Turkish Journal of Agriculture - Food Science and Technology, 4(5): 352-355, 2016

Turkish Journal of Agriculture - Food Science and Technology

www.agrifoodscience.com,
Turkish Science and Technology

Determination of Toll-Like Receptor 1 Gene Polymorphisms in Zavot, Turkish Grey, East Anatolian Red, Anatolian Black and South Anatolian Red Cattle Breeds

Mehmet Ulaş Çınar[1], Korhan Arslan[2], Esma Gamze Ilgar[2], Bilal Akyüz[2*]

[1] *Department of Animal Science, Unit of Biometry and Genetics, Faculty of Agriculture, Erciyes University, 38039 Kayseri, Turkey*
[2] *Genetics Department, Faculty of Veterinary Medicine, Erciyes University, 38039 Kayseri, Turkey*

A R T I C L E I N F O

Article history:
Received 16 November 2015
Accepted 22 March 2016
Available online, ISSN: 2148-127X

Keywords:
TLR1 gene
Turkish cattle breeds
Allele frequency
Genotype frequency
Genetic polymorphism

*Corresponding Author:
E-mail: bakyuz@erciyes.edu.tr

A B S T R A C T

Toll-like receptors (TLRs) play an important role in non-specific immunity against different infectious agents such as bacterium or parasite. The aim of this work was to investigate the allele and genotype frequencies of three single-nucleotide polymorphisms (SNPs) in bovine TLR1 gene in native Turkish cattle breeds. DNA samples were extracted using the phenol chloroform protocol from 77 Zavot, 60 Turkish Grey, 51 East Anatolian Red, 69 Anatolian Black and 46 South Anatolian Red cattle. Target regions of the TLR1 gene were digested *Bsp*I and *Hpy188*III restriction enzymes. Results showed that the (A) allele frequency had higher in all native Turkish cattle breeds of the TLR1-G1409A locus. The (F) allele frequency was found to be higher compared to (E) allele in the TLR1-G1550A site. The frequencies of both (C) and (T) alleles were close to each other in the TLR1-C632T site. In conclusion genetic polymorphisms exist in Turkish native cattle populations in terms of known TLR1 variants.

Introduction

Livestock diseases may have harmful effects on farm animal fertility and production, on trade in breeding animals, meat and milk yield and other animal products, on human health and, as a result, on the process of economic development. The exploitation of the effects of the genetic factors on the health status of livestock represents an important approach for controlling and protecting the diseases caused by bacterial agents (Novák, 2014). The innate immune system is the first steps of defense against invading pathogen agents and is activated by conserved pathogen associated molecular patterns (PAMPs) (Jann et al., 2009). Toll-like receptors (TLRs) are a family of signaling molecules that bind to PAMPs and thus trigger an immune response, play an important role within the innate immune system (Takeda et al., 2003). The main tasks of TLRs are simulating of inflammation and building of adaptive immunity. This function is mainly orchestrated via TLRs signaling pathway (O'Neill et al., 2013). TLR signaling can simulate production of inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). These cytokines then activate receptors in the cell membrane to produce chemokines or adhesion molecules, thus recruiting different leukocytes into the infection areas (Kaisho and Akira, 2006).

While the number of TLR members ranges from 10 to 17 (Novák, 2014) among different organisms, ten different Toll-like genes have been determined within the cattle genome (McGuire et al., 2006). Among them TLR1, 5, 6, and 10 (the so-called antibacterial TLRs) assistance the recognition of the cell walls of bacterial, on the other hand TLR3, 7, 8, and 9, recognize to viruses (the so-called anti-viral TLRs) (Werling et al., 2009). TLR2 and TLR4 recognise structures specific to both pathogen groups (Novák, 2014). Polymorphisms of the TLR1 those were subjected to this study, were associated previously with brucellosis and tuberculosis in cattle (Prakash et al. 2014; Sun et al., 2012). Bovine TLR1 gene is located on *Bos taurus* chromosome 6 (BTA 6) together with TLR 6 and 10 as 69 kb gene cluster (Opsal et al., 2006). Different QTL studies reveal that this gene cluster situated within a dense QTL region for milk production traits (Klungland et al., 2001; Ogorevc et al., 2009) and QTL for disease resistance such as mastitis (Jann et al., 2009). Enhancement in the innate immunity is feasible due to range of infections affected by TLR polymorphisms (Netea et al., 2012; Novák, 2014). Since TLR variants associated with various diseases have been identified in cattle, breeding of animals in terms of Toll-like receptor-TLR proteins could help in cattle disease

control. However, for this purpose the native livestock breeds should be accepted as the sources of genetic diversity (Novák, 2014).

The Zavot breed has been bred for more than 150 years in the Kars and Ardahan provinces which are located in the northeast of Turkey (Yüksel et al., 2010). Turkish Grey cattle originated from the Balkan Peninsula and this breed are raised in Marmara Region of Turkey. Turkish Grey cattle have strong horns and mostly a grey color. Their height at withers is relatively higher than other Turkish native cattle breeds (Ağaoğlu et al., 2015). East Anatolian Red (EAR) is a native breed adapted to the cold climate, poor quality grassland in the hills and high plateaus of especially North East Anatolia region which is 1300-2000 m high above sea level. The EAR breed has been raised for meat and milk yield and light red coat color is common, but coat color may vary from dark red to light (Ağaoğlu et al., 2015). The Anatolian Black cattle have been raised in central Anatolia and the coat color is black. This breed has relatively high adaptability for inadequate conditions, disease resistant, adverse climate conditions, tolerant of poor care and feeding (Ağaoğlu et al., 2015). The South Anatolian Red (SAR) cattle are raised in the southern Anatolia region of Turkey, Syria, Iraq, Jordan, Israel and Egypt. The SAR breed has a higher milk yield according to other Turkish native cattle breeds. For several years, the SAR breed adapted for poor quality pasture conditions, hot climatic conditions and especially resistance to parasitic diseases (Ağaoğlu et al., 2015). Therefore, the purpose of this work was to investigate the allele and genotype frequencies of three SNPs of bovine TLR1 gene in five native Turkish cattle breeds, know to be resistant to some diseases.

Materials and Methods

A total 303 cattle belonging to five different Turkish local cattle breeds Zavot-ZV (n=77, from Kars and Ardahan provinces), Turkish Grey-TG (n=60, Balıkesir and Edirne provinces), East Anatolian Red-EAR (n=51, Kars, Ardahan and Erzurum provinces), Anatolian Black-AB (n=69, Kayseri, Sivas and Yozgat provinces) and South Anatolian Red-SAR (n=46, Adana, Hatay and Kilis provinces) were included in this study. These native cattle breeds belonged to different ecological and geological zones of Turkey. Genomic DNA was extracted from whole blood using the standard phenol chloroform protocol. Five ml of blood were drawn from the jugular veins into vacuum tubes containing 1.5% EDTA. Stock DNA was diluted to working concentration of 50 ng/µl and the stock DNA stored at -20°C for further analysis. Genotyping of the TLR1 was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers to amplify the bovine TLR1 exon 1e was selected from the study of Sun et al. (2012). Detailed information on the primers and amplification conditions is shown in (Table 1).

The PCR reaction volume was 20 µl, which included 10 µL of PCR master mix, 0.5 µl of forward and reverse primers, 1 µl of genomic DNA template, and 8 µl of ultra pure water. The PCR amplification conditions were optimized in MyGenie96 Thermal Block (Bioneer). The cycling program consisted of an initial denaturation (94°C for 5 minutes) followed by 35 cycles of 40 seconds at 94°C, 30 seconds at the annealing temperature (Table 1), 30 seconds at 72°C, and a final extension of 7 minutes at 72°C. For RFLP, each of the PCR products was digested with a restriction enzyme in a reaction including 3 µl of the PCR product, 5 U of the restriction enzyme (Fermentas) and 1 µl of the corresponding 10x reaction buffer. The digestion reaction was then incubated at the appropriate temperature for at least 4 hours. The restriction fragments were electrophoresed in 3% agarose gel.

The genotype frequencies of examined animals in each breed were computed. The Hardy-Weinberg equilibrium of the examined breeds for three SNPs in TLR1 gene was analyzed using the Chi-square test. All statistical analysis was performed by using FSTAT v.2.9.3.2 software.

Results

The allele and genotype frequencies of three SNPs of TLR1 gene obtained for five Turkish native cattle breeds are shown in the Table 2. Digestion of the 179-bp fragment of TLR1 gene with *Bsl*I restriction enzyme revealed a polymorphism with 2 alleles (Table 2).

Two genotypes, GG (97 and 82 bp) and GA (179, 97 and 82 bp), were found at the TLR1-G1409A site (Figure 1).

Results showed that the (G) allele had higher frequency in all breeds (Table 2). Most of the animals had GG genotype compared to GA genotype. None of the animals had AA genotype (Table 2). Significant deviation was observed from HWE in the ZV and AB breeds (Table 2).

Digestion of the 203-bp fragment of TLR1 gene with *Bsl*I restriction enzyme a polymorphism with two alleles was detected (Table 2). The TLR1-G1550A site had three genotypes: EE (156 and 47 bp), FF (87, 69 and 47 bp) and EF (156, 87, 69 and 47 bp) (Figure 2).

Except ZV breed genotype frequencies were not consistent with the HWE ($P<0.05$). The frequency of (F) allele was found to be higher compared to (E) allele (Table 2).

The 402-bp fragment of TLR1 gene was digested with *Hpyl88*III restriction enzyme and a polymorphism with two alleles was detected. The TLR1-C632T site had three genotypes: CC (220, 93, 49 and 40 bp), CT (220, 140, 93, 80, 49 and 40 bp) and TT (140, 93, 80, 49 and 40 bp) (Figure 3).

Due to low agarose gel resolution, 40 and 49 bp bands could not be observed on gel. Band sizes 80, 140 and 220 were found to be genotype specific therefore genotypes without seeing 40 and 49 bp bands were successfully identified for TLR1-C632T site (Figure 3). The frequencies of both (C) and (T) alleles were close to each other (Table 2).

Table 1 Primers and amplification conditions

Site	Primer Sequence	A[1] (°C)	P[2](bp)	PP[3]	R[4]
TLR1–G1409A	F: 5'-TTTAGCAGCCTTTCCATACT-3' R: 5'-TCTACCACGTCACTGGATACT-3'	60.8	179	Exon 1e (1311 – 1489)	*Bsl*I
TLR1–G1550A	F: 5'-TAGGCCAAGTATCCAGTGAC-3' R: 5'-CAGATCCAGGTAGATACAGAG-3'	60.8	203	Exon 1e (1462 – 1664)	*Bsl*I
TLR1–C632T	F: 5'-GGAGATACTTATGGGGAAAGAGAA-3' R: 5'- GTGTATAGACAAGGCCTTCAGTGA-3'	52	402	Exon 1e (1311 – 1489)	*Hpyl88*III

[1]A: Annealing temperature; [2]P: Product size; [3]PP: Product position; [4]R: Restriction enzyme

Table 2 The distribution of TLR1 gene genotypes and allele frequencies in ZV, TG, EAR, AB and SAR cattle breeds

Locus	Breed	n	Allele frequency (%)		Genotype			HWE[1]
			G	A	GG	GA	AA	(χ^2)
TLR1-G1409A (Bs1I)	ZV[2]	77	0.792	0.208	45	32	0	5.3*
	TG[3]	60	0.883	0.117	46	14	0	1.05Ns
	EAR[4]	51	0.824	0.176	33	18	0	2.34Ns
	AB[5]	69	0.804	0.196	42	27	0	4.08*
	SAR[6]	46	0.826	0.174	30	16	0	2.04Ns
			E	F	EE	EF	FF	
TLR1-G1550A (Bs1I)	ZV[2]	77	0.227	0.773	0.01(1)	0.43(33)	0.56(43)	3.73Ns
	TG[3]	60	0.342	0.658	0.02(1)	0.65(39)	0.33(20)	11.88***
	EAR[4]	51	0.255	0.745	0	0.51(26)	0.49(25)	5.97*
	AB[5]	69	0.246	0.754	0.02(1)	0.46(32)	0.52(36)	4.27*
	SAR[6]	46	0.315	0.685	0	0.63(29)	0.37(17)	9.75**
			C	T	CC	CT	TT	
TLR1-C632T (Hpy188III)	ZV[2]	77	0.468	0.532	9	54	14	12.85***
	TG[3]	60	0.45	0.55	3	48	9	22.78***
	EAR[4]	51	0.44	0.56	5	34	11	7.22**
	AB[5]	69	0.552	0.448	13	48	6	13.49***
	SAR[6]	46	0.488	0.512	6	30	7	6.75**

[1]Hardy-Weinberg equilibrium (HWE); [2]ZV: Zavot; [3]TG: Turkish Grey; [4]EAR: East Anatolian Red; [5]AB: Anatolian Black; [6]SAR: South Anatolian Red; Ns: Not significant; *P<0.05; **P<0.01; ***P<0.001

Discussion

The present study evaluated the genetic polymorphism of the TLR1 gene in ZV, TG, EAR, AB and SAR cattle breeds. The PCR-RFLP analysis allowed us to identify different genotypes of each exon 1e region for TLR1 gene. The TLR1-(G1409A) SNP was genotyped and two alleles (G and A) and three genotypes (GG, GA and AA) were observed in which the frequency of allele G was found higher compared to allele A in native Indian cattle (Prakash et al., 2014). Prakash et al. (2014) observed that the frequency of GA genotype was found higher than the other two genotypes. The same SNP was genotyped in Chinese Holstein cattle population and only two genotypes (GG and GA) were indentified and frequency of GA genotype was found higher than frequency of genotype GG (Sun et al., 2012). In the same study frequency of allele G was observed higher compared to allele A (Sun et al., 2012). In other study which investigated Chinese Holstein population in terms of TLR1-(G1409A) polymorphism found also two genotypes (GG and GA) but genotype GG frequency was found higher compared to genotype GA (Li et al., 2009). Similar to findings of Li et al. (2009), in our experiment frequency of genotype GG was also found higher compared to the frequency of genotype GA. Likewise Holstein populations, reared in China (Li et al., 2009; Sun et al., 2012) AA genotype was not observed in five native Turkish cattle breed. Additionally, the frequency of allele G was observed higher than A allele in all investigated populations including five Turkish native cattle breeds. Presence of genotype AA in Indian native cattle (Prakash et al. 2014) but both Turkish native cattle and Holsteins (Li et al., 2009; Sun et al., 2012), European originated cattle breeds, are lack of this genotype. However, studies concerning larger animal populations and breeds could be designed to investigate the origin specificity of this genotypic condition.

Another SNP of TLR1 gene which named as G1550A, Sun et al. (2012) found that the frequencies of E and F alleles were close to each other in Chinese Holstein population and all three genotypes were detected. Additionally, it was found that genotype EF was the most frequent genotype in Chinese Holsteins (Sun et al., 2012). However, in another experiment in which Chinese Holstein population was investigated, found that frequency of F allele was found higher compared to E allele and the frequency of genotype FF was higher compared to other two genotype (Li et al., 2009). In our study frequency of F allele was found higher than E allele in investigated Turkish native cattle breeds. In addition to this, unlike Holstein breed the frequency of genotype EE was found lowest among three detected genotypes in Turkish native cattle breeds (Table 2).

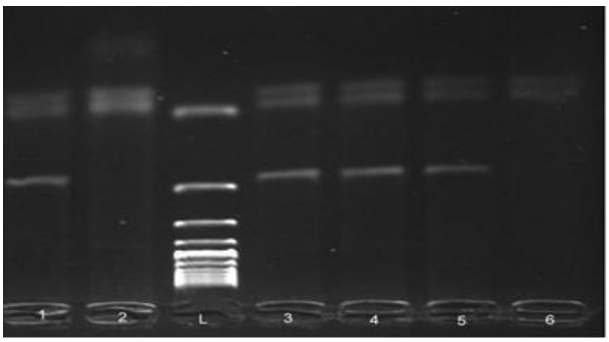

Figure 1 Lanes; 2 and 6: GG, Lanes; 1, 3, 4 and 5: GA, L: 100 bp DNA ladder

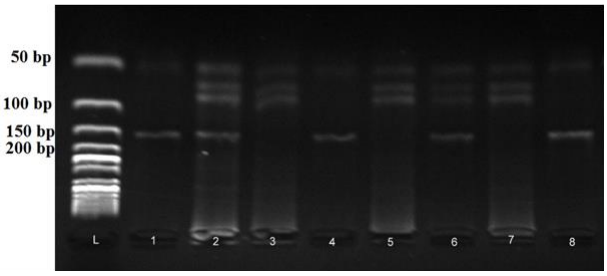

Figure 2 Lanes; 1, 4 and 8: EE, Lanes; 3, 5 and 7: EF, Lanes; 2 and 6: FF, L: 50 bp ladder

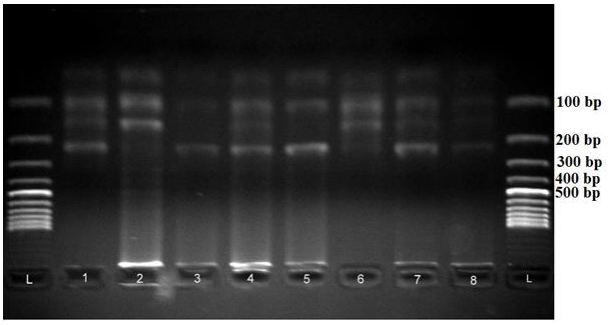

Figure 3 Lanes; 3 and 5: CC, Lanes; 1, 4 and 7- 8: CT, Lanes; 2 and 6: TT, L: 100 bp Ladder

Sun et al. (2012) investigated another SNP of TLR1 gene in terms of C632T mutation in Holstein cows. They found that allele frequencies were close to each other for the TLR1- C632T site. Similarly, we also observed very close allele frequencies in all Turkish native cattle breeds. In Holstein cattle all three genotypes were detected and genotype CT was found highest compared to other two genotypes (Sun et al., 2012). In our experiment the frequency of CT genotype was found higher compared to CC and TT genotypes (Table 2). The observed deviation from HWE in this study may also occur due to reduction in genetic diversity among Turkish native cattle breeds. Because these breeds are not preferred by farmers due to their low yielding (eg. milk yield and growth traits) capacity and this may lead to decrease in the population number of native cattle breeds in Turkey.

Taken together, results of the current study indicated that genetic polymorphisms exist in Turkish native cattle populations. Conserving the gene variants in the genetic pool is very crucial for genotyping the livestock for selection against various infections in cattle. New studies which are investigating association between genotype and brucellosis and tuberculosis diseases are required to understand the genetic background of disease resistance in native cattle breeds.

References

Ağaoğlu ÖK, Akyüz B, Kul BÇ, Bilgen N, Ertuğrul O. 2015. Genetic polymorphism of five genes associated with meat production traits in five cattle breeds in Turkey. Kafkas Univ. Vet. Fak. Derg., 21: 489-497.

Jann OC, King A, Corrales NL, Anderson SI, Jensen K, Ait-Ali T, Glass EJ. 2009. Comparative genomics of Toll-like receptor signalling in five species. B.M.C. Genomics, 10: 216.

Kaisho T, Akira S. 2006. Toll-like receptor function and signaling. J. Allergy. Clin. Immun., 117: 979–987.

Klungland H, Sabry A, Heringstad B, Olsen HG, Gomez-Raya L, Våge DI, Lien S. 2001. Quantitative trait loci affecting clinical mastitis and somatic cell count in dairy cattle. Mamm. Genome., 12: 837–42.

Li C, Shi W, Chu M, An Y, Chen H, Di R, Fang L. 2009. Polymorphisms of tlr1 gene and their relationship with somatic cell score in holstein cows. Sci. Agr. Sinica., 42: 2118–2125.

McGuire K, Jones M, Werling D, Williams JL, Glass EJ, Jann O. 2006. Radiation hybrid mapping of all 10 characterized bovine toll-like receptors. Anim. Genet. 37: 47–50.

Netea MG, Wijmenga C, O'Neill LAJ. 2012. Genetic variation in Toll-like receptors and disease susceptibility. Nat. Immun. 13: 535–542.

Novák K. 2014. Functional polymorphisms in Toll-like receptor genes for innate immunity in farm animals Vet. Immunol. Immunop., 157: 1–11.

O'Neill LAJ, Golenbock D, Bowie AG. 2013. The history of Toll-like receptors - redefining innate immunity. Nat. Rev. Immunol., 13: 453–60.

Ogorevc J, Kunej T, Razpet A, Dovc P. 2009. Database of cattle candidate genes and genetic markers for milk production and mastitis. Anim. Genet., 40: 832–51.

Opsal MA, Våge DI, Hayes B, Berget I, Lien S. 2006. Genomic organization and transcript profiling of the bovine toll-like receptor gene cluster TLR6-TLR1-TLR10. Gene., 384: 45–50.

Prakash O, Kumar A, Sonwane A, Rathore R, Singh RV, Chauhan A, Sharma D. 2014. Polymorphism of cytokine and innate immunity genes associated with bovine brucellosis in cattle. Mol. Biol. Rep., 41: 2815-25.

Sun L, Song Y, Riaz H, Yang H, Hua G, Guo A,Yang L. 2012. Polymorphisms in toll-like receptor 1 and 9 genes and their association with tuberculosis susceptibility in Chinese Holstein cattle. Vet. Immunol. Immunop.,147: 195–201.

Takeda K, Kaisho T, Akira S. 2003. Toll-like receptors. Annu. Rev. Immunol., 21: 335–76.

Turner ML, Cronin JG, Healey GD, Sheldon IM. 2014. Epithelial and stromal cells of bovine endometrium have roles in innate immunity and initiate inflammatory responses to bacterial lipopeptides in vitro via Toll-like receptors TLR2, TLR1 and TLR6. Endocrinology., 155: 1453-1465.

Werling D, Jann OC, Offord V, Glass EJ, Coffey TJ. 2009. Variation matters: TLR structure and species-specific pathogen recognition. Mod. Trends. Immunol., 30: 124–30.

Yüksel S, Sezgin E, Kopuzlu S. 2011. One of the native animal genetic resources and its importance in animal husbandry: Zavot cattle. RBI 8th Global Conference on the Conservation of Animal Genetic Resources, Tekirdag, Turkey, 4–8.10.2010, pp. 193–197.