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RESEARCH ARTICLE

The Comparison of Polymorphisms in the Heat Shock Transcription Factor 1 Gene of Turkish Grey Cattle and Holstein Cattle

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ABSTRACT

Turkish Grey cattle (*B. taurus*) are the only native cattle breed of Türkiye's Thrace and Western Anatolia regions. They can spend the whole year as free herds, including the hottest and coldest months, and can survive, feed, and reproduce without any human intervention. Therefore, Turkish Grey cattle are of interest for genetic marker studies on heat stress tolerance. This study was aimed to identify genetic polymorphism in the DNA binding domain (DBD) encoding region of the heat shock transcription factor 1 (HSF1) gene in Holstein (n: 50) and Turkish Grey (n: 50) cattle. It was determined that the 121 amino acid long sequence encoded by the first three exons of the HSF1 gene was the same in Turkish Grey and Holstein cattle. Two intronic and one synonymous SNPs were identified in Turkish Grey (rs719296338, rs522980029, rs17880386) and one inronic SNP (rs17870386) in Holstein cattle by DNA sequencing. These SNPs were searched in the Bovine Genome Variation Database (BGVD). Interestingly, according to BGVD, rs522980029 was conserved in *B. indicus* but absent in *B. taurus*. Moreover, the rs719296338 locus is monomorphic in western European and Eurasian taurine but is polymorphic in East Asian taurines and *B. indicus*. The results support the view that *B. indicus* is genetic introgression into *B. taurus* cattle in Anatolia. This hybridization may have contributed to Turkish Grey cattle ability to adapt to harsh environmental conditions and heat stress.

Keywords: Genetic introgression, Grey cattle, Heat stress

INTRODUCTION

Global warming is a major concern for the livestock industry worldwide [1]. High ambient temperatures and relative humidity cause heat stress by limiting the capacity of livestock to dissipate heat into their surrounding environment [2]. Decreased feed intake due to heat stress in all livestock species leads to a decrease in egg, milk and meat production [3,4]. Heat stress related economic loss is high in the dairy industry. Moreover, heat stress reduces the immune response against diseases in cattle [5]. Intensive selection has been applied for many years to increase the productivity of livestock. However, these high yielding breeds may be more susceptible to heat stress as they produce higher metabolic heat [6]. The development of a high-yielding breed of cattle that can tolerate heat stress has been an important goal of the researchers, as extreme temperatures are predicted to occur more frequently [7]. Heat stress also reduces of reproductive success, animal welfare and product quality. Heat stress causes a decrease in the content of milk fat and protein and changes in the meat colour and water-holding capacity [8-10].

Cattle breeds show genetic variation in thermotolerance, some being more tolerant than others [11]. This diversity may be due to many different biological mechanisms such as cellular, morphological (coat structure), behavioral and neuroendocrine systems. However, the molecular mechanism of environmental adaptation has not been fully elucidated today [12]. The genes involved in mammalian coping with heat stress have been investigated for decades and many candidate genes have been reported [13-15]. The main biomarker of heat stress in cattle is heat shock proteins (HSP). HSPs are highly conserved protein superfamily that are activated in many different stress situations such as oxidative stress, heat stress, injury, etc. HSPs are important for the maintenance of protein homeostasis. HSPs increase the ability of the cell to survive by reducing the accumulation of damaged or abnormal polypeptides within cells [6]. They restore misfolded proteins, direct irreparable proteins to the degradation pathway, and prevent protein aggregation and apoptosis [16].

HSF1, the major coordinator in the regulation of the heat stress response (HSR), plays a role in physiological processes such as metabolism, development and aging in non-stress situations [17]. HSR is also triggered by different stress factors such as infectious diseases, heavy metals, oxidative stress, in addition to elevated temperature [11,18,19]. HSF1 induces the transcription of heat shock proteins (HSP), also known as stress proteins, by binding to heat shock elements (HSE). HSE is specific DNA sequences (nGAAn) found in the promoter regions of HSPs [20]. HSF1 basically consists of four functional domains: trimerization domain, DNA binding domain (DBD), regulatory domain, and transcriptional activation domain [21]. HSF1, which is a monomeric conformation (inactive form) in the cytoplasm in the absence of stress, trimerizes under stress and migrates from the cytoplasm to the nucleus. Trimeric HSF1 has the ability to bind to DNA. However, it can upregulate HSP transcription after undergoing phosphorylation [22]. DBD plays a key role in HSF1 recognizing and binding to HSEs [23]. It has been reported that amino acid substitutions in DBD may cause physicochemical changes that alter the ability of HSF1 to bind to HSEs [24].

The HSF1 gene is located on chromosome 14 in cattle. It contains 13 exons and encodes the HSF1 protein contains 515 amino acids. The DBD is the best conserved domain of the HSF1 protein in cattle. The first three exons of the HSF1 gene encode the 106 amino acid long DBD between amino acids 15 to 120 of the HSF1 protein [24].

Native cattle breeds are an important target for genetic marker research. Unlike cultivated breeds that go through rigorous breeding programs, they can carry more genetic variations associated with survival and adaptation to different environmental conditions. Turkish Grey cattle are the only native cattle breed of Türkiye's Thrace and Western Anatolia regions. This breed can spend the whole year as free herds, including the hottest and coldest months, and can survive, feed, and reproduce without any human intervention [25]. Turkish Grey cattle are highly resistant to heat, cold and parasites and can be fed low quality feeds. It is preferred for meat and milk production in rural areas due to its very low breeding costs [25]. Holstein cattle are very vulnerable to heat stress and parasites [26]. Identification of genetic variations that confer superiority in stress tolerance in Turkish Grey cattle may provide the opportunity to apply them in breeding strategies such as marker assisted selection for cattle production systems. This study was designed to identify variations in the DNA loci encoding the DBD of the HSF1 protein in Turkish Grey and Holstein cattle.

MATERIAL AND METHODS

Sample Collection

Tissue samples were collected from 100 animals in two different breeds of *B. taurus*; Holstein cattle (n = 50) and Turkish Grey cattle (n=50). Holstein and Turkish Grey cattle tissue samples were collected from randomly selected carcasses after slaughter in the Thrace region of Türkiye. The muscle tissue samples were obtained from the neck of each carcass. Because the samples were collected on different dates, cattle samples from different farms were included in the study. The samples were stored at -20°C until molecular genetic studies.

Molecular Genetics Analysis

DNA extraction from muscle and blood tissue samples was performed using the Hybrigen (MG-DHDNA-01) DNA extraction kit. The two primer pairs were designed

Table 1. *Primer sequences, amplification regions and PCR fragment lengths*

Primers Name	Primer Sequences	Amplification Region	PCR Product (bp)
Primer E1	F: GTCGAACAACGCCCTCCAA	633178 - 633196	458
	R: CTTTGCGGTCGCTACCTCCT	633635 - 633616	
Primer E23	F: ACAGGCACCTGGTAGAAAGC	618336 - 618355	718
	R: TGAAAACTGTCACCCAGCCT	619053 - 619034	

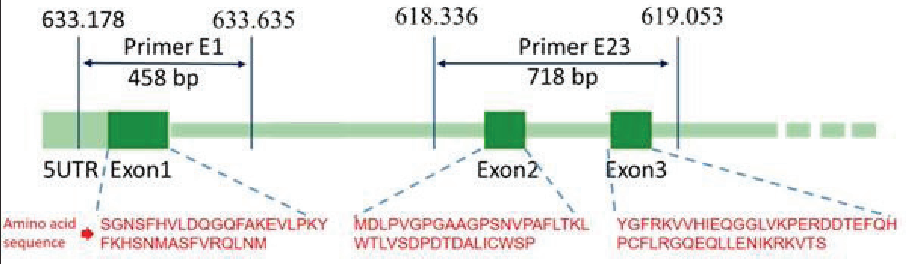

Fig 1. The loci amplified (458 bp and 718 bp) by the primer pairs on the cattle chromosome 14. The encoded amino acid sequences first three exons of the cattle HFS1 gene are written in red letters

using NCBI primer blast (*Table 1*). The primer pair E1 amplifies exon 1 and partially the 5' UTR and intron 1 regions. The primer pair E23 amplifies exon 2, intron 2, exon 3 and partially intron 1 and intron 3 regions. The HSF1 gene regions amplified by the primers are shown in *Fig. 1*.

PCR amplifications were performed by Proflex thermal cycler (Applied Biosystem). The PCR analyses were performed in a total volume mixture of 25 µL consisting of 12.5 µL of PCR mastermix (K0171, Thermo Scientific), 5 µL of gDNA, 1 µL (10 pmol) of each primer and 5.5 µL of nuclease free water. Sequencing reactions were carried out using a DTCS Quick Start sequencing kit and GenomeLab GeXP Genetic Analysis System (Beckman Coulter, USA). The DNA sequences were analyzed by BioEdit v7.2.5 (Hall, 1999) and Chromas v2.6.6 (Technelysium Pty Ltd, ASTL).

Statistical Analysis

The frequencies of SNPs in cattle breeds worldwide were examined using the Bovine Genome Variation Database (BGVD) [27]. The BDGV includes genomic variation data (SNP, indel, CNV) for 432 samples from 54 modern cattle breeds worldwide. Furthermore, the 54 cattle breeds can be grouped into six early differentiated ancestral populations (Indian indicine, Chinise indicine, East Asian taurine, European taurine, Eurasian taurine, Africa taurine). Distribution patterns of these variations can be obtained using dbSNP ID. Hardy Weinberg equilibrium (HWE) was tested using the HardyWeinberg v1.6.3 package [28] in the R platform.

RESULTS

The exon 1, 2 and 3 loci were amplified in 100 cattle samples using the designed primer pairs (*Table 1*). The DNA sequencing results were aligned and assembled with v7.2.5 (Hall, 1999). It was determined that the 121 amino acid long sequence encoded by the first three exons of the HSF1 gene was the same in Turkish Grey and Holstein cattle (*Fig. 1*). However, three SNPs were detected in intron 1 (rs719296338), exon 2 (rs522980029) and intron 3 (rs17880386) (*Fig. 2*). While rs7192966338 A>G and rs522980029 C>T were detected in Turkish Grey cattle, they were not observed in Holstein cattle. The rs17880386 A>G was identified in both breeds (*Table 2*). All SNP frequencies were in Hardy Weinberg equilibrium (HWE) in Turkish Grey and Holstein cattle (*Table 2*).

The three SNPs (rs719296338, rs522980029, rs17880386) were screened in BGVD and map plots were generated for each SNP (*Fig. 3*). Allele frequencies were obtained for each SNP according to the ancestral populations. The rs719296338 A>G was highly conserved in *B. indicus* but absent in European taurine, Eurasian taurine and African taurine. The frequency of the G allele was 0.014 in East Asian taurine (*Fig. 3-A*). rs522980029 C>T was absent in *B. taurus* but conserved in *B. indicus*. The T allele frequency was 0.091 in the Indian indicine and 0.684 in the Chinese indicine (*Fig. 3-B*). The rs17880386 A>G locus was polymorphic in both *B. taurus* and *B. indicus* according to the BGVD database. The G allele frequency was higher in African taurine, Chinese indicine and Indian indicine, and the A allele frequency was higher in other *B. taurus* (*Fig. 3-C*).

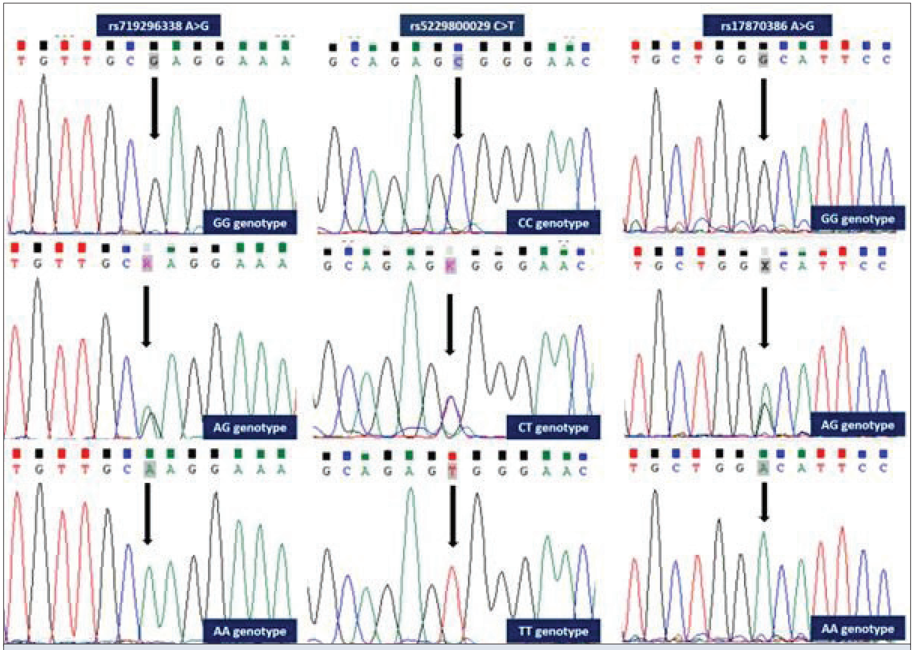

Fig 2. Electropherogram of DNA sequencing results of three SNPs identified in cattle HSF1 gene

Table 2. *Allele and genotype frequencies of three SNPs identified in HSF1 gene in Turkish Grey and Holstein cattle*

SNP/Breed	**Genotype Frequencies**			**Alleles Frequencies**		**HWE P value**
rs719296338	AA	AG	GG	A	G	
Turkish Grey	0.580	0.320	0.010	0.740	0.260	0.234
Holstein	1.000	0.000	0.000	1.000	0.000	-
rs522980029	CC	CT	TT	C	T	
Turkish Grey	0.620	0.340	0.040	0.790	0.210	0.861
Holstein	1.000	0.000	0.000	1.000	0.000	-
rs17870386	AA	AG	GG	A	G	
Turkish Grey	0.240	0.580	0.180	0.530	0.470	0.246
Holstein	0.780	0.220	0.000	0.890	0.110	0.382

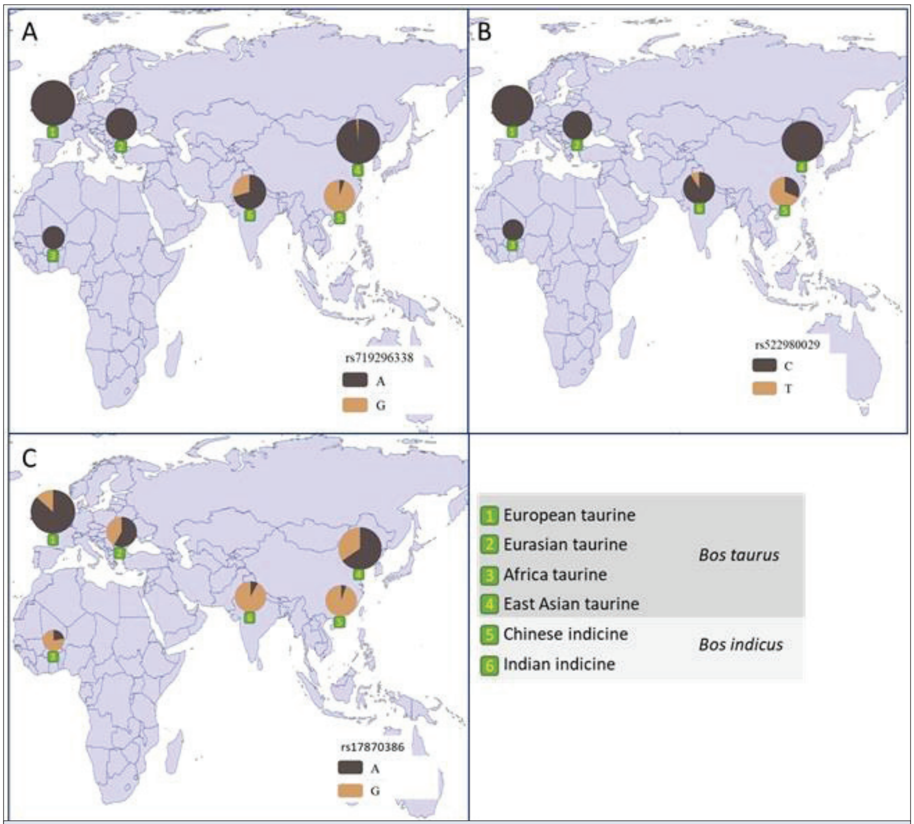

Fig 3. Allele frequency distribution of rs719296338 (A), rs522980029 (B) and rs17870386 (C) in six ancestral cattle groups according to Bovine Genome Variation Database and Selective Signatures (BGVD)

DISCUSSION

Anatolian native breeds, relatives of the first domesticated cattle, are considered the ancestors of many European breeds [29]. One of them, Turkish Grey cattle, also known as Plevne breed in Türkiye, originated from the *B. taurus primigenius* [30]. Although Turkish Grey cattle was a preferred breed by farmers in Thrace and Western Anatolia in the last century, unfortunately, it is under threat of extinction recently. Turkish Grey breeds show high tolerance to cold and heat and high resistance to ecto- and endoparasites [29,31]. They are preferred by poor farmers due to their low breeding costs in the Marmara region in Türkiye [32]. Due to their very high adaptive ability to survive under harsh environmental conditions [33], they are an interesting target for the identification of genetic variants associated with thermotolerance.

Thermotolerance in cattle is a quantitative trait under the influence of multiple genes [34]. Susceptibility to heat stress varies according to genetic potential, species, breed, nutritional status, life stage and body size. Dairy cows are more vulnerable to heat stress than beef cattle. *B. indicus* breeds and their crosses are better adapted to high environmental temperatures than *B. taurus* [1,35,36]. Traditional breeding methods to improve thermotolerance in cattle have not achieved the expected success. Therefore, genetic marker assisted selection methods can be used to increase the efficiency of traditional selection methods [37].

Maintaining protein homeostasis is essential for the survival of eukaryotic cells. Under stress conditions, proteostasis is dependent on HSR, a cytoprotective mechanism. HSR is triggered by HSF1 upregulating the transcription of a group of chaperones. These chaperones called HSPs, support cell survival by preventing the agglutination of misfolded or defective proteins. HSF1 upregulates the transcription of HSPs by binding to specific sequences called HSEs in their promoter region. The fact that HSF1 expression is higher in cattle in summer than in winter and autumn [38] indicates its relationship with heat stress. The SNPs in the HSF1gene have been reported to be related to heat tolerance in Chinese indigenous cattle [13], Chinese Holstein cattle [39,40] and Angus cattle [41].

In this study, the DBDs of HSF1 protein of two cattle breeds with different thermotolerance capacities were determined to have the same amino acid sequence *(Fig. 1)*. However, two intronic and one synonymous SNPs were detected at the analyzed loci. Three SNPs were observed in Turkish Grey cattle, which is known to have a higher adaptive ability, and one SNP was observed in Holstein cattle. Genetic diversity provides important information about both the evolutionary past and future of a species [42]. Homozygosity increased (decreased genetic diversity) in the Holstein population as a result of intensive selection and inbreeding. Homozygosity is generally detrimental to populations [13,43] and may have increased vulnerability to changing environmental conditions in Holstein cattle.

The synonymous SNP rs522980029 C>T in exon 2 of the HFS1 gene is located at position 618.972 on chromosome 14. It causes an AGC-AGT substitution at the codon 40 encoding the serine residue of the HSF1 protein. All three genotypes were identified in Turkish Grey cattle and the genotypic frequencies of CC, CT, and TT were 0.620, 0.340, and 0.040, respectively. The C>T locus was monomorphic for Holstein cattle, only the CC genotype was observed *(Table 2)*. Interestingly, based on the BGDB distribution models, the variation rs522980029 C>T was absent in *B. taurus* but conserved in *B. indicus*. T allele frequency was 0.091 in the Indian indicine and 0.684 in the Chinese indicine *(Fig. 3-B)*.

The rs719296338 A>G in the intron 1 in the HSF1 gene is located at position 633,222 on chromosome 14. Two alleles and three genotypes (AA, AG, GG) were observed for the rs719296338 in Turkish Grey cattle. Homozygote AA (0.580) had the greatest frequency in these genotypes, and homozygote GG (0.010) had the lowest frequency. The A allele frequency was determined as 0.740 and the G allele frequency as 0.260. The rs719296338 A>G locus was monomorphic for Holstein cattle, only the AA genotype was observed *(Table 2)*. The rs719296338 A>G was screened in the BGVD database. It was highly conserved in *B. indicus* but absent in European taurine, Eurasian taurine and African taurine. According to BGDB, the frequency of the G allele was 0.295 in Indian indicine, 0.947 in Chinese indicine, and 0.014 in East Asian taurine *(Fig. 3-A)*.

The rs17870386 A>G in the intron 2 in the HSF1 gene is located at position 618.721 on chromosome 14. This variation was observed in Turkish Grey and Holstein cattle. While AA (0.240), AG (0.580), GG (0.180) genotypes were determined in Turkish Grey cattle, GG genotype was not determined in Holsteins cattle. The genotypic frequencies of AA and AG in Holstein cattle were 0780 and 0.220, respectively *(Table 2)*. This locus was polymorphic in both *B. taurus* and *B. indicus* according to the BGVD database. The G allele frequency was higher in African taurine, Chinese indicine and Indian indicine, and the A allele frequency was higher in other *B. taurus (Fig. 3-C)*.

These results suggest that there may be a relationship between Turkish Grey cattle and *B. indicus*. Similar results were found in previous studies examining casein gene polymorphisms, and it was reported that genetic introgression of *B. indicus* into Turkish Grey cattle [31,44]. Anatolia, one of the centers where cattle were first domesticated, is accepted as a center of significant introgression with *B. indicus* [45,46]. It has been suggested that Mesopotamia suffered from prolonged drought as a result of significant climatic changes around 4000-3000 years before present [46,47]. It is thought that ancient herders may have brought arid-adapted *B. indicus* populations into the Near East at this time [48].

In order to provide food for the growing world population, it is necessary to have cattle breeds that can cope with the negative effects of global warming. In terms of genetic and physiological adaptability, native cattle breeds are superior to cultivated cattle breeds [26,49]. The study of cattle breeds that respond differently to heat stress can provide very important information to elucidate the molecular mechanisms of adaptation in highly adaptable cattle breeds [50]. SNPs can alter the gene expression transcriptionally and posttranscriptionally and missense SNPs may affect the functionality and stability of proteins [24]. SNPs may cause disruption in the program of biochemical adaptive responses [13,41].

Identification of SNPs in genes involved in thermoregulation may provide important data for marker assisted selection. The genetic variations found in the Turkish Grey cattle, which has high adaptability to harsh environmental conditions, can have the potential to be used as molecular marker candidates in future studies.

Availability of Data and Materials

Datasets analyzed during the current study are available in the corresponding author (S. Atalay) on reasonable request.

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Conflict of Interest

The authors declare that have no conflict of interest.

Ethica Approval

Ethical approval is not required for this study

Author Contributions

SA and SK designed the study. SA performed the laboratory analysis and wrote the paper. SK reviewed and revised the paper. All authors have read and agreed to the published version of the paper.

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