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Genotypic structure of four cattle breeds raised in Turkey by loci related to several diseases*

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ABSTRACT

This study aims to reveal the genotypic structure of four cattle breeds; Holstein (HS), Turkish Grey Steppe (TGS), Anatolian Black (AB) and East Anatolian Red (EAR), raised in Turkey in terms of CD14, MBL, ITGB6, SLC11A1 and TLR2 genes and to evaluate their usefulness in Marker Assisted Selection (MAS). It also assesses whether the loci associated with resistance to diseases are suitable for phylogenetic analysis. Desired alleles and/or genotypes were detected in native Turkish cattle breeds at different frequencies in terms of polymorphisms of CD14, MBL, ITGB6, SLC11A1 and TLR2 genes which were previously reported to be associated with mastitis, foot-and-mouth disease and tuberculosis. These variations offer opportunities to improve selection strategies against diseases in the future. These results preliminary indicate that associated studies between these variations and disease resistance in native Turkish cattle breeds should be conducted. On the other hand, phylogenetic tree constructed based on genetic distance clearly separated native Turkish cattle breeds from HS breed. The gene regions related to diseases can be used to distinguish native cattle breeds from exotic ones.

1. Introduction

Cattle breeding is mainly centred on exotic breeds in Turkey in which Holstein Friesian and its crossbreeds are the most commonly raised breed with approximately 12 million individuals. The comparative population size of native Turkish cattle breeds is low due to their lower economical yields. According to official data, the current population of Anatolian Black is represented by 650000 heads, while East Anatolian Red and Turkish Grey Steppe are estimated at 135000 and 25000 heads, respectively (HBS 2019, TUIK 2019). On the other hand, since native Turkish cattle breeds are highly resistant to harsh environmental conditions and some diseases (Yilmaz et al. 2012; Demir and Balcioglu 2019), genetic diversity in these breeds must be identified and conserved to meet the current production level under various environmental conditions as well as for adaptation to the potential changes in breeding purposes (Mahmoudi et al 2010; Ramadan et al 2012).

It is well known that economic production in livestock breeding depends on raising healthy animals with high breeding value. Health problems may cause economic losses in livestock breeding. Although, preventative vaccines and drugs are available for numerous diseases, there are increasing consumer concerns about drug residue in animal-derived products. Also, the economic burden may not be affordable for smallholder farmers. Therefore, alternative methods, such as obtaining animals genetically resistant to diseases, are being sought by researchers and some breeders (Morris 2007). Thanks to the advances in DNA technologies in the last decades, several gene regions including *CD14*, *MBL*, *ITGB6*, *SLC11A1* and *TLR2* have been reported to be associated with various diseases such as mastitis, foot-and-mouth disease and tuberculosis in cattle (Kumar et al. 2014; Sadana et al. 2015; Singh et al. 2015). According to polymorphisms of gene regions, individuals resistant to diseases can be easily detected by using fast developing molecular tools. The selection of animals with resistant genes as breeding material and its use in MAS programs can make significant contributions to getting these diseases under control. In this regard, this study aims to investigate polymorphisms of CD14, MBL, ITGB6, SLC11A1 and TLR2 genes which were reported to be associated with resistance/susceptibility to mastitis, foot-and-mouth disease and tuberculosis in four cattle breeds raised in Turkey and to evaluate their possible usefulness in MAS in the future. Additionally, these polymorphisms were tested to see whether they are suitable for phylogenetic analysis according to the genetic origins of the cattle breeds studied.

2. Materials and Methods

2.1. Ethic Statement

This research was approved by the Akdeniz University Animal Experiments Ethics Committee, Antalya, Turkey (Protocol No: B.30.2.AKD.0.05.07.00/1).

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2.2. Sample collection and DNA extraction

A total of 210 animals from HS (n= 64), AB (n= 54), TGS (n= 48) and EAR (n= 44) cattle breeds were chosen from different representative farms. HS samples were obtained from the dairy farm of the Faculty of Agriculture, Akdeniz University; Antalya, Turkey; AB samples from 4 different farms located in Eskisehir and Antalya province, Turkey; TGS samples from 3 different farms in Balıkesir province, Turkey; and EAR samples from breeders in Erzurum province. Genomic DNA was extracted from blood samples by using the salting-out method described by Miller et al (1988).

2.3. Determination of polymorphisms of candidate genes

In this study, the polymorphisms of CD14 and MBL1 (Exon 2; 2534G>A and 2651G>A), SLC11A1 (5411G>A and 7400G>A) and TLR2 genes were detected by PCR-RFLP; the polymorphisms of ITGB6 receptor (5'UTR region 29G>A; 2145T>C) gene by ARMS-PCR; and the variation in the repetitive region of the SLC11A1 gene by the microsatellite marker technique. Some descriptive information about candidate genes is given in Table 1.

2.4. PCR-RFLP analysis

To determine SNPs associated with diseases, gene regions were amplified by PCR using specific primers (Table 1). The PCR reaction mixture consisted of in total 30 µL including 10X PCR buffer (3 µl, pH 9.0); 5 pmol each primer; 1 U Taq DNA polymerase; 10 mM $MgCl_2$; 7.5 µl dNTPs and deionized water. PCR amplification was carried out as follows; the first denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing (Table 1) for 45 seconds, extension at 72°C for 50 seconds and the last extension at 72°C for 5 minutes.

Then, PCR products were digested by restriction enzymes to detect genotypes (Table 2). PCR products were digested by using 9 µL PCR products, 9 µL H2O, 1.1 mL enzyme buffer and 0.3-0.5 µL (10 U μl^{-1}) restriction enzyme.

Agarose gel electrophoresis was applied to visualise the possible genotypes for PCR-RFLP process (Table 3).

2.5. Microsatellite analysis

In order to determine the size of the repetitive region of the SLC11A1 gene associated with resistance to tuberculosis, PCR was performed with primers in Table 1. The PCR program and PCR components were the same as RFLP analysis. The lengths of PCR products were determined by using 96 automated capillary electrophoresis systems (Advanced Analytical Technologies-AATI, Ames, Iowa, USA). After capillary electrophoresis process, PROSize® 2.0 version 1.3.1.1 (Advanced Analytical Technologies, Inc., Ames, IA, USA) was used to visualise the bands.

Table 1. Some descriptive information on the gene regions studied

Diseases	Gene	Method	Primers	Annealing Temp. (°C)	References
Mastitis	CD14	PCR-RFLP	F: CTTCCTGTTATAGCCCCTTTCC R: CACGATACGTTACGGAGACTGA	60	Kumar et al. (2014)
	MBL1Exon2 (2651G>A)	PCR-RFLP	F: GGTGGCAAATGTTGGCTA R: GTCTTCTGAGCATCCTCCA	54	Wang et al. (2011)
	MBL1Exon2 (2534G>A)	PCR-RFLP	F: GTATCCTTCTCAAATACAAAAGAC R: CCCCTGTCTCTATGCTAGAC	54	Yuan et al. (2013)
Foot-and-Mouth Disease	ITGB6 5'UTR (29G>A)	ARMS-PCR	Outer F: CTTTCCCTAGCCTGCCTTCT Outer R: GTTCAATCCCCATCCGTTT Inner F: ATCATGTTGGAGTTGCTCATG Inner R: GGTAAAGAAGAAAAGCTGTGATT	-	Singh et al. (2014)
	ITGB6 (2145T>C)	ARMS-PCR	Outer F: TGCATAATAAAACTCAATAC Outer R: ATTCATCAGCCACCTTTTTG Inner F: CAGATTTCTCAAAGGATAGCTT Inner R: CTTGCAGAGAACAGGAAACAG	-	Singh et al. (2015)
Tuberculosis	SLC11A1	Microsatellite	F: GTGGAATGAGTGGGCACAGT R: TCTCCGTCTGCTGTGCAT	55	Kadarmideen et al. (2011)
	SLC11A1 (7400C>G)	PCR-RFLP	F: TGTGCTTCACATCTCCTTCCTA R: AGCACATTGAGCAGGTCGTT	60	Liu et al. (2017)
	SLC11A1 (5411G>A)	PCR-RFLP	F: TGAGGATCAGTGAGGGAAAAGA R: AAACTGCTTGCATATTCCCAAC	58	Liu et al. (2017)
	TLR2	PCR-RFLP	F: TTAACCTCCATCCCCTCTGG R: TAAAGGGACCTGAACCAGG	55	Sadana et al. (2015)

Table 2. Digestion conditions and enzymes used in RFLP process

Gene	Restrictions Enzyme	Enzyme Catalog Number	Digestion time (hour)	Digestion temperature
CD14	*Hinf*I	Thermo ER0801	3	37°C
MBL1, Exon 2 (2651G>A)	*Sty*I	Thermo ER0411	3	37°C
MBL1, Exon 2 (2534G>A)	*Mae*II (Tail)	Thermo ER1142	3	65°C
SLC11A1 (7400C>G)	*Pst*I	Thermo ER0611	3	37°C
SLC11A1 (5411G>A)	*Mae*II (Tail)	Thermo ER1142	3	65°C
TLR2	*Eco*RV (Eco32I)	Thermo ER0301	3	37°C

Table 3. Possible band sizes after RFLP process

Gene	PCR products (bp)	Digestion products (bp)
CD14	832	CC: 377, 272,183 CD: 377, 272, 225, 183, 47 DD: 377, 225, 183,47
MBL1, Exon 2 (2651G>A)	162	GG: 162 GA:162, 141, 21 AA: 141,21
MBL1, Exon 2 (2534G>A)	217	GG: 194,23 GA: 217, 194, 23 AA: 217
SLC11A1 (7400C>G)	936	GG: 633, 303 CG: 709, 633, 303, 227 CC:709;227
SLC11A1 (5411G>A)	998	GG: 631, 226, 141 AG: 631, 367, 226, 141 AA: 631,367
TLR2	245	CC: 245 CA: 245, 182, 63 AA: 182,63

2.6. Statistical analysis

The POPGENE 1.31 (Yeh et al. 1997) program was used to generate the phylogenetic tree by determining the gene and genotype frequencies. In addition, Hardy-Weinberg equilibrium was checked by using the chi-square (χ^2) statistic in each population (Hartl and Clark 1989).

3. Results and Discussion

Two alleles (C and D) leading to three different genotypes (CC, CD and DD) were detected in CD14/*Hinf*I polymorphism among the cattle breeds studied (Figure 1). No animals with C allele were observed in HS breed and CC genotype in all populations (Table 4). On the other hand, C allele was present in native Turkish cattle breeds with low frequencies ranging from 0.037 (AB) to 0.133 (TGS). According to polymorphisms of CD14, animals with CC genotype have been reported to be more resistant to mastitis (Kumar et al 2014; Selvan et al 2016). In this study, both desired genotype (CC) and allele (C) for CD14 gene were not detected in HS cattle breed. On the contrary, CD genotype and desired C allele were observed in native Turkish cattle breeds. The frequencies of C allele detected in AB (0.037), TGS (0.133) and EAR (0.091) breeds were lower than the values reported in Sahiwal (0.65) (Kumar et al 2014) and Karan Fries (0.29) cattle breed (Selvan et al 2016). Although it was lower than the values reported in the literature, it is important to detect desired allele in native Turkish cattle breeds in order to provide new opportunities for selection studies to be done for resistance to mastitis. The reason for monomorphism (DD genotype) of HS breed for the CD14 gene can be considered as collecting the samples from a single farm or the small numbers of samples (64). Samples for the HS breed were obtained from one single farm (the dairy farm of the Faculty of Agriculture, Akdeniz University; Antalya, Turkey) however, the animals in the farm were collected a very short time ago (four years ago) from about ten different farms in three different cities in Turkey. The number of samples were sufficient compared to similar studies. Therefore, the results obtained in the study for the CD14 gene were thought to be reflective of the actual case in the HS breed.

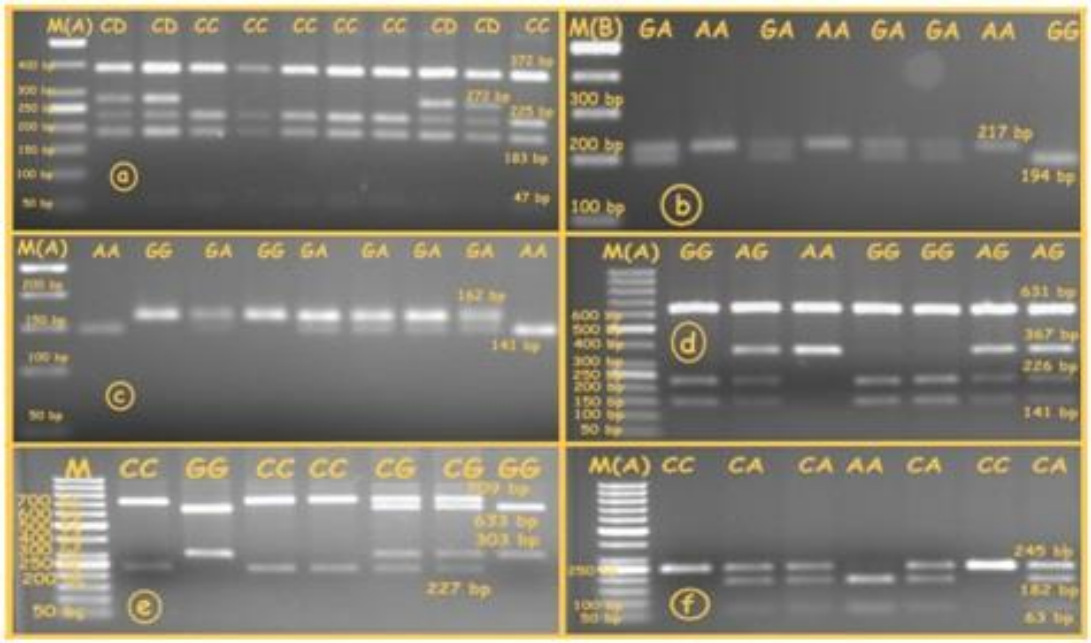

Figure 1. Agarose gel images for PCR-RFLP process. (M(A): Marker A (Thermo 50 bp; Cat. No: SM0371); M(B): Marker B (Thermo 100 bp; Cat. No: SM0241); a) Digestion of CD14 PCR products by *Hinf*I (3% agarose gel); b) Digestion of MBL1 (Ekzon 2, 2534G>A) PCR products by *Mae*II (2% agarose gel); c) Digestion of MBL1 (Ekzon 2, 2651G>A) PCR products by *Sty*I (3.5% agarose gel); d) Digestion of SLC11A1 (5411G>A) PCR products by *Mae*II (3% agarose gel); e) Digestion of SLC11A1 (7400C>G) PCR products by *Pst*I (3% agarose gel); f) Digestion of TLR2 PCR products by *Eco*RV(3% agarose gel)).

Table 4. Allele and genotype frequencies for the genes studied

Gen	Breed	n	Allele Frequencies		Genotype Frequencies			χ^2
			C	D	CC	CD	DD	
CD14	HS	61	0.000	1.000	0.000	0.000	1.000	-
	AB	53	0.037	0.962	0.000	0.075(4)	0.925 (49)	0.081[a]
	TGS	45	0.133	0.867	0.000	0.267(12)	0.733 (33)	1.065[a]
	EAR	44	0.091	0.909	0.000	0.181(8)	0.819 (36)	0.440[a]
MBL1 (Ekzon 2, 2534G>A)	**Breed**	**n**	A	G	AA	AG	GG	χ^2
	HS	61	0.590	0.410	0.327(20)	0.525(32)	0.148(9)	0.434[a]
	AB	54	0.333	0.667	0.093(5)	0.481(26)	0.426(23)	0.375[a]
	TGS	37	0.432	0.568	0.216(8)	0.433(16)	0.351(13)	0.524[a]
	EAR	44	0.262	0.738	0.068(3)	0.387(17)	0.545(24)	0.023[a]
MBL1 (Ekzon 2, 2651G>A)	**Breed**	**n**	A	G	AA	AG	GG	χ^2
	HS	59	0.745	0.255	0.610(36)	0.271(16)	0.119(7)	4.787[b]
	AB	54	0.518	0.482	0.444(24)	0.148(8)	0.408(22)	26.709[b]
	TGS	48	0.552	0.448	0.458(22)	0.188(9)	0.354(17)	18.504[b]
	EAR	44	0.500	0.500	0.477(21)	0.046(2)	0.477(21)	36.363[b]
SLC11A1 (5411G>A)	**Breed**	**n**	G	A	GG	GA	AA	χ^2
	HS	59	0.788	0.212	0.644(38)	0.288(17)	0.068(4)	1.110[a]
	AB	54	0.731	0.269	0.537(29)	0.389(21)	0.074(4)	0.005[a]
	TGS	45	0.655	0.345	0.423(19)	0.467(21)	0.111(5)	0.051[a]
	EAR	44	0.841	0.159	0.682(30)	0.318(14)	0.000	1.575[a]
SLC11A1 (7400G>A)	**Breed**	**n**	G	C	GG	GC	CC	χ^2
	HS	59	0.203	0.797	0.050(3)	0.305(18)	0.645(38)	0.201[a]
	AB	53	0.245	0.755	0.000	0.491(26)	0.509(27)	5.592[b]
	TGS	44	0.284	0.716	0.000	0.569(25)	0.431(19)	6.928[b]
	EAR	44	0.159	0.841	0.000	0.318(14)	0.682(30)	1.575[a]
TLR2	**Breed**	**n**	C	A	CC	CA	AA	χ^2
	HS	54	0.287	0.713	0.093(5)	0.389(21)	0.518(28)	0.134[a]
	AB	54	0.546	0.454	0.315(17)	0.462(25)	0.223(12)	0.235[a]
	TGS	47	0.383	0.617	0.149(7)	0.468(22)	0.383(18)	0.004[a]
	EAR	44	0.557	0.443	0.227(10)	0.659(29)	0.114(5)	4.950[b]
ITGB6 reseptör (5'UTR, 29G>A)	**Breed**	**n**	G	A	GG	GA	AA	χ^2
	HS	61	0.393	0.607	0.213(13)	0.361(22)	0.426(26)	3.640[a]
	AB	54	0.296	0.704	0.093(5)	0.407(22)	0.500(27)	0.029[a]
	TGS	48	0.281	0.719	0.063(3)	0.437(21)	0.500(24)	0.323[a]
	EAR	44	0.477	0.523	0.205(9)	0.545(24)	0.250(11)	0.381[a]
ITGB6 reseptör (5'UTR, 2145T>C)	**Breed**	**n**	T	C	TT	TC	C	χ^2
	HS	62	0.500	0.500	0.000	1.000	0.000	-
	AB	54	0.500	0.500	0.000	1.000	0.000	-
	TGS	48	0.500	0.500	0.000	1.000	0.000	-
	EAR	44	0.500	0.500	0.000	1.000	0.000	-

$\chi^2_{0.05;1}$: 3.84; a: Nonsignificant deviation from H-W equilibrium, b: Significant deviation from H-W equilibrium (P<0.05).

As expected, two alleles (A and G) leading to three different genotypes (AA, AG and GG) were observed in the cattle breeds studied in MBL1/*Mae*II polymorphism (Figure 1). The A allele frequency was ranged from 0.262 (EAR) to 0.590 (HS), whereas the frequency of G allele were between 0.410 (HS) and 738 (EAR) (Table 4). The frequency of GG genotype, which is reported to be resistant to tuberculosis (Yuan et al. 2013) were ranged from 0.148 (HS) to 0.545 (EAR) (Table 4). Yuan et al (2013) reported GG genotype frequency as 0.542 in Holstein, 0.378 in Sanhe and 0.479 in Simmental cattle breeds raised in Chine in which G allele frequency ranged from 0.544 (Sanhe) to 0.633 (Holstein). Although G allele and GG genotype frequencies detected in EAR cattle breed were higher than the values by Yuan et al (2013), similar allele and genotype frequencies were observed in TGS and AB breeds. On the other hand, a higher GG genotype frequency was detected in native Turkish cattle breed than in the HS breed. It was revealed that MBL1 could be used in MAS studies for resistance to mastitis in all breeds.

All the cattle breeds studied were polymorphic and held three different genotypes (AA, AG and GG) in terms of MBL/*Sty*I polymorphism (Figure 1). The A allele frequency varied from 0.500 (EAR) to 0.745 (HS), while frequency of G allele ranged from 0.255 (HS) to 0.500 (EAR) (Table 4). A study conducted by Wang et al (2011) showed that the number of somatic cells in the milk of the animals with AG and GG genotypes was significantly lower (P<0.05) than that of AA genotype according to polymorphisms of MBL1 (Exon 2; 2651G>A) gene. They have stated that the animals with AG and GG genotype were more resistant to mastitis. In the present study, the AA genotype frequency ranged from 0.444 (AB) to 0.610 (HS) and a higher frequency of desired genotypes for

resistant to mastitis (AG and GG) was detected in native Turkish cattle breeds than in the HS breed. There is sufficient genetic variation in local cattle breeds for MBL1 (Exon 2; 2651G>A) gene and it can be used in MAS studies for resistance to mastitis.

Two alleles (G and A) together with three genotypes (GG, GA and AA) were detected in ITGB6 receptor (5'UTR, 29G>A) gene polymorphism by ARMS-PCR across the cattle breeds studied (Figure 2). All breeds were polymorphic in terms of ITGB6 receptor (5'UTR, 29G>A) gene polymorphism, in which G allele frequency ranged from 0.281 (TGS) to 0.477 (EAR), whereas the A allele frequency was between 0.523 (EAR) and 0.719 (TGS) (Table 4). AA genotype, which is more resistant to FMD (Singh et al 2014), and the desired allele A were detected in all breeds. In this study, the frequencies of A allele in HS, AB, TGS and EAR breeds were 0.607, 0.704, 0.719 and 0.523 respectively and were similar to the value reported in HS breed (0.626) raised in India (Singh et al 2014), while they were lower than the values reported in Sahiwal (0.833), Kankrej (1.000) and Ongole (1.000) cattle breed. It is not surprising, since the genetic roots of Sahiwal, Kankrej and Ongole breeds is *Bos indicus*, while the cattle breeds studied originated from *Bos taurus*. It is known that *Bos indicus* is more resistant to FMD than *Bos taurus* (Singh et al 2014; 2015).

All breeds studied were monomorphic (TC) in terms of another mutation (2145T>C) in the ITGB6 receptor gene related to the resistance to FMD (Figure 2). No TT genotype, which is the FMD-resistant genotype (Singh et al 2015), was detected in HS, AB, TGS and EAR breeds (Table 4). Therefore, ITGB6 receptor gene (5'UTR, 2145T>C) cannot be used in MAS studies on Turkish native cattle breeds.

Two alleles (G and A) and three genotypes (GG, GA and AA) were detected in SLC11A1/*Mae*II polymorphism in the cattle populations studied (Figure 1). The lowest and highest G and A allele frequencies were 0.655 (TGS) – 0.841 (EAR) and 0.159 (EAR) – 0.345 (TGS), respectively. In the present study, the frequency of GG genotype is related to resistance to tuberculosis (Liu et al. 2017) ranged from 0.423 (TGS) to 0.682 (EAR) according to SLC11A1 (5411G>A) gene polymorphism. No AA genotype, which is a susceptible genotype for tuberculosis, was detected in EAR breed in which GG and AG genotype frequencies were 0.682 and 0.318, respectively. These values were quite similar to the frequencies of genotype (GG: 0.72; AG: 0.28 and AA: 0.00) obtained by Liu et al (2017) in healthy individuals of HS breed. The fact that all the breeds under analyses in Hardy-Weinberg equilibrium for the corresponding gene indicates that the populations have sufficient genetic variation.

As expected, two alleles (G and C) leading to three different genotypes (GG, GC and CC) were observed in SLC11A1/*Pst*I polymorphism (Figure 1). G allele frequencies ranging from 0.159 (EAR) and 0.284 (TGS) were comparatively lower than C allele frequencies ranging from 0.841 (EAR) and 0.716 (TGS) in all the cattle breeds studied (Table 4). Among the CG and GG genotypes, the genotypes for resistance to tuberculosis for this gene (Liu et al. 2017), GG genotype, was not found in the native breeds (AB, TGS and EAR). GG genotype was identified in the HS breed with a very low frequency (0.050). This value was much lower than the value reported by Liu et al (2017) in the HS breed raised in China. Although the GG genotype in SLC11A1 (7400G>A) gene frequencies of four different cattle breeds raised in Turkey were low, the frequency of CG, the second genotype for resistance to tuberculosis, was found at a moderate level. The frequencies of CG genotype in HS, AB, TGS and EAR breeds were found to be 0.305, 0.491, 0.569 and 0.318 respectively. However, the deviation of Hardy-Weinberg equilibrium of AB and TGS populations for SLC11A (5411G>A) gene may be the sign of lower genetic variation. This could be attributed to the fast reduction in the number of these breeds.

Possessing two alleles (C and A) and three genotypes (CC, CA and AA) (Figure 1), all cattle breeds studied were polymorphic for TLR2/*Eco*RV polymorphism, which were previously reported to be associated with tuberculosis in cattle (Sadana et al. 2015). C allele frequency was between 0.287 (HS) and 0.557 (EAR), whereas A allele frequency ranged from 0.443 (EAR) to 0.713 (HS) (Table 4). HS, AB and TGS were in Hardy-Weinberg equilibrium, while significant deviation was detected in EAR breed for TLR2 gene. The frequencies of tuberculosis-resistant AA genotype (Sadana et al. 2015) in HS, AB, TGS and EAR breeds were 0.518, 0.223, 0.383 and 0.114, respectively. AA genotype frequency (0.260) reported in Sahiwal, a native Indian cattle breed (Sadana et al. 2015), was similar to values detected in AB and TGS. On the other hand, a significantly higher AA genotype frequency was detected in HS breed than in native Turkish cattle breeds indicating that the success rate of MAS studies for tuberculosis is higher in HS breed. Bhaladhare et al (2016) reported CC, CA and AA genotype frequencies as 0.651, 0.269 and 0.080, respectively in native Indian cattle breeds for TLR2 gene polymorphisms. In the present study, the frequencies of the desired genotype (AA) for tuberculosis were higher than the value (0.080) reported by Bhaladhare et al (2016). The underlying reason may be the genetic origins of these cattle breeds.

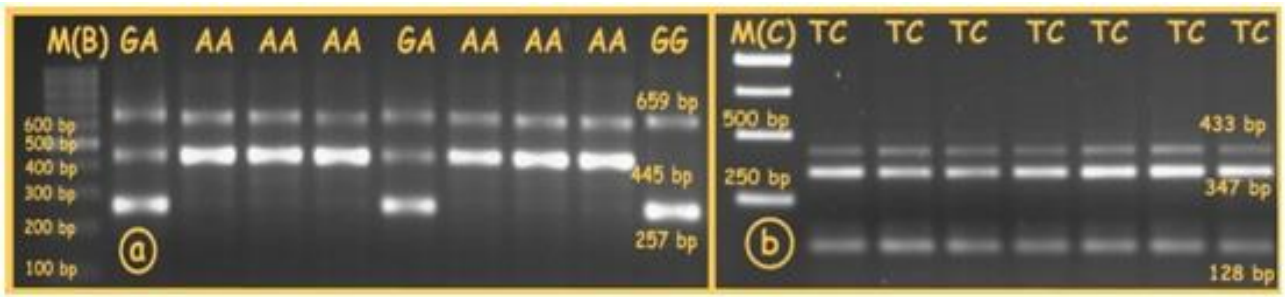

Figure 2. Agarose gel images for ARMS-PCR process. (M(B): Marker B (Thermo 100 bp; Cat. No: SM0241); M(C): Marker C Thermo 1kb; Cat. No: SM0311); a) ITGB6 receptor (5'UTR region, 29G>A; 1.5 % agarose gel) gene b) ITGB6 receptor (5'UTR region, 2145T>C, 2 % agarose gel) gene)

In this study, a total of 9 different repetitive fragments were detected in SLC11A1 gene across the cattle populations studied via microsatellite marker (Table 5). A higher number of alleles was detected in HS (8) than native Turkish cattle breeds (4-6). Observed heterozygosity was between 0.520 (AB) and 0.909 (EAR), while expected heterozygosity ranged from 0.637 (TGS) to 0.840 (HS) (Table 5). Kadarmideen et al. (2011) investigated the repetitive region in the bovine gene of SLC11A1 (solute carrier family 11), using the microsatellite method, and obtained four alleles including 211, 213, 215 and 217 bp length. The animals with the alleles of 211, 215 and 217 bp length were reported to be more resistant to BTB disease ($P<0.001$). In the present study, we found more alleles than Kadarmideen et al (2011). The identification of fragment sizes in the microsatellite marker is very precise. The differences may result from using different devices for fragment analyses. This situation prevented us from reaching exact results when identifying the resistant or susceptible genotypes in our study. Therefore, this locus was only involved determination of phylogenetic relation only.

Table 5. Allele frequencies obtained on microsatellite locus in the SLC11A1 gene.

Allele	HS	AB	TGS	EAR
201	0.020	0.000	0.000	0.000
203	0.030	0.000	0.000	0.000
205	0.098	0.160	0.418	0.523
207	0.206	0.000	0.000	0.000
209	0.245	0.230	0.427	0.204
211	0.156	0.470	0.107	0.182
213	0.000	0.050	0.012	0.091
215	0.137	0.070	0.036	0.000
217	0.108	0.020	0.000	0.000
Na	8	6	5	4
Ne	5.939	3.255	2.701	2.806
Ho	0.745	0.520	0.761	0.909
He	0.840	0.699	0.637	0.651

The phylogenetic tree that was constructed based on disease resistance loci has grouped the four breeds into two different clusters according to their genetic origins (Figure 3). The first branch separated HS breed from native Turkish cattle breeds, while the second branch separated TGS breed from AB and EAR breeds. Similarly, Demir and Balcioglu (2019), who used 20 microsatellite markers, separated native Turkish cattle breeds from HS breed according to phylogenetic analysis. The results of this study indicate that disease resistance loci may be used for the phylogenetic analysis of breeds. Microsatellite markers and next generation sequencing (NGS) analyses are generally used for phylogenetic analyses. These methods, and particularly the NGS analyses, are more informative for phylogenetic analyses, but are also costly procedures that require better technical infrastructure and more time. Therefore, disease resistance loci can be used in breed segregation, if not in ecotype or subtype segregation, when results need to be obtained in a shorter time and for lower costs.

In conclusion, for the MAS studies performed in Turkey for mastitis, CD14 gene is not appropriate for the HS breed but may be used in AB, TGS and EAR breeds. MBL1 (Exon 2; 2534G>A) and MBL1 (Exon 2; 2651G>A) genes may be used in four cattle breeds. However, it should not be forgotten that all populations deviated from the Hardy-Weinberg equilibrium for MBL1 (Exon 2; 2651G>A) gene. ITGB6 receptor gene (5'UTR, 29G>A) is very useful for the MAS studies to be performed on FMD. This gene can be used in MAS for FMD in all four cattle breeds. Since the resistant TT genotype could not be identified in the four breeds under analysis for another mutation in ITGB6 receptor gene (5'UTR, 2145T>C), it is not suitable to be used in MAS studies. SLC11A1 (5411G>A), SLC11A1 (7400G>A) and TLR2 genes can be included in the MAS programs for tuberculosis resistance in HS, AB, TGS and EAR cattle breeds. Additionally, the loci of disease resistance are very suitable for the genetic separation of breeds. Disease resistance loci for cattle breeds separation may be preferred because it is cost effective, quick and offers easy laboratory and statistical analyses.

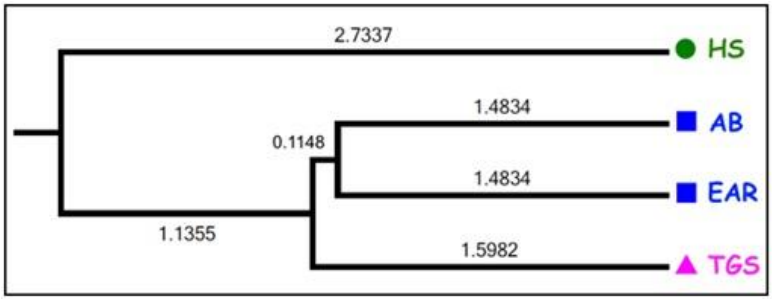

Figure 3. UPGMA dendogram among four cattle breeds raised in Turkey based on the loci of resistance to diseases.

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References

Bhaladhare A, Sharma D, Kumar A, Sonwane A, Chauhan A, Singh R, Kumar P, Yadav R, Baqir M, Bhushan B, Prakash O (2016) Single nucleotide polymorphism in toll-like reseptor genes and case control association studies with bovine tuberculosis. Veterinary World 9: 458-464.

Demir E, Balcıoğlu MS (2019) Genetic diversity and population structure of four cattle breeds raised in Turkey using microsatellite markers. Czech Journal of Animal Science 64: 411-419.

Hartl DL, Clark AG (1989) Principles of Population Genetics. Second Edition. Sinauer Associates, Sunderland.

HBS Hayvan Bilgi Sistemi (2019) Republic of Turkey Ministry of Agriculture and Forestry. Ankara, Turkey.

Kadarmideen HN, Ali AA, Thomson PC, Müller B, Zinsstag J (2011) Polymorphism of the SLC11A1 gene and resistance to bovine tuberculosis in African Zebu cattle. Animal Genetics 42: 656-658.

Kumar V, Gupta ID, Verma A, Kumar SR, Chaudhari MV (2014) CD14 gene polymorphism using HinfI restriction enzyme and its association with mastitis in Sahiwal cattle. Indian Journal of Animal Science 48: 11-13.

Liu K, Zhang B, Teng Z, Wang Z, Wang Y, Dong G, Xu C, Qin B, Song C, Chai J, Li Y, Shi X, Shu X, Zhang Y (2017) Association between SLC11A1 (NRAMP1) polymorphisms and susceptibility to tuberculosis in Chinese Holstein cattle. Tuberculosis 103: 10-15.

Mahmoudi B, Bayat M, Sadeghi R, Babayev MS, Abdollahi H (2010) Genetic diversity among three goat populations assessed by microsatellite DNA markers in Iran. Global Veterinary 4: 118-124.

Miller S, Dykes D, Plesky HA (1988) Simple salting out procedure for extracting DNA from human cells. Nucleic Acids Research 16: 1215.

Morris CA (2007) A review of genetic resistance to disease in *Bos taurus* cattle. The Veterinary Journal 174: 481-491.

Ramadan S, Kayang BB, Inoue E, Nirasawa K, Hayakawa H, Ito S, Inoue-Murayama M (2012) Evaluation of genetic diversity and conservation priorities for Egyptian chickens. Journal of Animal Science 2: 83-90.

Sadana T, Singh RV, Singh SV, Saxena VK, Sharma D, Singh PK, Kumar N, Gupta S, Chaubey KK, Jayaraman S, Tiwari R, Dhama K, Bhatia AS, Sohal JS (2015) Single nucleotide polymorphism of SLC11A1, CARD15, IFNG and TLR2 genes and their association with *Mycobacrterium avium* subspecies *paratuberculosis* infection in native Indian cattle population. Indian Journal of Biotecnology 14: 469-475.

Selvan AS, Gupta ID, Verma A, Chaudhari MV, Magotra A (2016) Molecular characterization and combined genotype association study of bovine cluster of differentiation 14 gene with clinical mastitis in crossbred dairy cattle. Veterinary World 9: 680-684.

Singh R, Deb R, Singh U, Alex R, Kumar S, Chakraborti S, Sharma S, Sengar G, Singh R (2014) Development of a tetra-primer ARMS PCR-based assay for detection of a novel single-nucleotide polymorphism in the 50 untranslated region of the bovine ITGB6 receptor gene associated with foot-and-mouth disease susceptibility in cattle. Archives of Virology 159: 3385-3389.

Singh R, Deb R, Singh U, Raja TV, Alex R, Kumar S, Chakraborti S, Alyethodi RR, Sharma, S, Sengar G (2015). Heterozygosity at the SNP (rs136500299) of ITGB6 receptor gene possibly influences the susceptibility among crossbred bull to foot and mouth disease infection. Virus Diseases 26: 48-54.

TUIK Turkish Statistical Institute (2019) Livestock Statistics, Ankara, Turkey: http://www.tuik.gov.tr/UstMenu.do?metod=temelist, Accessed 1 September, 2021.

Wang C, Liu M, Li Q, Ju Z, Huang J, Li J, Wang H, Zhong J (2011). Three novel single-nucleotide polymorphisms of MBL1 gene in Chinese native cattle and their associations with milk performance traits. Veterinary Immunology and Immunopathology 139: 229-236.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997) POPGENE, The user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Yilmaz O, Akin O, Yener SM, Ertugrul M, Wilson RT (2012) The domestic livestock resources of Turkey: cattle local breeds and types and their conservation status. Animal Genetics Resources 50: 65-73.

Yuan Z, Li J, Li J, Gao X, Xu S (2013) SNPs identification and its correlation analysis with milk somatic cell score in bovine MBL1 gene. Molecular Biology Reports 40: 7-12.

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