

Growth hormone (*GH*), prolactin (*PRL*), and diacylglycerol acyltransferase (*DGAT1*) gene polymorphisms in Turkish native cattle breeds

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Received: 05.09.2014

Accepted/Published Online: 16.12.2014

Printed: 30.09.2015

Abstract: The aim of this study was to determine the genetic diversity of 4 native Turkish cattle breeds, based on the growth hormone (*GH*), prolactin (*PRL*), and diacylglycerol acyltransferase (*DGAT1*) genes. In order to study the polymorphisms in these genes, the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method was performed. A 329-bp fragment and a 223-bp fragment of *GH*, a 156-bp fragment of *PRL*, and a 411-bp fragment of *DGAT1*, thus 4 loci of 3 genes, were amplified via PCR. These fragments were then restricted with the enzymes *MspI*, *AluI*, *RsaI*, and *CfrI*, respectively. In this study, 2 types of alleles, (+) and (-) for the *GH-MspI*, L and V for the *GH-AluI*, A and B for the *PRL*, and K and A for the *DGAT1* loci, were observed. The results of the present study will contribute to the polymorphism data on the world's cattle breeds. Furthermore, the above-mentioned allele frequencies of Turkish native breeds are evaluated in relation to their genetic relatedness, and to infer their milk production properties on the basis of the available literature. In turn, these results can be utilized for future breeding programs of dairy cattle in Turkey.

Key words: Milk protein, PCR-RFLP, *GH*, *PRL*, *DGAT1*, Turkish native cattle breeds

1. Introduction

Genetic research in farm animals focuses mainly on the identification of genes influencing economically important traits that could be useful in breeding programs. In dairy cattle, most studies have researched the genes that may determine variations in milk yield and milk composition (Komisarek and Dorynek, 2009). The polymorphisms of several genes including growth hormone (*GH*), prolactin (*PRL*), and diacylglycerol acyltransferase (*DGAT1*) have been shown to affect milk yield and milk composition traits in dairy cattle.

GH belongs to a family of somatotrophic hormones including prolactin, placental lactogen, and a number of hematopoietic growth factors (Cosman et al., 1990). *GH* is located on the 19th chromosome in the q26-qter band region (Hediger et al., 1990). The *GH* gene of cattle is approximately 1800 bp in size and contains 5 exons and 4 introns. This gene encodes an mRNA with a size of 786 bp (Woychik et al., 1982). Although a number of polymorphisms have been observed in the *GH* gene of cattle, the 2 polymorphisms that are located in intron 3 and exon 5 have been found to be significant in their effects on milk and meat yield parameters (Lucy et al.,

1991, 1993; Høj et al., 1993). The polymorphism in intron 3 of *GH* is observed when *GH* is digested by the restriction enzyme *MspI* (*GH-MspI*) (Zhang et al., 1993). Two alleles are observed as a result of digestion with this enzyme. The first allele, *GH-MspI* (-) contains a T insertion at the +837 position, and the second allele has a C-G transition at the +837 position (Lee et al., 1994). Zhang et al. (1993b) reported that the polymorphism in exon 5 could be observed when digested by the *AluI* enzyme (*GH-AluI*) and the 2 alleles called L (leucine in the 127th codon) and V (valine in the 127th codon) occur.

The *PRL* gene is located on chromosome 23 and is composed of 5 exons and 4 introns (Camper et al., 1984, as cited by Skinkyte et al., 2005). A silent A-G mutation of the 103rd codon in exon 3 of the bovine *PRL* gene led to a polymorphic *RsaI* site (Lewin, 1992, as cited by Skinkyte et al., 2005). The polymorphism leading to the *RsaI* enzyme restriction site of the bovine *PRL* gene determines milk production traits including milk yield, milk fat, and milk protein contents (Lewin et al., 1992). The significant effect of the *PRL* locus on milk production traits was later shown by Chung et al. (1996, as cited by Dybus, 2002a) as well.

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DGAT1 is a candidate gene related to variation in milk fat content in dairy cattle. This gene is localized on the centromeric end of the 14th bovine chromosome. A base substitution (*K232A*) in the *DGAT1* gene in exon number 8 led to the quantitative trait loci variation (Grisart et al., 2002). While the lysine variant (K allele) of the *DGAT1* gene was associated with high fat content of the milk, the alanine variant (A allele) of the *DGAT1* gene was associated with high milk yield. The lysine encoding variant is considered to be the ancestral state of *DGAT1*, and mutation is believed to have taken place early in the history of the domestication of cattle or even before domestication (Grisart et al., 2002; Winter et al., 2002).

It is believed that taurine cattle were first domesticated in southeastern Anatolia (Zeder, 2008). Thus, because of their geographic proximity to the domestication center, it was first assumed that Turkish native cattle breeds were the closest descendants of the first domesticated taurine cattle. However, a recent study (Decker et al., 2014) examining more than 43,000 SNPs and 134 domestic cattle breeds clearly indicated that modern Turkish cattle breeds exhibit taurine introgression, as had already been proposed by Loftus et al. (1999), Ibeagha-Awemu and Erhardt (2005), Özkan (2005), and Freeman et al. (2006). The *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* gene polymorphisms have been well studied in Turkish cattle breeds (Baklacı, 2005; Kepenek, 2007; Yardibi et al., 2009; Ağaoğlu and Akyüz, 2013; Bal and Akyüz, 2014; Cerit et al., 2014).

In the present study, polymorphisms in terms of the above-mentioned alleles of *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* loci in Turkish native cattle breeds were assessed. The purpose of the study was to contribute to the polymorphism data on cattle breeds with respect to the loci under consideration by reinforcing the available data from Turkish native cattle breeds. Genetic relatedness between Turkish native cattle breeds in terms of the studied loci will be determined. Furthermore, the allele frequencies of Turkish native breeds will be used to tentatively infer their milk production properties on the basis of the available literature covering allele frequencies and milk production properties of cattle. The latter information can be employed for future breeding programs of dairy cattle in Turkey.

2. Materials and methods

In the present study, the 4 native Turkish cattle breeds that were examined and their geographic distributions in Turkey are as follows: South Anatolian Red (SAR) is found south of the Taurus Mountains and in southern Anatolia, Turkish Grey (TG) is distributed in Thrace and other cities of northwestern Anatolia, East Anatolian Red (EAR) is distributed in eastern and northeastern Anatolia, and Anatolian Black (AB) is mainly found in central and northern Anatolia. A total of 175 individuals from these

native breeds were examined. Samples of the SAR breed ($n = 47$) were obtained from the Çukurova Agricultural Research Institute. The AB breed samples ($n = 42$) were collected from villages around Çankırı and Kastamonu. The EAR breed samples ($n = 41$) were obtained from the Institute of East Anatolian Agricultural Research. The TG breed samples ($n = 45$) were obtained from the Marmara Agricultural Institute and from villages around Kesan in Thrace. Genomic DNA was extracted by using a standard phenol-chloroform extraction method (Sambrook and Russell, 2001). DNA concentrations were assessed by comparison with a standard DNA marker (Fermentas GeneRuler 50-bp DNA ladder) on agarose gel. The quality of the DNA was checked on 0.8% agarose gel prepared with Tris-boric acid-EDTA (TBE) buffer. To investigate the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), *MspI* and *AluI* restriction enzymes were used for the 2 amplified *GH* regions. The enzymes *RsaI* and *CfrI* were used to restrict the amplified regions of the *PRL* and *DGAT1* genes, respectively (Dybus, 2002a; Spelman et al., 2002; Dybus et al., 2004) in the 4 Turkish native cattle breeds (Kepenek, 2007). The primer sequences, annealing temperatures of the primers, expected size of the amplification products, size of the RFLP bands, and corresponding genotypes are summarized in Table 1.

Some of the data concerning *DGAT1* and *PRL* loci were obtained by EŞ Kepenek (Kepenek, 2007) during her MSc studies. However, for the present study, the number of observations was increased. For instance, 155 and 122 were previously observed for the *PRL* and *DGAT1* loci, respectively, while the total number in the present study is 175. Thus, the minimum number of observations for each breed was 41.

2.1. Statistical analysis

The calculation of the allele frequencies and the observed heterozygosities and the test for Hardy-Weinberg equilibrium (by Fisher's exact test) were performed using the Arlequin v.3.11 package program (Excoffier et al., 2006). Within-population inbreeding estimates (F_{IS}) and the pairwise F_{ST} values, according to Weir and Cockerham's (1984) approach, were obtained by using the FSTAT v.2.9.3.2 package program (Goudet, 2002). The data were permuted 1000 times in order to test the significance of the F_{IS} and F_{ST} values. For multiple tests in relation to Hardy-Weinberg equilibrium, F_{IS} , and F_{ST} , the level of significance ($P < 0.05$) was adjusted with the Bonferroni correction. Since there were 4 loci, the information obtained from all of them was analyzed by a multivariate method, principal component analysis (PCA), which was used for visualizing population relationships among the 4 breeds in 3-dimensional space. PCA was performed by using the NTSYS package program (Rohlf, 1993).

Table 1. Primers used for the partial PCR amplification of the *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* loci; annealing temperatures used for the amplifications; PCR product size after the amplification; restriction enzyme used; and genotypes in terms of the obtained digestion products.

Locus	Primers (5'-3')	Annealing temp. (°C)	PCR product size (bp)	Restriction endonuclease	Digestion product size (bp)
<i>GH-MspI</i>	F (5'-CCCACGGGCAAGAATGAGGC-3')R (5'-TGAGGAACTGCAGGGGCCCA-3')	60	329	<i>MspI</i>	(+/+) 329 (+/-) 329, 224, 105 (-/-) 224, 105
<i>GH-AluI</i>	F (5'-GCTGCTCCTGAGGGCCCTTCG-3')R (5'-GCGGGCGCACTTCATGACCCCT-3')	60	223	<i>AluI</i>	(LL) 223 (LV) 223, 171, 52 (VV) 171, 52
<i>PRL</i>	F (5'-CGAGTCCTTATGAGCTTGATTCTT-3')R (5'GCCTTCCAGAAGTCGTTTGTTC-3')	53	156	<i>RsaI</i>	(AA) 156 (AB) 156, 82, 74 (BB) 82, 74
<i>DGAT1</i>	F (5'-GCACCATCCTCTCCTCAAG-3')R (5'-GGAAGCGCTTTCGGATG-3')	60	411	<i>CfrI</i>	(AA) 411 (AB) 411, 208, 203 (BB) 208, 203

3. Results and discussion

In this study, 175 individuals from 4 native Turkish cattle breeds (TG, EAR, AB, and SAR) were genotyped for *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* loci. The observed allele frequencies, expected genotype frequencies, observed and expected heterozygosities, and P values indicating the significance of deviations from Hardy-Weinberg equilibrium for the 4 loci from each of the 4 different cattle breeds are shown in Table 2. In 16 comparisons, only 3 (18.75%) significant deviations from Hardy-Weinberg equilibrium were observed. Deviations were not accumulated within one breed or on one locus. In addition, the within-population F_{IS} values (inbreeding coefficient) were not significant (Table 3), except for that of *DGAT1* in AB ($P < 0.001$). Therefore, it can be concluded that genotyping was done appropriately and samples were not from an inbred gene pool.

There are accumulated data from many cattle breeds about the allele frequencies of the 4 loci (3 genes) mentioned above. These data and observed allele frequencies for *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* loci, together with those observed in the present study, are summarized in Tables 4, 5, 6, and 7, respectively. Information about the possible association between the alleles and milk production trait properties is also available. Observed associations between the alleles of the *GH*, *PRL*, and *DGAT1* genes and milk traits (milk yield, protein, and fat contents of milk) are summarized in Table 8. It seems that *GH-MspI* (+) and *PRL* (A) alleles have been associated with high milk yield in at least 2 independent studies (Table 8). Similarly, *GH-MspI* (-), *GH-AluI* (V), and *DGAT1* (K) alleles seem to be associated with high fat content of cattle milk. The protein

content of the milk, however, may be associated with *GH-MspI* (+) and *GH-AluI* (V) alleles in cattle (Table 8).

3.1. *GH-MspI* and *GH-AluI* polymorphisms

The frequency of the *GH-MspI* (-) allele ranged from 0.268 to 0.357. It was found that the (-) allele had the lowest frequency in EAR (0.268) and the highest frequency in AB (0.357). For these breeds, the frequencies of *GH-MspI* alleles obtained in this study are similar to those reported for the Turkish native cattle breeds in earlier studies (Table 4) (Baklaci, 2005; Yardibi et al., 2009). It has been shown that the *MspI* (-) allele frequencies in zebu cattle are different (relatively high frequencies) from those reported for taurine breeds (*Bos taurus*) from northern Europe, Mediterranean countries, and North America. For instance, 0.000 and 0.026 were reported in the Holstein breed (Zhang et al., 1993a; Lagziel et al., 2000; Zhou et al., 2005), 0.000 in Hereford and in N'Dama, 0.150 in Jersey, 0.140 in Angus, 0.190 in Red Danish (Lagziel et al., 2000), and 0.050 in Norwegian Red and in Red Danish (Høj et al., 1993). Table 4 suggests that the frequency range of the *MspI* (-) allele is 0.100 and 0.390 for cattle breeds of northern Europe, the United States, Europe, and the Mediterranean (Lagziel et al., 2000; Öner et al., 2011), but 0.670 to 0.940 in indicine (zebu) cattle breeds (Sodhi et al., 2007). The frequencies observed in the present study are between the frequencies ranges that were observed for taurine and indicine breeds.

The frequencies of the *GH-AluI* alleles obtained in this study (0.133–0.31) are similar to those reported in earlier studies (Table 5) for Turkish cattle breeds (0.147–0.570) (Yardibi et al., 2009; Ağaoglu and Akyüz, 2013; Akyüz et al., 2013). The *GH-AluI* (V) allele distribution of the *GH-*

Table 2. Distribution of the observed allele frequencies for *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* loci; expected genotype frequencies in accordance with Hardy–Weinberg equilibrium; P values for the fitness to Hardy–Weinberg equilibrium; and observed (H_o) and expected heterozygosities (H_e) in Turkish Grey (TG), East Anatolian Red (EAR), Anatolian Black (AB), and South Anatolian Red (SAR) cattle breeds of Turkey.

Locus	Population	n	Allele frequency		Genotypes (expected)			P value	H_o	H_e
			(+)	(-)	(+ / +)	(+ / -)	(- / -)			
<i>GH-MspI</i>	TG	45	0.700	0.300	0.444	0.511	0.044	0.284 ^{NS}	0.5110	0.4247
	EAR	41	0.732	0.268	0.488	0.488	0.024	0.232 ^{NS}	0.4878	0.3975
	AB	42	0.643	0.357	0.405	0.452	0.143	1.000 ^{NS}	0.4762	0.4647
	SAR	47	0.660	0.340	0.404	0.511	0.085	0.518 ^{NS}	0.5106	0.4539
			L	V	LL	LV	VV			
<i>GH-AluI</i>	TG	45	0.867	0.133	0.778	0.178	0.044	0.153 ^{NS}	0.1778	0.2337
	EAR	41	0.866	0.134	0.756	0.171	0.073	0.232 ^{NS}	0.1707	0.2352
	AB	42	0.690	0.310	0.405	0.571	0.024	0.065 ^{NS}	0.5714	0.4326
	SAR	47	0.787	0.213	0.617	0.340	0.043	1.000 ^{NS}	0.3404	0.3386
			A	B	AA	AB	BB			
<i>PRL</i>	TG	45	0.700	0.300	0.400	0.600	0.000	0.004 ^{**}	0.6000	0.4247
	EAR	41	0.683	0.317	0.366	0.634	0.000	0.003 ^{**}	0.6341	0.4384
	AB	42	0.524	0.476	0.262	0.524	0.214	1.000 ^{NS}	0.5238	0.5049
	SAR	47	0.713	0.287	0.532	0.362	0.106	0.476 ^{NS}	0.3617	0.4139
			K	A	KK	KA	AA			
<i>DGAT1</i>	TG	45	0.700	0.300	0.533	0.333	0.133	0.1676 ^{NS}	0.3333	0.4247
	EAR	41	0.780	0.220	0.561	0.439	0.000	0.163 ^{NS}	0.4390	0.3469
	AB	42	0.583	0.417	0.476	0.214	0.310	0.0003 ^{***}	0.2143	0.4920
	SAR	47	0.798	0.202	0.596	0.404	0.000	0.167 ^{NS}	0.4043	0.3260

NS, Not significant; *** P < 0.001, ** P < 0.01.

Table 3. Within-population inbreeding estimates (F_{IS} values) of the 4 Turkish native cattle breeds (TG, EAR, AB, SAR) for each of the 4 loci (*GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1*).

Locus	TG	EAR	AB	SAR
<i>GH-MSP1</i>	-0.206	-0.231	-0.025	-0.127
<i>GH-ALU1</i>	0.241	0.276	-0.326	-0.005
<i>PRL</i>	-0.419	-0.455	-0.038	0.127
<i>DGAT1</i>	0.217	-0.270	0.567 ^{***}	-0.243
All	-0.077	-0.225	0.058	-0.056

*** P < 0.001.

Table 4. *GH-MspI* (-) allele frequencies in cattle breeds from different countries.

Breeds	A	n	Frequency	References
Red Danish	2	58	0.05–0.28	Høj et al., 1993
Norwegian Red	2	32		
Black-and-White cattle breed (from different countries)		1128	0.09–0.26	Zhang et al., 1993a Yao et al., 1996 Falaki et al., 1996 Sabour et al., 1997 Vukasinovic et al., 1998
Breeds from different countries	20	1338	0.00–0.89	Lagziel et al., 2000
Brazilian Nellore	6	211	0.150	Unanian et al., 2002
Polish Black-and-White	5	900	0.019–0.157	Dybus et al., 2004
Gyr breed	1	40	0.810	Mattos et al., 2004
Yaroslavl breed	2	120	0.020	Khatami et al., 2005
Russian Black-and-White	1	32	0.083–0.125	
German Black-and-White	1	32		
Beijing Holstein	5	543	0.125	Zhou et al., 2005
Turkish native breeds	6	125	0.220–0.525	Baklaci, 2005
Iran native breeds	3	296	0.450–0.560	Zakizadeh et al., 2006
Black and White	1	110	0.170	
China Holstein	5	543	0.080–0.200	Zhou et al., 2006
Indian zebu cattle (<i>Bos indicus</i>) breeds	17	750	0.670–0.940	Sodhi et al., 2007
Turkish native cattle breeds	2	100	0.330–0.410	Yardibi et al., 2009
Iranian Holstein cattle breed	2	183	0.117	Gorbani et al., 2009
Bali, Limousine, Simmental	3	87	0.111–1.00	Jakaria et al., 2009
Indian cattle breed	1	57	0.860	Mitra et al., 1995
Turkish Brown Swiss and Holstein	5	110	0.037–0.184	Öner et al., 2011
Turkish native cattle breeds	4	175	0.268–0.357	Present study

A: Number of sampled breeds, n: total number of individuals.

AluI locus in different cattle breeds from different countries (Table 5) showed that the frequency was high (0.070–0.604) in the taurine cattle group (Reis et al., 2001; Dybus, 2002b; Khatami et al., 2005) and low (0.000–0.100) in the indicine cattle group (Mitra et al., 1995; Kemenes et al., 1999; Mattos et al., 2004; Curi et al., 2006; Jakaria Noor, 2011). Similar to the *GH-MspI* allele frequencies, the *GH-AluI* (V) allele frequencies in Turkish native breeds are between the frequency ranges of taurine and indicine, perhaps indicating the presence of indicine introgression into Turkish native breeds, as has been observed several times in previous

studies (Ibeagha-Awemu and Erhardt, 2005; Özkan, 2005; Freeman et al., 2006; Decker et al., 2014).

In our study, simultaneous records of genotypes and milk trait properties for Turkish native breeds were not available. Assuming that the associations between the allele frequencies and milk trait properties shown in Table 8 are valid for most cattle breeds, some inferences for the Turkish breeds can be made. In this line of argument, the high milk fat content of the AB breed (GDAR, 2011) might be attributed to the maximum frequencies observed in the *GH-MspI* (-) allele and *GH-AluI* (V) allele in the AB

Table 5. *GH-AluI* (V) allele frequencies in cattle breeds from different countries.

Breeds	A	n	Frequency	References
German Black-and-White bulls Bavarian Brown bulls Bavarian Simmental bulls	3	84	0.100–0.290	Schlee et al., 1994
Breeds from different countries		950	0.070–0.470	Schlee et al., 1994 Chung et al., 1996 Sabour et al., 1997 Chrenek et al., 1998 Chrenek et al., 1999 Dybus et al., 2002a
Breeds from different countries		3840	0.070–0.390	Sabour and Lin., 1996 Lovendahl et al., 1997 Shariflou et al., 1998 Zwierzchowski et al., 1998 Oprzadek et al., 1999 Shariflou et al., 2000 Kovács et al., 2006
Nelore, Gyr, Guzerá, Caracu, Charolais, Canchim, and Santa Gertrudis cattle	7	283	0.000–0.280	Kemenes et al., 1999
Portuguese cattle breeds	8	195	0.045–0.604	Reis et al., 2001
Polish Black-and-White breed		1086	0.135–0.216	Dybus, 2002b
Gyr breed	1	40	0.000	Mattos et al., 2004
Yaroslavl breed	2	120	0.430	Khatami et al., 2005
Russian Black-and-White	1	32	0.190–0.375	
German Black-and-White	1	32		
Brahman	1	324	0.360	Beauchemin et al., 2006
Iran native cattle breeds	3	296	0.080–0.160	Zakizadeh et al., 2006
Black-and-White cattle breed	1	110		
Angus and Shorthorn		1027	0.230–0.240	Barendse et al., 2006
Nellore		79	0.000	Curi et al., 2006
Turkish native breeds	2	100	0.440–0.570	Yardibi et al., 2009
Bali, Limousine, Simmental	3	87	0.000–0.306	Jakaria et al., 2009
Bali cattle	2	232	0.000–0.030	Jakaria, 2011
Indian cattle breed	1	57	0.040	Mitra et al., 1995
Holstein Friesian (Iran)	1	268	0.531	Mehmannavaz and Ghorbani, 2012
Cattle breeds in Turkey (Zavot, East Anatolian Red, Simmental, Brown Swiss)	4	246	0.219–0.266	Ağaoğlu and Akyüz, 2013
Holstein, Brown Swiss, Simmental	3	250	0.1467–0.34	Akyüz et al., 2013
Slovak spotted cattle	4	353	0.220–0.360	Moravčíková and Trakovická, 2013
Turkish native breeds	4	175	0.133–0.310	Present study

A: Number of sampled breeds, n: total number of individuals.

breed of Turkey. In another study, individuals from both SAR and EAR possessing VV genotypes of the *GH-AluI* locus had higher milk fat content than those having other genotypes for the same locus (Yardibi et al., 2009). In the same study, those individuals from SAR having (-/-) genotypes on the basis of the *GH-MspI* locus had higher milk fat content. Thus, observations from a previous study (Yardibi et al., 2009) seem to confirm that *GH-MspI* (-) and *GH-AluI* (V) alleles might be increasing the fat content of the milk in Turkish native cattle breeds.

3.2. *PRL* polymorphism

Within the bovine *PRL* gene, several polymorphisms have been reported (Cowan et al., 1989; Hart et al., 1993; Zhang et al., 1993b; Chung and Kim, 1997). The genotype and allele frequencies of the *PRL* gene for the studied cattle breeds are presented in Table 2. The BB genotype frequency (0.000–0.214) of the *PRL* locus was lower than those of the AA genotype (0.262–0.532) and AB genotype (0.362–0.634). The heterozygous AB genotype was present in the highest frequency across all of the breeds. The *PRL* A allele was found with a high frequency (0.713–0.683) in TG, SAR, and EAR cattle and low frequency (0.524) in AB cattle breeds. In contrast to our *PRL* A allele frequency results (0.524–0.713), several studies reported a higher frequency (0.80–0.95) for the *PRL* A allele in Holstein populations from different countries (Table 6). The *PRL* A allele was accepted as the predominant allele in cattle breeds, which is believed to have originated from *Bos taurus* (Sodhi et al., 2011). The *PRL* A allele frequency was found to be higher than the B allele frequency in all the native cattle breeds of Anatolia that were investigated in this study (Table 2), but the frequency is not as high as was observed in taurine breeds, presumably because of the introgression from the indicine breeds.

The frequencies of *PRL* alleles obtained in this study are similar to those reported in some earlier studies (e.g., Mitra et al., 1995; Chung et al., 1996; Chrenek et al., 1998b, 1999; Dybus, 2002a; Alipanah et al., 2007, 2008; Kumari et al., 2008) for the different taurine cattle breeds as indicated in Table 6 (Swiss Brown, Slovak Pinzgauer, Korean cattle breeds, Red Angus, Russian Red Pied, Jersey, Russian Black Pied, Red Pied, etc.). Studies of the *PRL* gene of cattle from different countries reported that the frequency of the A allele was higher in some European *Bos taurus* cattle breeds (Mitra et al., 1995; Chung et al., 1996; Chrenek et al., 1998a, 1998b) and lower in the *Bos indicus* cattle breeds (Mitra et al., 1995; Kumari et al., 2008; Sodhi et al., 2011; Dayal Das et al., 2012; Mahajan et al., 2012; Ishaq et al., 2013), as shown in Table 6.

In summary, among the Turkish cattle breeds, the frequency of the A allele is intermediate between the frequency ranges of taurine and indicine breeds but closer to the frequencies observed in taurine breeds.

3.3. *DGAT1* polymorphism

The allele frequencies of the *DGAT1* gene for TG, EAR, AB, and SAR cattle are presented in Table 7. The *DGAT1* (K) allele was observed with high frequencies in SAR (0.798), EAR (0.780), and TG (0.700) and low frequency in the AB (0.583) breeds, while the (A) allele frequency of the *DGAT1* gene was 0.417 and 0.300 in AB and TG cattle, respectively. The frequency of the (A) allele in EAR (0.220) and SAR (0.202) was lower compared to AB and TG (Table 3). The *DGAT1* gene K allele is related to a decrease in protein and milk yield. The increase in fat content is related to the alanine variant. The A allele is effective on the increase in milk and protein yield and the decrease in fat content (Grisart et al., 2002; Thaller et al., 2003; Kaupé et al., 2004; Cerit et al., 2014).

In the present study, the observed frequencies of the (K) allele (0.583–0.798) are higher than those observed (0.210–0.380) for the native Turkish breeds previously (Kaupé et al., 2004), and they are in the range of zebu breeds' frequencies (Table 7). Moreover, the frequencies of the *DGAT1* (K) allele obtained in this study are higher than the reported values of earlier studies for the Anatolian Black (0.350) and East Anatolian Red (0.640) breeds (Bal and Akyüz, 2014; Cerit et al., 2014). European *Bos taurus* breeds, with the exception of the Turkish native and Jersey breeds, showed the lowest frequency of the (K) allele, while the highest (K) allele frequencies were harbored by *Bos indicus* type cattle. In addition, unselected South American Creole cattle breeds and the synthetic Brangus breed had intermediate allele frequencies (Table 7).

To reiterate, the high (K) allele frequency observed in Turkish native breeds might be attributed to zebu cattle introgression. Alternatively, the K allele might have been an ancient allele in the taurine breeds. As regards the selection of high milk yield, it was lower in European breeds, but because the breeds have not been subjected to systematic selection in Anatolia, high frequencies of the (K) allele might still be seen in Anatolian cattle breeds.

3.4. Genetic differentiation of SAR, EAR, AB, and TG cattle breeds on the basis of *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* loci

The multilocus (*GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1*) pairwise F_{ST} values were used to analyze the degree of genetic differentiation between the Turkish cattle breeds. Pairwise F_{ST} values between EAR and AB (0.0483), SAR and AB (0.041), and TG and AB (0.0340) were high and statistically significant ($P < 0.01$), indicating that these breeds are genetically quite distinct from each other with respect to the 4 studied loci. SAR and EAR were genetically the most similar breeds based on the smallest nonsignificant pairwise F_{ST} value observed between these breeds (-0.001).

Table 6. PRL (A) allele frequencies in cattle breeds from different countries.

Breeds	A	n	Frequency	References
German Black-and-White			0.800	
Swiss Brown			0.610	Mitra et al., 1995
Sahiwal (<i>Bos indicus</i>)			0.490	
Korean cattle	1	320	0.678–0.767	Chung et al., 1996
Holstein			0.730–0.950	Chung et al., 1996 Chrenek et al., 1998
Slovak Pied; Slovak Pinzgauer			0.680–0.870	Chrenek et al., 1998
Swiss Brown			0.670	Chrenek et al., 1999
Creole, Holstein-Friesian (Argentina)	9	293	0.871–0.956	Golijow et al., 1999
Red Angus			0.700	Dybus, 2002a
Russian Ayrshire, Gorbato Red		81	0.800–0.859	Udina et al., 2001
Black Pied			0.914	
Polish			0.862	Dybus, 2002a
Black-and-White–Polish Red			0.760–0.870	Klauzińska, 2002
Argentine and Bolivian Creole	8	463	0.816–1.000	Lirón et al., 2002
Yaroslavl breed	2	120	0.650	
Russian Black, White	1	32	0.954	Khatami et al., 2005
German Black, White	1	32	0.610	
Black-and-White, Jersey	3	427	0.845–0.861 0.308	Dybus et al., 2005
Black-and-White, Jersey	2	324	0.887 0.294	Brym et al., 2005
Russian Red Pied	1	125	0.794	Alipanah et al., 2007
Turkish native cattle breeds and Holstein	5	176	0.564–0.857	Kepenek, 2007
<i>Bos taurus</i>				
Holstein Friesian, Jersey		366	0.550–0.900	
<i>Bos indicus</i>				
Sahiwal, Khillari, Ongole, Kankrej, Gir, Red Sindhi, Hariana, Red Kandhari, Dangi, Deoni, Tharparkar		135	0.460–1.000	Kumari et al., 2008
Russian Black Pied, Red Pied		170	0.710–0.790	Alipanah et al., 2008
Montebeliard (Iran)	1	120	0.890	Ghasemi et al., 2009
Najdi cattle breed		84	0.571	Sharifi et al., 2010
Indian breed	1	57	0.490	Mitra et al., 1995
Indian native breeds	23	938	0.380–0.700	Sodhi et al., 2011
Deoni cattle breed			0.390	Dayal Das et al., 2012
Holstein Friesian (Iran)	1	268	0.931	Mehmannavaz and Ghorbani, 2012
Frieswal cattle	1	54	0.630	Vikas et al., 2012
American Swiss cattle	6	417	0.8765	Alfonso et al., 2012
Malvi, Nimari and Frieswal	3		0.450–0.630	Mahajan et al., 2012
Holstein, Brown Swiss, Simmental	3	250	0.760–0.873	Akyüz et al., 2013
Sahiwal and Achai	3	200	0.560–0.810	Ishaq et al., 2013
Yakut, Yaroslavl, Bestuzhev, Kostroma	4	335	0.646–0.750	Lazebnaya et al., 2013
Chinese Holstein	1	586	0.875	Dong et al., 2013
Turkish native cattle breeds	4	175	0.524–0.713	Present study

A: Number of sampled breeds; n: total number of individuals.

Table 7. DGAT1 (K) allele frequencies in cattle breeds from different countries and countries.

Breeds	A	n	Frequency	References
Holstein, Fleckvieh	39	1691	0.060–0.548	Thaller et al., 2003
<i>Bos taurus</i> , <i>Bos indicus</i> cattle	38	1748	0.010–0.99	Kaupe et al., 2004
Jersey	1	100	0.830	Komisarek et al., 2004
Polish Black and White cattle	3	502	0.480–0.680	Pareek et al., 2005
Uruguayan Creole cattle	1	115	0.114	Rincón et al., 2006
Brazilian cattle breeds		331	0.270–1.000	Lacorte et al., 2006
European breeds	6		0.000–0.350	
Creole cattle	5	296	0.350–0.500	Ripoli et al., 2006
Cebuine breeds	3		0.375–0.800	
Hanwoo (Korea cattle)		200	0.773	Kong et al., 2007
Turkish native cattle breeds and Holstein	5	122	0.779–0.925	Kepenek, 2007
Holstein		497	0.620	Oikonomou et al., 2009
Indian Holstein		281	0.590	Patel et al., 2009
Friesian			0.410	
Jersey		103	0.480	
Piedmontese-Valdostana			0.000–0.030	Signorelli et al., 2009
Nelore (<i>Bos indicus</i>)	3	357	0.850–0.990	Souza et al., 2010
Italian cattle breeds		651	0.000–0.254	Scotti et al., 2010
Iranian Holstein cattle	1	206	0.340	Abdolmohammadi et al., 2011
Holstein		315	0.190	Manga and Riha, 2011
Holstein	1	87	0.138	
Slovak Spotted cattle	1	42	0.047	Bauer et al., 2011
Polish Holstein	1	156	0.440	Urtnowski et al., 2011
Cal Poly Holstein			0.170–0.307	
Cal Poly Jersey		482	0.435–0.490	Laubscher et al., 2011
Girolando			0.736	Pereira et al., 2011
Holstein	2	398	0.370	Koopaei et al., 2012
	1	103	0.796	Mao et al., 2012
	2	1061		Mashhadi et al., 2012
Holstein		415	0.222	
Jersey		340	0.812	
Simmental		481	0.098	Anton et al., 2012
Isfahan Holstein	5	408	0.320–0.460	Nanaei et al., 2013
Holstein	7	300	0.64	Asmarasari, 2013
Slovak Spotted cattle		57	0.123	Lešková et al., 2013
Sahiwal (Indian zebu) Frieswal (Holstein Friesian × Sahiwal)		51	0.960	
		126	0.164	Indrajit et al., 2013
Holstein		100	0.250	
East Anatolian Red		50	0.640	Bal and Akyüz, 2014
Anatolian Black		50	0.700	
Holstein	1	278	0.260	Kadlecová et al., 2014
Native Black and Holstein	2	50	0.350–0.950	Cerit et al., 2014
Turkish native cattle breeds	4	175	0.583–0.798	Present study

A: Number of sampled breeds; n: total number of individuals.

Genetic differentiation of the cattle breeds based on the allele frequencies at 4 loci is presented through a 3-dimensional PCA in the Figure. The first principal component (PC1) (the first axis) of the PCA in the Figure displays 80.38% of the total genetic variation that is present among the breeds, whereas the second principal component (PC2) (the second axis of the PCA) accounts for 15.84% of the total genetic variation, and the third principal component (PC3) (the third axis) accounts for 3.77% of the total genetic variation. Therefore, the 3 axes of the PCA explain a total of 99.99% of the genetic variation of allele frequencies. On the 3-dimensional graph in the Figure, the high genetic similarity of the SAR and EAR breeds and the distinctness of AB, especially from EAR and SAR, can be seen clearly.

This study contributes to the genetic data on cattle breeds by increasing observation numbers for the allele frequencies of *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* loci in 4 Turkish native cattle breeds. It was observed that, in general, with respect to these loci, Turkish cattle breeds have intermediate allele frequencies between the frequency ranges of taurine and indicine cattle breeds. Among the 4 breeds, AB seemed to be the most distinct breed, as was expected since it had the maximum frequencies of *GH-MspI* (-), *GH-AluI* (V), *PRL* (A), and *DGAT1* (A) alleles.

Turkish native cattle breeds are considered to have low milk yields (Taşdan et al., 2008). Based on Table 8, it can be inferred that due to their generally high *GH-MspI* (-), *GH-AluI* (V), *PRL* (B), and *DGAT1* (K) allele frequencies all breeds seemed to have high fat content in their milk.

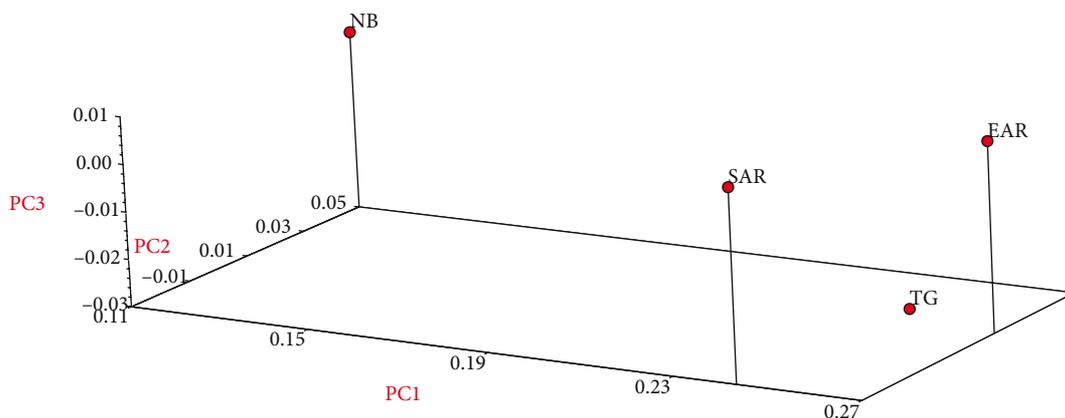


Figure. PCA analysis based on *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* loci in Turkish native cattle breeds (Turkish Grey - TG, East Anatolian Red - EAR, Anatolian Black - AB, and South Anatolian Red - SAR).

Table 8. Summary of the studies in relation to associations between *GH-MspI*, *GH-AluI*, *PRL*, and *DGAT1* polymorphisms and milk production traits (milk yield, protein content, fat content).

Loci	Alleles	Milk production traits			References
		Milk yield	Protein content	Fat content	
<i>GH-MspI</i>	(+)	↑	ND	ND	Høj et al., 1993; Lee et al., 1994
	(+)	↑	↑	↑	Yao et al., 1996
	(-)	ND	ND	↑	Høj et al., 1993; Lee et al., 1994
	(-)	ND	ND	↑	Falaki et al., 1996
<i>GH-AluI</i>	L	↑	ND	ND	Lucy et al., 1993
	V	ND	↑	↑	Sabour et al., 1997
	V	ND	↑	↑	Zwierzchowski et al., 2002
	V	ND	ND	↑	Høj et al., 1993; Lee et al., 1994
<i>PRL</i>	A	↑	ND	ND	Chung et al., 1996; Chrenek et al., 1998; Miceikienė et al., 2006
	B	↓	ND	↑	Khatami et al., 2005
<i>DGAT1</i>	A	↑	ND	ND	Grisart et al., 2002
	K	ND	ND	↑	Kaupe et al., 2003; Grisart et al., 2002

Arrows indicate increase (↑) or decrease (↓); ND: not determined

In a recent study, where the same samples but different milk trait genes (beta-casein, kappa-casein, and beta-lactoglobulin) were examined (Dinc et al, 2013), a high frequency of B allele of beta-lactoglobulin and the absence of the E allele of kappa-casein genes were observed. These were known to correlate with good cheese and yogurt properties (Dinc et al., 2013 and references therein). Since most of the milk (90%) produced in Anatolia is bovine milk and since it is mostly consumed as cheese (55.6%) and yogurt (19.6%) (Taşdan et al., 2008), perhaps in parallel to

the previous findings, the high fat content of milk among the native Turkish cattle breeds is another preferred feature of good cheese and yogurt properties in Turkey. In breeding studies, for instance for higher milk yield, it must be remembered that higher milk yield might be attained at the expense of lower-quality cheese and yogurt.

Acknowledgment

This study contains some data from the second author's thesis work.

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