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Research Article

Genetic Polymorphism of *STAT1* and *STAT5A* Genes in Holstein, Jersey, and Indigenous Cattle Breeds in Turkey [1][2]

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Abstract

This study aimed to determine genetic polymorphism in *STAT1* and *STAT5A* genes for dairy cattle and some native cattle breeds in Turkey. 283 Jersey and a total of 472 Holstein cows from two different herds and 93 Grey Steppe, 85 Anatolian Black Cattle, and 66 East Anatolian Red cattle were used in this research. Generally, C allele gene frequency was higher than T allele for *STAT1* in all breeds whereas C allele gene frequency was detected higher than G allele for *STAT5A* in Jersey and East Anatolian Red. On the other hand, G allele gene frequency was higher than C allele in Holstein, Grey Steppe, and Anatolian Black Cattle breeds. The expected deviations from the Hardy-Weinberg Equilibrium were significant only for Jersey breeds for *STAT1* gene. Meanwhile, the expected deviation from equilibrium was also significantly different for Holstein in Black Sea Region (BSR), Anatolian Black Cattle and Grey Steppe for the *STAT5A* gene. FIS values were determined to *STAT1* gene as negative for all breeds except for Holstein in Marmara Region (MR). Similarly, this value was determined to *STAT5A* gene as positive for all breeds except for Holstein in BSR. The genetic distances for two loci were calculated between 0.0029 and 0.1599 among all populations. Depending on the cluster analysis, Holstein in BSR and MR, Anatolian Black Cattle, East Anatolian Red were closely clustered to each other, while Grey Steppe and Jersey were located in completely different clusters. As a conclusion, based on the detected genetic diversity in *STAT1* and *STAT5A* genes, it is possible to make a genetic improvement among bovine breeds raised in Turkey.

Keywords: *Cattle, Genetic polymorphism, Genetic relationships, STAT1, STAT5A*

Türkiye'de Holstein, Jersey ve Yerli Sığır Irklarında *STAT1* ve *STAT5A* Genlerinin Genetik Polimorfizmi

Öz

Bu çalışma, Türkiye'de süt sığırları ve bazı yerli sığır ırklarında *STAT1* ve *STAT5A* genlerine ait genetik polimorfizmin belirlenmesini amaçlamaktadır. Araştırma kapsamında, 283 Jersey ve iki farklı sürüden toplam 472 Siyah Alaca inekleri ile 93 Boz Irk, 85 Yerli Kara ve 66 Doğu Anadolu Kırmızısı sığırları kullanılmıştır. Genel olarak, tüm ırklarda C allel gen frekansı STAT1 için T allelinden daha yüksek olurken Jersey ve Doğu Anadolu Kırmızısı'n da ise C allel gen frekansı *STAT5A* için G allelinden daha yüksektir. Diğer yandan ise, G allel gen frekansı Siyah Alaca, Boz Irk ve Yerli Kara ırklarında C allelinden daha yüksektir. Hardy-Weinberg Dengesinden beklenen sapmalar sadece *STAT1* genin için Jersey ırkında önemlidir. Ayrıca, *STAT5A* geni için dengeden beklenen sapma Karadeniz Bölgesi'nde (KB) ki Siyah Alacalar, Yerli Kara ve Boz Irkları için de anlamlı derecede farklıdır. FIS değerleri, *STAT1* geni bakımından Marmara Bölgesi'nde (MB) ki Siyah Alacalar dışında bütün ırklar için negatif olarak belirlenmiştir. Benzer şekilde, bu değer, *STAT5A* geni bakımından KB'de ki Siyah Alacalar hariç bütün ırklar için pozitif olarak belirlenmiştir. İki lokusun genetik mesafeleri tüm popülasyonlar bakımından 0.0029 ile 0.1599 arasında hesaplanmıştır. Kümeleme analizine bağlı olarak, KB ve MB'de ki Siyah Alacalar, Yerli Kara, Doğu Anadolu Kırmızısı birbirlerine çok yakın kümelenmişken, Boz Irk ve Jersey ırkları tamamen farklı kümeler de yer almıştır. Sonuç olarak, *STAT1* ve *STAT5A* genlerinde tespit edilen genetik çeşitliliğe dayanarak Türkiye'de yetiştirilen büyükbaş hayvan ırkları arasında genetik bir iyileştirme yapılması mümkün görülmektedir.

Anahtar sözcükler: *Sığır, Genetik polimorfizm, Genetik ilişkiler, STAT1, STAT5A*

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INTRODUCTION

Determination of the genes with an effect on economically important yield traits in livestock species will provide faster progress in animal breeding as compared with the traditional selection practices. Besides knowing and preserving genetic diversity among farm animals, it is essential for the continuation of the existence of these species in the future and will also provide the implementation of right strategies [1]. Molecular genetic markers are widely used to achieve these purposes because molecular markers were reported to be more reliable in determining the genetic structure and widely used to discover the phylogenetic relationships among species as well as among breeds [2].

One of the essential genes that are thought to affect the yield traits of animals is the *STAT* gene family, which is known as Signal Transducers and Activators of Transcription Factors. This gene family has several different known forms as *STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5*, and *STAT6* [3]. There are two isoforms of *STAT5* which are described as *A* and *B* form due to the difference of few amino acids at the carboxcylic end of protein molecule. Of this large gene family, the *STAT1* and *STAT5A* genes are frequently used in the candidate gene analyses. The bovine *STAT1* gene is located at 60 to 63 cM in chromosome 2 [4]. However, *STAT5A* maps to chromosome 19 containing 19 exons with 794 amino acid chains [5].

The different genomic regions discovered to be active on milk yield traits in the last decade were mapped in many dairy cattle breeds [6]. In many studies conducted, the relationship between the phenotypic traits and different alleles of the candidate gene in the population were investigated [7]. There are some evidence that *STAT1* plays an essential role in developmental process and a differentiation of the mammary gland [8,9]. Within this concept, *STAT1* gene was searched to determine the relationships between the genetic structure and yield traits of the herd in Czech Fleckvieh cattle. In that study, the frequencies of the *CC*, *CT,* and *TT* genotypes were as 71.60%, 26.75%, and 2.15%, respectively. Furthermore, significant differences in milk protein contents were observed among the all genotypes [10]. In a previous study performed for the *STAT1* gene, the effects of *CC* and *CT* genotypes calculated as a deviation from *TT* genotype had a significant impact of increasing milk yield as well as milk protein and fat yields in North American Holstein cows [11].

In a study where the relationships between meat yield traits and the *STAT5A* gene were investigated in Holstein cows reared in China, the frequencies of *CC*, *CT,* and *TT* genotypes were 0.79, 0.21, and 0.0 for this gene, respectively. It was reported that animals with *CC* genotype were more advantageous in terms of meat yield traits [12]. On the other hand, the *STAT5A* gene is also expressed to play a significant role in carrying signals, particularly from prolactin to milk protein genes [13]. The studies about *STAT5A* gene revealed that the significant effects on milk fat content and milk yield traits were observed in the Holstein cows reared in Poland and USA, respectively [14,15].

The effects of the *STAT5A* polymorphism on milk yield traits in Jersey cattle were also studied by Dario and Selvaggi [16]. They reported that *CC* genotype was expressed to be selectively advantageous in milk yield traits as compared to those with *CT* genotype. However, the herd was not in Hardy-Weinberg Genetic Equilibrium. On the contrary, the other study proved that animals carrying *TT* genotype for *STAT5A* gene turned out to be significantly different from those with *CT* genotype in term of milk fat content in Jersey cattle [17].

Although there are many studies conducted about the polymorphic effect of *STAT* gene family in many countries, there are not many studies conducted about these genes in cattle breeds raised in Turkey. Therefore, the purpose of this study was to precisely determine genetic polymorphism and genetic diversity of the *STAT1* and *STAT5A* genes in two different dairy breeds; as Holstein and Jersey cattle, and also in some indigenous cattle breeds; as Anatolian Black Cattle, East Anatolian Red, and Grey Steppe raised in Turkey.

MATERIAL and METHODS

Dairy and native cattle breeds were used as the animal material in this study. The dairy breeds were composed of 283 Jersey from TIGEM - Karakoy Agricultural Management in Bafra, Samsun, and 163 Holstein cattle from a commercial farm in Carsamba, Samsun at the Black Sea Region and 309 Holstein cattle from a commercial farm in Bursa at the Marmara Region. On the other hand, the native breeds were comprised of 93 Grey Steppe cattle from Bandirma Sheep Research Institute in Balıkesir and a commercial farm at Thrace Region, 85 Anatolian Black Cattle from Lalahan Livestock Central Research Institute in Ankara at Central Anatolia Region, and 66 East Anatolian Red cattle reared from breeders in Ardahan at Eastern Anatolia Region of Turkey. All the animals used in the study were chosen randomly from the herds.

Based on the study, blood samples from the jugular vein were collected into 10 mL vacuum tubes coated with K_2EDTA anticoagulant and DNA extractions were performed using a standard phenol/chloroform method [18]. Isolated DNA samples were used in PCR reaction for a technique of restriction fragment length polymorphism *(PCR-RFLP)*. The quality and quantity of DNA samples were evaluated using NanoDrop Spectrophotometer (Thermo Fisher Scientific Inc., USA).

In the amplification of genomic DNA, 50 ng of genomic DNA, 50 µM of each primer (forward and reverse), 200 µM of each dNTP, 2.5 µL of 10x PCR buffer solutions and 0.3 U (unit) of the *Taq* Polymerase were used in the PCR to have a total volume of 25 µL. According to the PCR protocol, the

Table 1. *Primer information for the STAT1 and STAT5A genes*

Gene	Primer Sequence (5′ to 3′)	Enzyme Digestion	PCR Product Size (bp)	Gen Bank #	References
STAT1	GCCTCAAGTTTGCCAGTGGC GGCTCCCTTGATAGAACTGT	*BspHI* 5′T/CATGA3′	314	AW289395	[11]
STAT5A	GAGAAGTTGGCGGAGATTATC CCGTGTGTCCTCATCACCTG	*BstEII* 5′G/GTNACC3′	820	NW_001493678	[15]

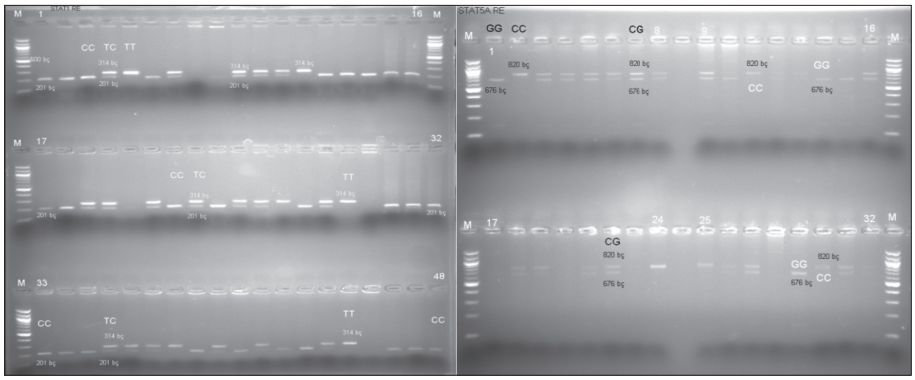

Fig 1. The patterns of restriction fragments of *STAT1* and *STAT5A* genes after digestions with *BspHI* and *BstEII* enzymes, respectively

amplification was carried out by an initial denaturation at 95°C for 5 min, 30 cycles a denaturation at 94°C for 45 s, an annealing at 50°C for 45 s, an elongation at 72°C for 45 s and a final extension at 72°C for 7 min. PCR products were digested with 10 U/µL of each *BspHI* and *BstEII* enzymes (Thermo Fisher Scientific Inc., USA) at 37°C for about four hour to determine allelic polymorphism in *STAT1* and *STAT5A* genes, respectively. A list of the primers used in the amplification of the target gene regions is provided in *Table 1*. A total of 999 cattle, 755 animals from imported dairy breeds and 244 animals from native breeds were genotyped within the scope of this study.

The allelic and genotypic frequencies were calculated with the method of direct gene counting, whether the distributions of genotypic frequencies were following the Hardy-Weinberg genetic equilibrium by the chi-square test. F-statistics are used to compare genetic variability in the total population, intra-subpopulation, and individual structures. These *F* criteria are known as F_{IT}, F_{IS}, and F_{ST}. The F_{IT} value was calculated as the difference of the total actual level of heterozygosity in all populations. The F_{ST} value indicates the genetic diversity among populations. The N_m value was estimated from the F_{ST} value according to the equation $(F_{ST}: 0.25(1-F_{ST})/F_{ST})$ to determine gene flow. The observed values were calculated as the ratio of the genotypes with such a trait to the total number of genotypes, while the expected values were determined, the fixation index (F_{IS}) values, N_e, the effective number of alleles and N_{ei} values were detected, respectively. Furthermore, the unweighted pair group method average *(UPGMA)* analysis was conducted to display the relationship among the bovine breeds phylogenetically [19].

RESULTS

In the study, first of all, the digested PCR products were distinguished by the alleles of *C* and *T* for *STAT1* and the alleles of *C* and *G* for *STAT5A*. The *T* allele was indicated by a band of 314 bp and the *C* allele was indicated by two bands of 201 and 113 bp for *STAT1*. On the other hand; the digestion products were determined by 820 bp for *C* allele and 626 bp for *G* allele in *STAT5A (Fig. 1)*. Based on the results, all breeds were found to be polymorphic for the marker loci in *STAT1* and *STAT5A* genes. The allelic gene and genotypic frequencies, as well as the χ2 results determined for the *STAT1* gene in Jersey, Holstein in BSR, Holstein in MR, Grey Steppe, East Anatolian Red, and Anatolian Black Cattle are presented in *Table 2*. The frequencies of *C* allele of the *STAT1* gene were found as 0.70, 0.74, 0.66, 0.92, 0.86, and 0.82 for Jersey, Holstein in BSR, Holstein in MR, Grey Steppe, East Anatolian Red, and Anatolian Black Cattle breeds, respectively. Of all populations, only Jersey was not in genetic equilibrium due to the various environmental effects, like sampling or inbreeding pressure. In general, the rate of *C* allele was found high for the *STAT1* gene in all populations as compared with *T*.

The allelic gene and genotypic frequencies, as well as the χ2 results determined for the *STAT5A* gene in Jersey, Holstein in BSR, Holstein in MR, Grey Steppe, East Anatolian Red, and Anatolian Black Cattle breeds, are presented in *Table 3*. The frequencies of *C* of the *STAT5A* gene were calculated as 0.79, 0.47, 0.48, 0.39, 0.54, and 0.45 for Jersey, Holstein in BSR, Holstein in MR, Grey Steppe, East Anatolian Red, and Anatolian Black Cattle breeds, respectively. Of all populations, only Grey Steppe breed was not in genetic equilibrium more likely due to the environmental influences over the observed allelic frequencies. In general, the rate of *C* allele was higher than that of *G* allele for the *STAT5A* gene in the populations for Jersey and East Anatolian Red. In case of Holstein, it is almost the same frequency between the alleles but for Grey Steppe and Anatolian Black Cattle breeds the frequencies of *G* allele had higher than that of *C* allele.

Table 2. *The gene, genotypic frequencies, and chi-square results for the STAT1 gene*

Population	Allele #	Allelic Gene Frequency (%)		Genotypic Frequency (%)			χ2
		C	T	CC	CT	TT	
Jersey	566	0.70	0.30	0.43	0.53	0.04	17.25**
Holstein in BSR	326	0.74	0.26	0.55	0.39	0.06	0.08
Holstein in MR	618	0.66	0.34	0.45	0.41	0.14	2.37
Grey Steppe	186	0.92	0.08	0.84	0.16	---	0.66
East Anatolian Red	132	0.86	0.14	0.73	0.26	0.01	0.09
Anatolian Black Cattle	170	0.82	0.18	0.66	0.33	0.01	1.40
Overall	1998	0.73	0.27	0.53	0.40	0.07	0.71

BSR: *Black Sea Region,* ***MR:*** *Marmara Region; ** P<0.01*

Table 3. *The gene, genotypic frequencies, and chi-square results for the STAT5A gene*

Population	Allele #	Allelic Gene Frequency (%)		Genotypic Frequency (%)			χ2
		C	G	CC	CG	GG	
Jersey	496	0.79	0.21	0.63	0.32	0.05	0.56
Holstein in BSR	276	0.47	0.53	0.18	0.59	0.23	4.14*
Holstein in MR	584	0.48	0.52	0.25	0.46	0.29	1.98
Grey Steppe	178	0.39	0.61	0.22	0.34	0.44	7.98**
East Anatolian Red	130	0.54	0.46	0.31	0.46	0.23	0.40
Anatolian Black Cattle	164	0.45	0.55	0.26	0.38	0.36	4.75*
Overall	1828	0.56	0.44	0.34	0.42	0.24	19.93**

BSR: *Black Sea Region,* ***MR:*** *Marmara Region; * P<0.05, ** P<0.01*

Table 4. *The F-statistics results of the STAT1 gene for breeds*

Population	Na[1]	Ne[2]	I[3]	PIC[4]	Obs-Hom[5]	Obs-Het[5]	Exp-Hom[5]	Exp-Het[5]	Ave-Het[6]	Nei[7]	F_{IS}[8]
Jersey	2	1.72	0.61	0.332	0.47	0.53	0.57	0.43	0.53	0.42	-0.248
Holstein in BSR	2	1.61	0.57	0.311	0.60	0.40	0.61	0.39	0.40	0.38	-0.026
Holstein in MR	2	1.81	0.64	0.348	0.58	0.42	0.54	0.46	0.42	0.44	0.086
Grey Steppe	2	1.17	0.28	0.136	0.83	0.17	0.85	0.15	0.17	0.14	-0.087
East Anatolian Red	2	1.32	0.41	0.212	0.74	0.26	0.75	0.25	0.26	0.24	-0.045
Anatolian Black Cattle	2	1.40	0.46	0.252	0.67	0.33	0.71	0.29	0.33	0.29	-0.133
Overall	2	1.63	0.57	0.317	0.60	0.40	0.61	0.39	0.40	0.38	-0.027

[1] Na: Observed number of alleles, [2] Ne: Effective number of alleles,[3] I: Shannon's information index, [4] PIC:P olymorphism Information Content, [5] Observed and expected homozygosity and heterozygosity, repectively, [6] Average heterozygosity, [7] Nei's expected heterozygosity, [8] Fixation index, BSR: Black Sea Region, MR: Marmara Region

The results of the F-statistics determined for the *STAT1* gene in all breeds are presented in *Table 4*. When the F_{IS} values of the populations for the *STAT1* gene were considered, the amount concerned was seen as 8% in Holstein in MR with the dominance of homozygous individuals, while it was displayed as 24% in Jersey, 2% in Holstein in BSR, 8% in Grey Steppe, 4% in East Anatolian Red, and 13% in Anatolian Black Cattle breeds with the dominance of heterozygous individuals. The expected deviations from the Hardy-Weinberg ratio in terms of the *STAT1* locus in these populations were found to be a significant in the Jersey population only (P<0.01). The dominance of heterozygous individuals at a rate of 2% was seen on the general population basis. Expected homozygosity was 57% in Jersey, 61% in Holstein in BSR, 54% in Holstein in MR, 85% in Grey Steppe, 75% in East Anatolian Red, and 71% in Anatolian Black Cattle breeds, with the most homogeneous genes observed in Grey Steppe breed. However, this value was detected as 61% in general. In all populations, the expected heterozygosity was estimated as 42% in Jersey, 38% Holstein in BSR, 44% in Holstein in MR, 14% in Grey Steppe, 24% in East Anatolian Red, and 29% in Anatolian Black Cattle breeds, whereas it was calculated as 38% in general. As a result of the statistical analyses, the highest polymorphism information content *(PIC)* value for *STAT1* gene was observed to be 0.348 for Holstein in MR, and the lowest one was found as 0.136 for Grey Steppe cattle *(Table 4)*. The average *PIC* value for the same gene was 0.317. On the other hand, the highest *PIC* value in terms of *STAT5A* gene was calculated as 0.375 in Holstein in MR, and this value was determined as the lowest of 0.277 in

Table 5. *The F-statistics results of the STAT5A gene for breeds*

Population	Na[1]	Ne[2]	I[3]	PIC[4]	Obs-Hom[5]	Obs-Het[5]	Exp-Hom[5]	Exp-Het[5]	Ave-Het[6]	Nei[7]	F_{IS}[8]
Jersey	2	1.50	0.51	0.277	0.68	0.32	0.67	0.33	0.32	0.33	0.045
Holstein in BSR	2	1.99	0.69	0.374	0.41	0.59	0.50	0.50	0.59	0.49	-0.176
Holstein in MR	2	1.99	0.69	0.375	0.54	0.46	0.50	0.50	0.46	0.49	0.080
Grey Steppe	2	1.91	0.67	0.363	0.66	0.34	0.52	0.48	0.34	0.47	0.293
East Anatolian Red	2	1.98	0.69	0.373	0.54	0.46	0.50	0.50	0.46	0.49	0.071
Anatolian Black Cattle	2	1.97	0.98	0.373	0.62	0.38	0.50	0.50	0.38	0.49	0.234
Overall	2	1.97	0.68	0.371	0.58	0.42	0.51	0.49	0.42	0.49	0.147

[1] Na: Observed number of alleles, [2] Ne: Effective number of alleles, [3] I: Shannon's information index, [4] PIC: Polymorphism Information Content, [5] Observed and expected homozygosity and heterozygosity, repectively, [6] Average heterozygosity, [7] Nei's expected heterozygosity, [8] Fixation index, BSR: Black Sea Region, MR: Marmara Region

Table 6. *The gene flow (N_m) and F-statistics for the STAT1 and STAT5A genes*

Loci	Allele #	F_{IS}	F_{IT}	F_{ST}	N_m*
STAT1	1998	-0.077	-0.019	0.049	4.852
STAT5A	1828	0.092	0.1516	0.0652	3.582

** The N_m value was calculated from the F_{ST} value; F_{ST}: 0.25(1-F_{ST})/F_S*

Jersey breed. The average *PIC* value is 0.371 at this time *(Table 5)*.

The results of the F-statistics determined for the *STAT5A* gene in all breeds are also provided in *Table 5*. When the F_{IS} values of the populations for the *STAT5A* gene were considered, it was seen that the amount concerned was 17% and negative in Holstein in BSR population only; accordingly, the dominance of heterozygous individuals was observed. This value was 4% in Jersey, 8% in Holstein in MR, 29% in Grey Steppe, 7% in East Anatolian Red, and 23% in Anatolian Black Cattle breeds and had a positive sign; furthermore, the dominance of homozygous individuals was displayed. The expected deviations from the Hardy-Weinberg ratio in terms of the *STAT5A* locus in the populations were found significant in Holstein in BSR and Anatolian Black Cattle ($P<0.05$) and Grey Steppe ($P<0.01$) breeds. On general population basis, the dominance of homozygous individuals was found to be at the rate of 14%, and the expected deviations from the Hardy-Weinberg ratio were observed as significant ($P<0.01$). Expected homozygosity was 67% in Jersey, 50% in Holstein in BSR, 50% in Holstein in MR, 52% in Grey Steppe, 50% in East Anatolian Red, and 50% in Anatolian Black Cattle breeds, with the most homogeneous genes shown in Jersey breed. Nevertheless, this value was recorded as 51% in general. In all populations, the expected heterozygosity for the *STAT5A* gene was found as 33% in Jersey, 49% in Holstein in BSR, 49% in Holstein in MR, 47% in Grey Steppe, 49% in East Anatolian Red, 49% in Anatolian Black Cattle breeds and 49% in general. In this study, the average values of heterozygosity for the *STAT1* and *STAT5A* genes were calculated as 0.53, 0.40, 0.42, 0.17, 0.26, and 0.33 as well as 0.32, 0.59, 0.46, 0.34, 0.46, and 0.38 in Jersey, Holstein in BSR, Holstein in MR, Grey Steppe, East Anatolian Red, and Anatolian Black Cattle, respectively.

The results of the gene flow *(N_m)* and F-statistics for the *STAT1* and *STAT5A* genes when both loci analyzed together are given in *Table 6*. According to the F_{ST} value determined for the *STAT1* gene, there was a decrease in the genetic diversity among the subpopulations *(F_{ST}:* 0.049) and this value was 4.9%. According to the F_{IT} value calculated *(F_{IT}:* -0.019), the actual level of heterozygosity in the population for all individuals differed by 1.9% from what it should be according to the Hardy-Weinberg principle. According to the F_{ST} value found for the *STAT5A* gene, the decrease in the genetic diversity among the subpopulations was recorded as F_{ST}: 0.0652. According to the F_{IT} value calculated *(F_{IT}:* 0.1516), the actual level of homozygosity in the population for all individuals differed by about 15% from what it should be according to the Hardy-Weinberg principle.

Genetic similarity and distance values resulting from the analysis of both loci together for the *STAT1* and *STAT5A* genes are presented in *Table 7*. The genetic distance values were found to range from 0.0029 to 0.1599 among the populations. The lowest genetic distance values were detected between East Anatolian Red and Anatolian Black Cattle populations, whereas the highest values were observed between Holstein in BSR and Grey Steppe populations. As a result of the cluster analysis, two clusters occurred for the *STAT1* and *STAT5A* genes *(Fig. 2)*. While Holstein in BSR, Holstein in MR, Anatolian Black Cattle, and East Anatolian Red were found closely clustered together, Grey Steppe was more distant from this main cluster, and Jersey was located in a completely different cluster.

Based on the overall results, *C* allele is more favorable than *T* allele for all breeds in *STAT1* gene. However, *C* allele is more frequent than *G* allele for Jersey and East Anatolian Red, but this is the exact opposite cases for Holstein and other indigenous breeds in *STAT5A*.

DISCUSSION

In the study performed, the frequency of *C* allele was generally found high for the *STAT1* gene in all populations as compared with *T* allele and the ratios of *CC*, *CT*, and *TT* genotypes were found as 53%, 40%, and 0.07%, respectively. In one of the recent studies, the frequencies of *CC*, *CT* and

Table 7. *Genetic similarity and genetic distance for STAT1 and STAT5A genes*

Population	Jersey	Holstein in BSR	Holstein in MR	Grey Steppe	East Anatolian Red	Anatolian Black Cattle
Jersey	-----	0.9561	0.9749	0.9183	0.9950	0.9847
Holstein in BSR	0.0449	----	0.9818	0.8522	0.9797	0.9920
Holstein in MR	0.0254	0.0183	----	0.9360	0.9901	0.9928
Grey Steppe	0.0852	0.1599	0.0662	----	0.9149	0.8986
East Anatolian Red	0.0051	0.0205	0.0099	0.0890	-----	0.9971
Anatolian Black Cattle	0.0154	0.0080	0.0072	0.1069	0.0029	-----

Above the diagonal are the values of genetic similarity, while below it are the values of genetic distance. ***BSR:*** *Black Sea Region,* ***MR:*** *Marmara Region*

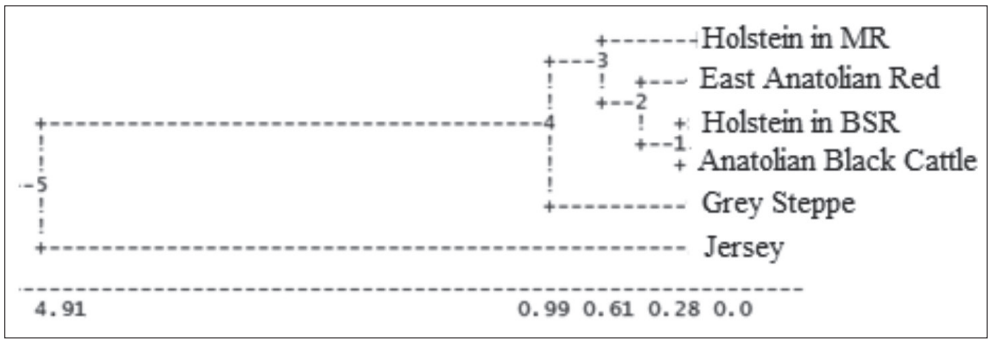

Fig 2. The *UPGMA* dendrogram showing relationships among the populations at the time of two locus process for *STAT1* and *STAT5A* polymorphisms

TT genotypes of the *STAT1* gene were reported similarly in the same pattern but somewhat lower as 45.24% for CC, 36.31% for *CT* genotypes, but a little higher as 18.45% for *TT* genotype in Holstein cows raised in the other farm also located to west part of Turkey, respectively [20]. They reported that the population did not follow Hardy-Weinberg Equilibrium for this locus which might be due to sampling, inbreeding or population stratification.

Many studies on the *STAT5A* gene have been performed in different cattle breeds in various environmental conditions. In these studies several polymorphic sites in bovine *STAT5A* were occupied for an association test with reproductive and productive traits in cow populations [14,16,17]. In a current study, the frequency of *C* allele was generally found high for the *STAT5A* gene in Jersey and East Anatolian Red populations as compared with *G*, which was its alternative allele. In this case, Holstein, Grey Steppe, and Anatolian Black Cattle breeds had higher frequencies of *G* allele than that of *C* allele. For the *STAT5A* gene, the rates of *CG* genotype were generally higher than the other genotypes; on the contrary, *CC* genotype was observed in high frequency in Jersey breed. One of the other studies, the *STAT5A* polymorphism was investigated in Holstein cow [21]. They reported that the population was found in genetic equilibrium. The frequencies of *CC*, *CT*, and *TT* genotypes were reported as 0.751, 0.234 and 0.015 for the gene concerned. Regarding this gene, heterozygosity and effective number of alleles (N_e) were reported as 0.229 and 1.298, respectively. One of the recent study, Oner et al.[22] investigated the effect of *STAT5A* and some other genes on fertility in Holstein-Friesian heifers. Even if the allele frequency of *G* was reported as higher than *C* allele, the association between *STAT5A* polymorphism and fertility was not significantly important. However Ouerghi et al.[23] reported that the substitution of *C* allele by *G* at *STAT5* might be an alternative for improving fertility rate in dairy cows of Tunisia.

The *STAT5A* gene polymorphism was also investigated in the Simmental cattle reared in Romania. For this gene, the frequencies of *CC*, *CT*, and *TT* genotypes were similarly found as 0.67, 0.33, and 0.00, respectively. Moreover, it was expressed that the population was in genetic equilibrium [24]. Arslan et al.[25] investigated the *STAT5A* gene and the other two gene polymorphisms in five different indigenous breeds reared in Turkey. For East Anatolian Red and Anatolian Black Cattle, the frequencies of *CC* genotypes were recorded as 63.1% vs. 62.5% and the frequencies of *C* allele as 71% and 72%; respectively nevertheless both breeds were not found in genetic equilibrium for *STAT5A*. Even if the genotypic frequency of *CC* genotype (75%) and allelic frequency of *C* (86%) was higher in Grey Steppe breed, it was found in genetic equilibrium on the contrary. In a fairly recent study, four different genes including *STAT5A* were searched to identify genetic polymorphism in 167 Turkish Holstein cows. Holstein cows were found to be polymorphic and three genotypes with *CC*, *CT* and *TT* were identified with regard to *SNP-AvaI* polymorphism in the 7^{th} exon of bovine *STAT5A* gene. The *CC* genotype was the most common with 74.2% followed by *CT* with 24% and *TT* with 1.8%, respectively. It was not detected any deviation from Hardy-Weinberg Equilibrium for this locus, either [26].

Based on the different polymorphic site at the position 12.743 in exon 16 for the *STAT5A* gene, the frequencies of *TT*, *CT*, and *CC* genotypes were found to be 0.72, 0.26 and 0.02 in the Polish Friesian herd, respectively [14]. They reported

the frequencies of 0.85 and 0.15 for *T* and *C* alleles in this herd, respectively. On the contrary, the frequencies of *CC*, *TT,* and *CT* genotypes for the *STAT5A* locus were 75.2%, 0.4%, and 24.4% and the *C* and *T* allele frequencies were 0.875 and 0.125 in Chinese Holstein cattle, respectively [27]. The inconsistencies observed among the studies are probably due to differences in cattle breeds. In another study carried out for the *STAT5A* polymorphism at the same chromosomal location in Jersey cattle, the genotypic frequencies for the gene concerned were similar as 73.68%, 23.16% and 3.16% for *TT*, *TC*, and *CC*, respectively and the herd was found in genetic equilibrium [17]. However, when the relationships between the *STAT5A/AvaI* polymorphism at position 6,853 within the exon 7 was investigated in another study; the genotypic frequencies concerning this gene were found as 51.83%, 47.12% and 1.05% for *CC*, *CT*, and *TT* genotypes in Jersey, respectively. The herd was not seen in genetic equilibrium according to the Hardy-Weinberg principle [16].

In contrast to the aforementioned study, allele frequencies in this study were determined as 0.75 for *C* and 0.25 for *T*, respectively. In a recent study, the relationships between the nucleotide polymorphism at position 12.743 in exon 16 of *STAT5A* gene and growth traits were investigated in Podolica bulls by Selvaggi et al.[28]. The frequencies of *TT*, *TC* and *CC* genotypes were recorded as 45.70%, 39.78% and 14.51%, respectively. The observed allele frequencies of *C* and *T* were 0.344 and 0.656, respectively. Unlike a previous study, the herd was found in genetic equilibrium. In another study, Selvaggi et al.[29] investigated two different polymorphic regions at *STAT5A* gene in 92 Agerolese cows belonging 15 different sires in Italy. They reported that all the genotypes of animals were *CC* at *STAT5A/AvaI* locus, there were no any animal with *CT* and *TT* genotypes. On the other hand, the animals with *TT* genotype was the most frequent (75%) followed by the animals with *CT* (25%) and there was no any animal observed with *CC* genotype at *STAT5A/MslI* locus in this herd. The frequencies of *T* and *C* were observed as 0.875 and 0.125, respectively which also indicated that the population was in Hardy-Weinberg Equilibrium for this locus. In a recent study, Coizet et al.[30] investigated the effect of *STAT5A* and two other genes on dairy production in Mediterranean Italian Buffalo. Based on the *SNP* marker polymorphism detected at intron 8-9 in *STAT5A* gene, the buffaloes with *TT* genotypes displayed higher percentages than the buffaloes with other genotypes.

In terms of *STAT1* gene, the observed homozygosity and heterozygosity values were 0.40 and 0.60 in Iranian Holstein cattle. The average value of heterozygosity was found as 0.56 [31]. In the present study, however, the observed homozygosity and heterozygosity values were 0.59 and 0.41, respectively and the average value of heterozygosity was found as 0.41 for *STAT1* in Holstein breed which is quite lower than the study with Iranian Holstein. Since the average value of heterozygosity is unaffected by the sampling error, it is acknowledged as one of the best indicators of genetic diversity [32].

According to the F_{ST} value found for the *STAT1* gene, there was a decrease in the genetic diversity among the subpopulations. Depending on this value, the genetic difference among the subpopulations was also at a low level. According to the F_{IT} value, the actual level of heterozygosity in the population is different than what it should be based on the Hardy-Weinberg. F_{ST} value was merely calculated for the *STAT5A* gene and depending on this value; the genetic difference among the subpopulations was at a moderate level. Based on F_{IT} value, the actual level of homozygosity in the population was different than what it should be according to the Hardy-Weinberg by 15%. In one of the nearly conducted study; various gene polymorphisms including *STAT5A* were investigated in Turkish native cattle breeds [25]. The F_{ST} values between the breeds for *STAT5A* were reported as 0.006 between Anatolian Black Cattle and East Anatolian Red, as 0.014 between Anatolian Black Cattle and Grey Steppe, and as 0.009 between East Anatolian Red and Grey Steppe which the results were not concordant to the findings of this current study.

When both loci were considered together, the lowest genetic distance values for the *STAT1* and *STAT5A* genes were between East Anatolian Red and Anatolian Black Cattle populations, whereas the highest values were between Holstein in BSR and Grey Steppe populations. These breeds were aggregated in two main clusters for the *STAT1* and *STAT5A* polymorphisms. Whilst Holstein in BSR, Holstein in MR, Anatolian Black Cattle, and East Anatolian Red were closely clustered together, the Grey breed was clustered separately from the main cluster. Jersey was most distant from all other breeds. Ozbeyaz et al.[33] identified three main clusters in their cluster analysis; South Anatolian Red and Jersey breeds formed two distant clusters and a third cluster between these two clusters was comprised of Brown Swiss, Holstein, East Anatolian Red, Anatolian Black Cattle, and Grey Steppe populations, similar to the findings in the present study.

With this study, the intention was to reveal the genetic diversity in terms of the *STAT1* and *STAT5A* genes and the genetic relationships among the breeds in the populations of two different dairy breeds; Holstein from Black Sea and Marmara Regions and Jersey, as well as of Anatolian Black Cattle, East Anatolian Red, and Grey Steppe out of other native cattle breeds. According to the results obtained about genetic variation among the breeds in terms of *STAT* gene families in this study, it is possible to make genetic progress for these breeds raised in Turkey based on animal selection methods like the Marker Assistant Selection (MAS). But it seems reasonable to continue study applying haplotype analysis by using various polymorphic regions, especially in *STAT5A* gene before using them in Turkish dairy selection programs extensively.

Conflicts of Interest

The authors declare no conflict of interest.

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