat populations based on MHC-linked microsatellite markers

A total of 65 alleles ranging from 11 (BM1258) to 15 (DYMS1) per marker were detected in all population (Table 2). Null allele frequency ranged from 0.00 (DYMS1) to 0.11 (BM1258) per marker with a mean of 0.05 indicating that all studied loci were highly amplificated. Mean D_{ST} and $G_{ST,}$ indicating the genetic diversity between breeds and the coefficient of gene differentiation, were 0.06 and 0.07, respectively. In this study, high number of alleles ranging from 8.20 (NRD) to 8.80 (HAI and KBK) were detected across studied goat populations by using a total of 5 different MHC-linked microsatellite markers (Table 3). Of 10 private alleles, 9 alleles were detected in NRD population, while only one private allele was observed in HNM populations (data not shown). Although, the frequency of private alleles was low (<0.3), the frequency of an allele (176 bp) at BF1 locus were at 0.60 for NRD population. Observed heterozygosity were between 0.68 (NRD) and 0.80 (KBK), whereas expected heterozygosity ranged from 0.74 (NRD) to 0.82 (KBK) across studied populations. Inbreeding coefficients were 0.04, 0.13, -0.01 and 0.09 for HAI, HNM, KBK and NRD populations, respectively (Table 3).

Table 2- Genetic diversity parameters across four native Turkish goat populations based on 5 MHC-linked loci

Loci	*AR*	*n*	*Na*	*Ne*	H_O	H_E	F_{IS}	D_{ST}	G_{ST}	*F (Null)*	*HWE*
BF1	154-180	110	13	4.52	0.82	0.78	-0.21	0.14	0.17	0.01	***
BM1818	246-270	100	12	7.58	0.68	0.87	0.18	0.02	0.03	0.09	***
BM1258	98-120	108	11	6.25	0.62	0.84	0.20	0.07	0.08	0.11	***
SMHCC1	91-199	112	14	6.20	0.69	0.84	0.15	0.01	0.01	0.06	*
DYMS1	175-205	109	15	7.53	0.89	0.87	-0.12	0.06	0.07	0.00	***
Mean	-	-	13	6.42	0.74	0.83	0.05	0.06	0.07	0.05	-

AR: Allele range, n: Number of genotyped individuals, Na: number of alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity, F_{IS}: inbreeding coefficient, D_{ST}: diversity between breeds, G_{ST}: coefficient of gene differentiation, F (Null): null allele frequency, HWE: Hardy-Weinberg equilibrium, *: P<0.05, **: P<0.01, ***: P<0.001.

Table 3- Genetic diversity parameters per studied goat populations based on 5 MHC-linked loci

Breeds	*MNA*	*MNE*	H_O	H_E	F_{IS}	*HWE*
HAI	8.80	4.83	0.74	0.80	0.04	-
HNM	8.40	5.10	0.76	0.79	0.13	1
KBK	8.80	5.36	0.80	0.82	-0.01	2
NRD	8.20	4.66	0.68	0.74	0.09	1

MNA: mean number of alleles, MNE: mean number of effective alleles, Ho: mean observed heterozygosity, He: mean expected heterozygosity, F_{IS}: mean inbreeding coefficient, HWE: number of loci deviated from Hardy-Weinberg equilibrium.

The same MHC-linked microsatellite markers were preferred by Guang-Xin et al. (2015) and Salles et al. (2011) to reveal genetic diversity in different goat breeds raised in China. Although, similar genetic diversity was reported for Dazu Black and Chuannan Black goat breeds, genetic diversity in native Turkish goat populations were higher than Chuanzhong, Enshi, Hechuan, Jining Qing, Tibetan and Yichang goat breeds raised in China (Guang-Xin et al. 2015). Additionally, mean observed heterozygosity values for native Turkish goat populations were higher than the values reported for Xuhuai, Pashmina, Changthangi, Vendi, Galla, Small East African goat breeds (Salles et al. 2011). On the other hand, higher genetic diversity was reported in previous studies conducted on native Turkish goats breeds in which Karsli et al. (2020) used 20 microsatellite markers, while Gül et al. (2020) preferred a total 22 microsatellite markers to reveal genetic structure of studied goat breeds. It is not surprising, since genetic diversity may increase by using more microsatellite markers with higher population size.

Compared to the goat breeds raised in China (Salles et al. 2011; Guang-Xin et al. 2015), lower inbreeding was detected in native Turkish goats via MHC-linked microsatellite markers. Similarly, low inbreeding was reported in previous microsatellite studies on native Turkish goats (Gül et al. 2020; Karsli et al. 2020; Tefiel et al. 2020). As highlighted by Argun Karsli et al. (2020), higher null allele frequency (>0.2) may affect estimates of observed heterozygosity and inbreeding coefficient parameters. Low null allele frequencies observed in the present study were the sign of that these parameters (observed heterozygosity and inbreeding coefficient) were calculated at high accuracy.

This study revealed that native Turkish goat populations are of high genetic diversity and low inbreeding which could be attributed to different phenomenon. As highlighted by Demir & Balcioglu (2019), Anatolia is a part of domestication centre

indicating that theoretically native Turkish goat breeds contain a large part of *C. hircus* gene pool. Additionally, sampling strategy may be another reason, since studied animals were provided from different representative herds in which animals were unrelated according to pedigree record.

Food and Agriculture Organization of the United Nations reported and recommended at least 30 microsatellite markers for molecular characterization of farm animals (FAO 2011). On the other hand, the main aim of the present study was to reveal genetic diversity based on MHC-linked microsatellite markers which are naturally less compared to other autosomal microsatellite loci. Despite of low number of used microsatellite markers, comparatively high genetic diversity (Na, Ne, Ho and He) were detected in native Turkish goat breeds. Similarly, Ceccobelli et al. (2020) reported that even decreased number of microsatellite markers from 16 to 12 was enough to distinguish a local Italian goat breed from exotic breeds.

3.2 Phylogenetic relationships among native Turkish goat populations based on MHC-linked microsatellite markers

In this study, UPGMA dendrogram (Figure 1), FCA (Figure 2) and structure (Figure 3) analysis were utilized to reveal phylogenetic relationships among native Turkish goat populations. Genetic distance and similarity values were calculated in order to construct UPGMA dendrogram. The highest genetic distance value (1.19) was observed between KBK and NRD populations, while the highest similarity value (0.87) was detected between HAI and KBK populations (Table 4).

Table 4- Nei's genetic distance (below the diagonal) and similarity (above the diagonal) values between studied populations

	HAI	***HNM***	***KBK***	***NRD***
HAI	****	0.84	0.87	0.34
HNM	0.16	****	0.86	0.33
KBK	0.13	0.13	****	0.30
NRD	1.06*	1.09*	1.19*	****

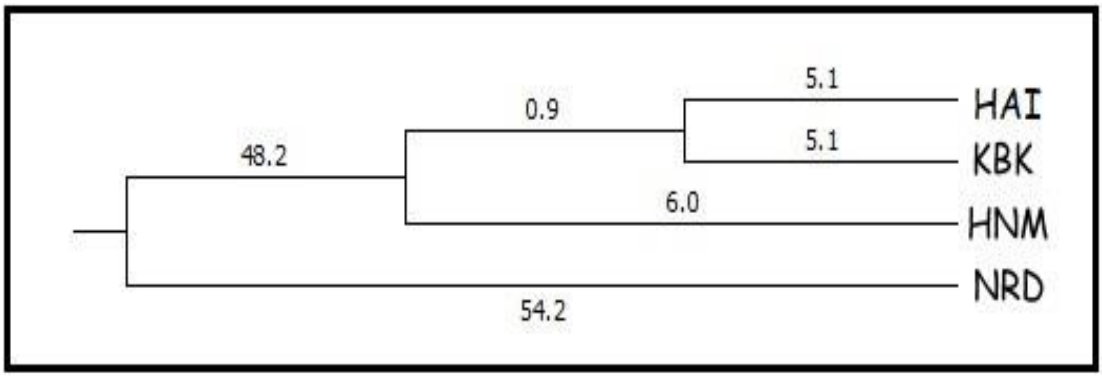

Figure 1- UPGMA denrogram among native Turkish goats based on genetic distance values (Nei 1978)

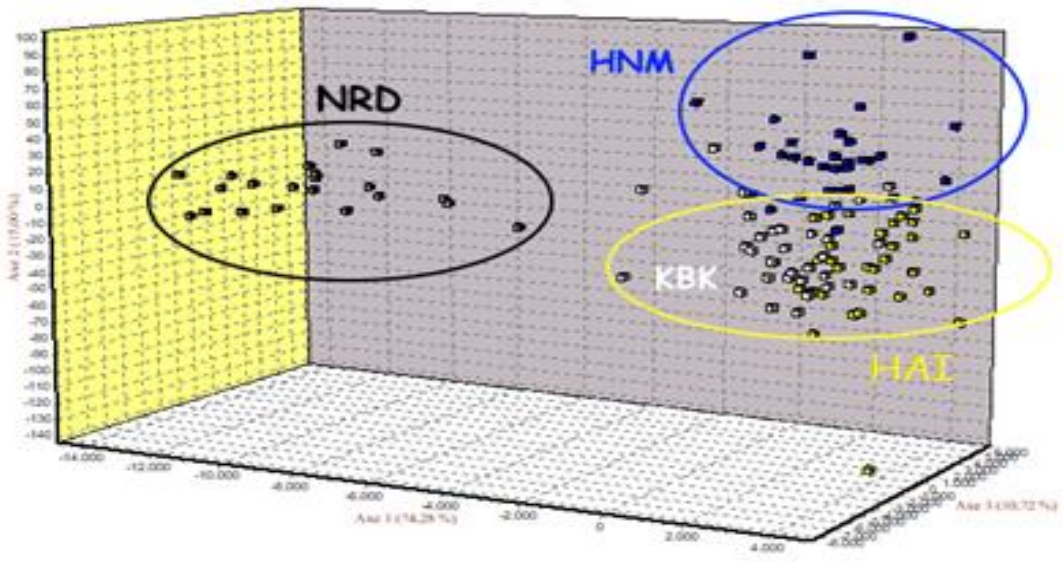

Figure 2- FCA analysis of native Turkish goats

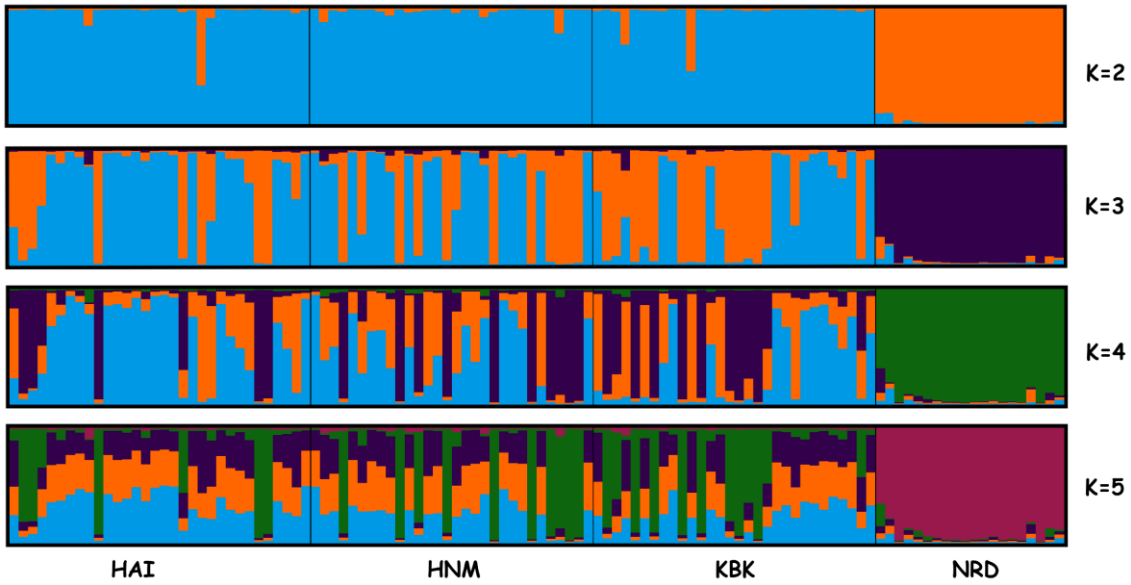

Figure 3- Population structure of native Turkish goats via STRUCTURE analysis

NRD population were clustered separately from the other populations in UPGMA dendrogram, while HAI and KBK were clustered together. HNM were found closer to HAI and KBK than NRD population. Results of UPGMA dendrogram was supported by FCA analysis in which NRD population were distinctively separate from the other populations in three-dimensional space, while KBK and HAI clustered together. Similarly, admixture was observed between HNM, HAI and KBK populations.

Although, four different Turkish goat populations were analysed in this study, the best K value was determined as 3 by Structure Harvester (Earl & vonHoldt 2012) indicating that studied individuals were assigned to three different clusters (Table 5). At K=2, NRD population were distinct clearly from the other populations, while HAI, KBK and HNM were assigned into the same clade. At K=3, similar results were obtained in which NRD was clustered separately from the other populations, while a high level of admixture was detected among HAI, KBK and HNM.

Table 5- Estimated posterior probabilities [LnPr (X|K)] and ΔK statistics for different number of clusters

Number of Cluster	*[LnPr (X|K)]*	*ΔK*
2	-2617.895	-
3	**-2530.851**	**47.860**
4	-2532.445	1.715
5	-2550.721	-

In this study, phylogenetic relationship analysis revealed that NRD population was genetically different from HAI, KBK and HNM populations in harmony with its breeding history. Indeed, NRD population is raised separately from the other goat breeds in a limited region of Turkey (Van province) and this isolation led to different genetic structure together with specific phenotypes such as lower body size. Similarly, NRD were reported to be genetically different from the other native Turkish goat breeds by Karsli et al. (2020) via 20 different microsatellite markers.

On the contrary, although they are of different morphological traits, a high level of admixture was detected among HAI, KBK and HNM populations based on phylogenetic analysis. Similarly, previous studies could not distinguish HNM and HAI breeds by microsatellite markers (Ağaoğlu & Ertuğrul 2012; Bulut et al. 2016). Conversely, a recently published study showed for the first time that KBK has become genetically different from HAI breed (Karsli et al. 2020).

3.3 Restriction enzyme-based polymorphisms of MHC loci in native Turkish goats

In order to assess the polymorphisms status of native Turkish goats, MHC Class II-DRB gene region was digested separately with *Pst*I (Figure 4a), *Taq*I (Figure 4b), *BsaH*I (Figure 4c) and *Alu*I (Figure 4d) restriction enzymes.

Two alleles (P and p) leading to three genotypes (PP, Pp and pp) were observed in native Turkish goat populations in terms of MHC Class II-DRB/*Pst*I polymorphism (Table 6). P allele frequency ranged from 0.73 (KBK) to 0.95 (NRD), while p allele frequency was between 0.05 (NRD) and 0.27 (KBK). PP was the most common genotype with ranging from 0.57 (KBK) to 0.90 (NRD), while pp was the least genotype varying between 0.00 (NRD) and 0.10 (HNM and KBK) in studied populations. The

highest and lowest frequency of Pp genotype were detected in KBK (0.33) and NRD (0.10) population, respectively. A significant deviation from HWE was detected in only HNM population. (Table 6).

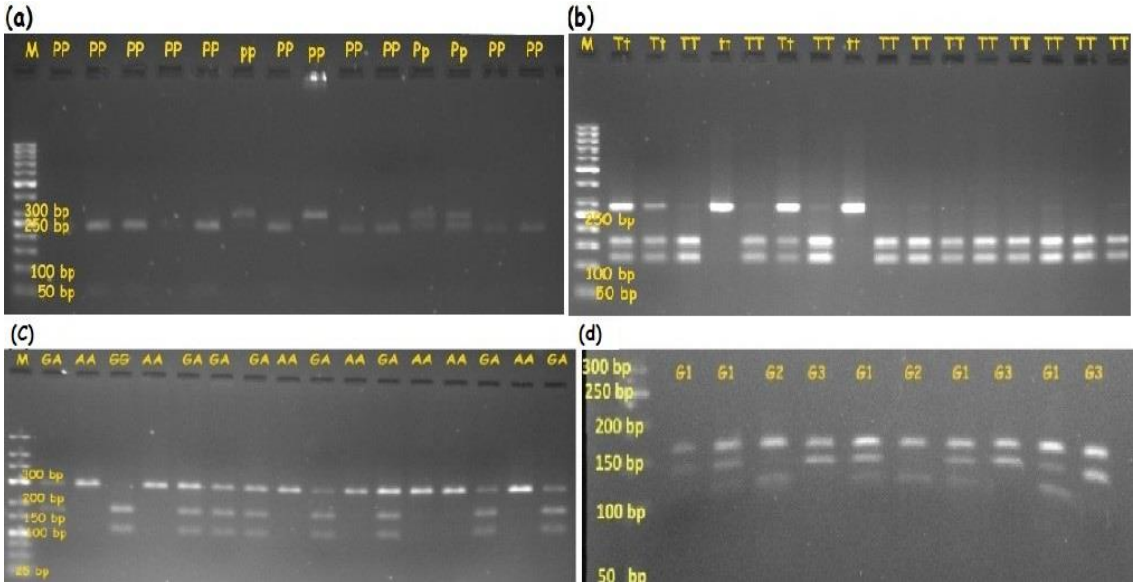

Figure 4- Digestion image of MHC Class II-DRB gene region using *Pst*I (a), *Taq*I (b), *Bsa*HI (c) and *Alu*I (d) restriction enzymes. M: Thermo 50 bp DNA ladder, Cat. No: SM0371

Table 6- Allele and genotype frequencies of *Pst*I, *Taq*I, *BsaH*I and *Alu*I, polymorphisms on MHC Class II-DRB gene region in studied populations

MHC Class II-DRB/PstI								***MHC Class II-DRB/TaqI***							
Breed	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2	**Breed**	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2
		P	**p**	**PP**	**Pp**	**pp**				**T**	**t**	**TT**	**Tt**	**tt**	
HAI	34	0.81	0.19	0.68	0.26	0.06	0.89[a]	**HAI**	39	0.65	0.35	0.36	0.59	0.05	3.58[a]
HNM	31	0.82	0.18	0.74	0.16	0.10	6.20*	**HNM**	30	0.80	0.20	0.67	0.27	0.06	0.83[a]
KBK	30	0.73	0.27	0.57	0.33	0.10	0.65[a]	**KBK**	30	0.49	0.51	0.20	0.57	0.23	0.54[a]
NRD	20	0.95	0.05	0.90	0.10	0.00	0.06[a]	**NRD**	20	0.60	0.40	0.45	0.30	0.25	2.82[a]
MHC Class II-DRB/BsaHI								***MHC Class II-DRB/AluI***							
Breed	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2	**Breed**	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2
		G	**A**	**GG**	**GA**	**AA**				**A_1**	**A_2**	**G1**	**G2**	**G3**	
HAI	40	0.39	0.61	0.00	0.78	0.22	16.01**	**HAI**	40	0.84	0.16	0.75	0.17	0.08	5.10*
HNM	31	0.16	0.84	0.00	0.32	0.68	1.14[a]	**HNM**	30	0.67	0.33	0.50	0.33	0.17	1.88[a]
KBK	30	0.28	0.72	0.03	0.50	0.47	1.60[a]	**KBK**	30	0.73	0.27	0.53	0.40	0.07	0.01[a]
NRD	20	0.30	0.70	0.05	0.50	0.45	0.73[a]	**NRD**	20	0.37	0.63	0.20	0.35	0.45	1.28[a]

$\chi^2_{0.01;1}$:3.84; $\chi^2_{0.05;1}$:6.63; * P<0.05; **P<0.01; a: Non-significant from HWE.

Similarly, presence of three different genotypes were reported in goat breeds raised in India (Jamunapari) (Singh et al. 2012) and Indonesia (Saanen, Etawah Grade and their crossbred) (Petlane et al. 2012) in MHC Class II-DRB/*Pst*I polymorphism. Moreover, Singh et al. (2012) highlighted that animals with pp genotype showed resistance to tuberculosis, while PP and Pp genotypes were commonly found in animals susceptible to tuberculosis. Frequency of pp genotype was reported as 65.04% and 90% in susceptible and resistant group for tuberculosis, respectively (Singh et al. 2012). On the other hand, pp genotype frequency was reported as 0.28, 039, and 0.35 in Saanen, Etawah Grade and their crossbred (Petlane et al. 2012). Frequency of p allele in native Turkish goat populations was lower than that of reported in goat breeds raised in India and Indonesia (Petlane et al. 2012; Singh et al. 2012). It is possible that they have developed resistance to tuberculosis during long history of breeding, since tuberculosis occurs more frequently in ruminants raised in South Asia and Africa (Humblet et al. 2009).

T and t allele together with TT, Tt and tt genotypes were detected by *Taq*I polymorphism in studied populations (Table 6). The highest and lowest frequency of T allele were detected in HNM (0.80) and KBK (0.49), respectively, whereas frequency of t allele was between 0.20 (HNM) and 0.51 (KBK) in studied populations. TT genotype frequency was between 0.20 (HNM) and 0.51 (KBK), while tt genotype frequency ranged from 0.05 (HAI) to 0.25 (NRD). The highest and lowest Tt genotype frequencies were detected in HAI (0.59) and HNM (0.27), respectively. All goat population were in HWE in terms of *Taq*I polymorphism.

Singh et al. (2012) reported that animals with Tt genotype had advantages in terms of resistance to tuberculosis than that of TT and tt genotypes in Jamunapari goat breeds in MHC Class II-DRB/*Taq*I polymorphism. Additionally, frequency of Tt genotype was reported as 0.70 in resistant group in Jamunapari (Singh et al. 2012) and 0.39, 0.15 and 0.17 in Saanen, Etawah Grade and their crossbred (Petlane et al. 2012). The lower frequency of desired genotype (Tt) in terms of tuberculosis was detected in native Turkish goat populations compared to Jumanapari (Singh et al. 2012), while higher Tt genotype frequencies were observed for HAI and KBK than Saanen goat breed (Petlane et al. 2012).

G allele frequency ranged from 0.16 (HNM) to 0.39 (HAI), while the highest and lowest A allele frequency were observed in HNM (0.84) and HAI (0.61), respectively in *BsaH*I polymorphism (Table 6). No GG genotype were detected in HAI and HNM populations, while it was present in KBK (0.03) and NRD (0.05) goats at very low levels. On the contrary, GA and AA genotypes were observed in all studied populations at variable levels ranging from 0.32 (HNM) to 0.78 (HAI) for GA genotype and ranging from 0.22 (HAI) to 0.68 (HNM) for AA genotype. Significant deviation from HWE was observed in only HAI population.

According to MHC Class II-DRB/*BsaH*I polymorphism, similar alleles and genotypes were reported by a previous study conducted on West African Dwarf, Red Sokoto and Shael goat breeds (Yakubu et al. 2017) in which animals with AA genotype had advantages in terms of heat tolerance. Yakubu et al. (2017) reported that AA allele frequencies were 0.45, 0.22 and 0.10 for West African Dwarf, Red Sokoto and Shael goat breeds, respectively. Desired genotype (AA) detected in HNM were significantly higher than the values reported by Yakubu et al. (2017) and the other native Turkish goat populations.

According to *Alu*I polymorphisms, Yakubu et al. (2017) reported three different genotypes such as GG (158, 84 and 42 bp), GC (200, 158, 84 and 42 bp) and CC (200 and 84 bp) in West African Dwarf, Red Sokoto and Shael goat breeds. Benefiting from the same primers, similar PCR fragment (284 bp) was detected in native Turkish goats. On the other hand, by using the same restriction enzyme and RFLP process, restriction fragments in native Turkish goats were different from genotype patterns reported by Yakubu et al. (2017). This variation must be further analysed by sequencing in order to obtain more information on MHC Class II-DRB/*Alu*I polymorphism.

Briefly, previous studies reported that restriction enzyme-based polymorphisms of MHC Class II-DRB gene were associated with tuberculosis resistance/susceptibility and heat tolerance in different goat breeds. Our preliminary results showed that MHC Class II-DRB gene were polymorphic in native Turkish goats. Association analysis between these polymorphisms and tuberculosis and heat tolerance should be investigated to keep sustainability of goat breeding at optimum level in Turkey.

4. Conclusions

In this study genetic diversity and phylogenetic relationships of native Turkish goat breeds were investigated via 5 different MHC-linked microsatellite markers, whereas polymorphism status of MHC Class II-DRB gene were assessed by *Pst*I, *Taq*I, *BsaH*I and *Alu*I restriction enzymes. High level of genetic diversity and low inbreeding were detected based on five MHC-linked microsatellite markers across the studied populations indicating that native Turkish goat breeds have enough genetic diversity for sustainability for the future. On the other hand, the temperature is raising globally, and diseases develop under suitable conditions in livestock species which are believed to negatively affect sustainability of livestock sector in the future. According to previous association studies on different goat breeds, native Turkish goat populations have desired genotypes for heat tolerance and resistance to tuberculosis at variable frequencies. Surprisingly, being a sub-type (variety) of HAI and raised in limited region of Southern Turkey (Elmali, Kas and Fethiye districts), KBK has the desired genotypes for tuberculosis resistance (pp and Tt) with higher frequencies than the other Turkish goat breeds. This result highlighted the importance of conserving genetic diversity not only at breeds but also sub-type level. Still, these genotypes should be investigated in terms of heat tolerance and resistance to tuberculosis and frequency of desired alleles and genotypes should be increased by applying related genes in MAS studies. Additionally, the current status of native Turkish goat breeds for the other environmental challenges and diseases should be monitored to obtain the best selection application. Additionally, a low number of MHC-linked microsatellite loci were found enough to distinguish the studied populations according to their breeding history and origins.

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Ethical Statement

This research was approved by the Akdeniz University Animal Experiments Ethics Committee, Antalya, Turkey (Protocol No: B.30.2.AKD.0.05.07.00/28).

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