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Genetic diversity of the 3' and 5' untranslated regions of the *HSP70.1* gene between native Turkish and Holstein Friesian cattle breeds

Y. Öner[1,#] A. Keskin[2,3], H. Üstüner[4], D. Soysal[5] & V. Karakaş[6]

[1] Department of Animal Science, Biometry and Genetics, Faculty of Agriculture, University of Uludag, TR-16059 Bursa, Turkey
[2] Uludag University, Faculty of Veterinary Medicine, Obstetrics and Gynaecology Department, 16059, Görükle, Bursa, Turkey
[3] Kyrgyz Turkish Manas University, Faculty of Veterinary Medicine, Obstetrics and Gynaecology Department Bishkek, Kyrgyzstan
[4] Department of Zootechnics, Faculty of Veterinary Medicine, Uludag University 16059 Nilufer, Bursa, Turkey
[5] Sheep Research Institute, Bandırma, Turkey
[6] International Centre for Livestock Research and Training (ICLRT), Ankara, Turkey

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Abstract

Heat stress proteins are important factors in protecting cells against environmental stress. The *HSP70.1* gene is one of the most important members of the heat stress protein family, which is essential for life, production and reproduction. In this study, partial regions of *HSP70.1* (3' and 5' untranslated regions (UTRs)) were sequenced in six cattle breeds. Blood samples of five native breeds, namely Yerli Kara (YK), Boz ırk (BI), Yerli Güney Sarısı (YGS), Güney Doğu Anadolu Kırmızısı (GAK) and Doğu Anadolu Kırmızısı (DAK) were collected from their native regions and blood samples of the Holstein Friesian (Siyah Alaca (SA)) breed were collected from each of these regions. Totals of 249 and 206 animals were analysed for the *HSP70.1*- 3' and 5' UTR regions, respectively. In the 3' UTR region, 13 single nucleotide polymorphisms (SNPs) and one indel were found, whereas this region was found to be monomorphic among animals of the Holstein Friesian breed. In the 5' UTR region, 43 SNPs and three indels were revealed in all of the investigated breeds. On the other hand, a new C983T nucleotide substitution was identified in this region, and is thought to disrupt the Sp1-hsp70 promoter binding site. The 5' UTR region was also more variable in the Turkish native breeds than in the Holstein Friesian. This study is the first to investigate the 3' and 5' UTRs of the *HSP70.1* gene in Turkish native breeds. The genetic structure of these gene regions in Turkish native cattle breeds was found to be quite different from those of other cattle breeds that had been studied in the past.

Keywords: Bovine, heat shock genes, heat stress, polymorphism
[#] Corresponding author: yaseminoner@yahoo.com

Introduction

Global warming affects climate change negatively, and has become a threat to food supply by affecting agricultural production systems adversely (Roush, 1994). For this reason, in recent years there has been increasing concern about the thermal comfort of livestock. The restrictive effects of heat stress on reproduction and other productive traits in livestock have been proved (Ealy *et al*., 1993; West, 2003). Various thermal tolerance levels of *Bos indicus* and *Bos taurus* indicate the importance of genotypic differences for thermal tolerance (Skinner & Louw, 1966; Ealy *et al*., 1985). Selecting thermotolerant animals when designing a breeding scheme may be more sustainable and less expensive than improving environmental and management conditions (Mader *et al*., 2006).

Heat shock proteins (HSPs), a group of proteins conserved in both prokaryotic and eukaryotic organisms, play a vital role in cell response to environmental stress (Lindquis, 1986; Morimoto *et al*., 1994). The HSP 70.1 protein is coded by the *HSP70.1* gene, also known as *HSPA1A* or *HSPA1*, which is located on 23q13 in the bovine genome (Anonymous, 2017). This protein is present under normal and stress conditions (Christians *et al*.,

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1997). Previous studies carried out in early embryonic and gametic stages have shown that the expression of *HSP* genes affects follicular development, embryonic survival, and pregnancy maintenance (Britt, 1992; Sağırkaya *et al*., 2006; Wilkerson & Sarge, 2009). The expression of *HSP* genes is related to thermal stress. For this reason, the functional characterization of these genes is important. In addition to this functional importance, close proximity to the major histocompatibility complex genes highlights the *HSP70.1* gene as a powerful candidate marker for health, reproduction and productive traits (Wurst *et al*., 1989). For these reasons, studies of polymorphisms in this gene and resultant phenotypic traits in both livestock and human beings have increased (Wu *et al*., 2004; Basiricó *et al*., 2011; Sodhi *et al*., 2013; Xiong *et al*., 2013).

Untranslated regions, in addition to coded regions, have attracted attention because of their importance in terms of expression level and stability. While the 5' UTR may affect the expression level of the transcript, the 3' UTR is thought to affect mRNA stability (Basiricó *et al*., 2011; Sodhi *et al*., 2013). Various studies have been carried out to detect mutations in these gene regions, and interest in the relationships between these mutations and reproductive traits has grown (Grosz *et al*., 1994; Schwerin *et al*., 2003; Adamowicz *et al*., 2005; Banks, 2007a; Rosenkrans *et al*., 2010; Basiricó *et al*., 2011; Sodhi *et al*., 2013; Xiong *et al*., 2013; Deb *et al*., 2013). It was also reported that mutations may occur at promoter regions of *HSP70.1* and may negatively affect spermatogenesis, embryonic mortality, and pregnancy (Rivera & Hansen, 2001; Hansen *et al*., 2001; Huang *et al*., 2002). Implementing the selection of thermotolerant animals in production systems would be important for both the agricultural economy and animal welfare. For sustainable livestock farming, native breeds should be characterized for the gene regions related to thermal adaptation. In this study, the 3' and 5' UTRs of *HSP70.1* were partially characterized in five Turkish native cattle breeds and the genetic structures of these regions were compared with those of the Holstein cattle breed, which is widely used in animal production systems in Turkey.

Materials and Methods

Blood samples were collected from five Turkish native cattle breeds in their original regions, namely YGS, BI, GAK, DAK and YK. Samples from the Holstein Friesian (SA) breed were collected from each geographical region from which the native breeds were sampled (Figure 1).

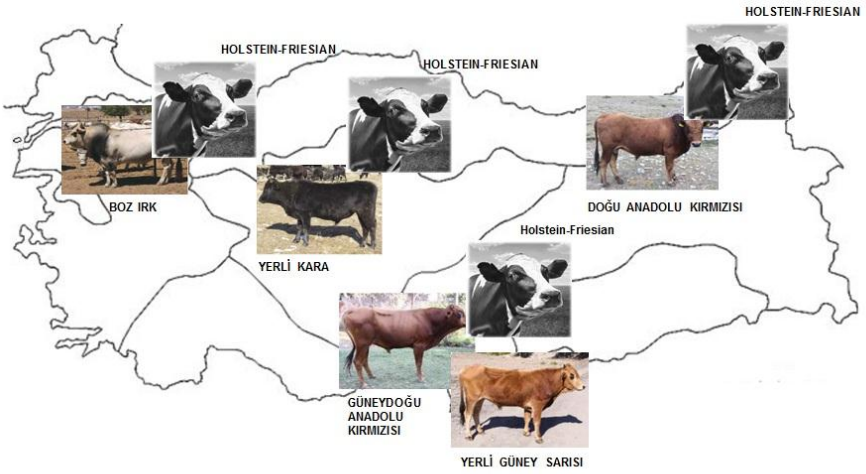

Figure 1 Distribution of breeds by geographical region in Turkey (Yerli Güney Sarısı (YGS), Boz Irk (BI), Güney Doğu Anadolu Kırmızısı (GAK), Doğu Anadolu Kırmızısı (DAK), Yerli Kara (YK), Holstein Friesian (Siyah Alaca (SA))

The distribution of animals sampled and analysed from each breed for each genomic regions is presented in Table 1.

Table 1 Distribution of animals sampled from five native Turkish and Holstein Friesian breeds for investigated 3' and 5' untranslated regions of the *HSP70.1* gene

Breeds	Gene regions	
	3' UTR	5' UTR
	n	
YGS	42	34
BI	46	32
GAK	37	37
DAK	36	37
YK	38	36
Native Breeds Total	199	176
SA	50	30
Total	249	206

UTR: Untranslated region; YGS: Yerli Güney Sarısı; BI: Boz Irk; GAK: Güney Doğu Anadolu Kırmızısı; DAK: Doğu Anadolu Kırmızısı; YK: Yerli Kara; SA: Holstein Friesian (Siyah Alaca)

For DNA isolation, 10-ml blood samples were obtained from the coccygeal vein of each animal. Total DNA was extracted with a genomic DNA extraction kit (NucleoSpin Blood, Macherey-Nagel GmbH & Co. KG) according to the instructions provided in the manual. The conditions used for PCR were as described by Grosz *et al*. (1994) and Starkey *et al*. (2007). Sequences of the primers that were utilized are presented in Table 2. The resulting PCR fragments were purified with a Macherey-Nagel purification kit (NucleoSpin PCR Clean-up, GmbH&Co., KG) and sequenced using the same primers that were used for the PCR reactions. Purified PCR products were sequenced using an automated genetic analyser ABI3130XL (Applied Biosystems, Calif, USA).

Table 2 Primers used for Polymerase Chain Reactions and DNA sequencing

Regions	Primer sequences (5' → 3')	Annealing temperature (°C)	Product size (bp)	Literature
HSP70-3'UTR	F: GGATTGCTCATGTTTGTTATGG R: CTTGGAAGTAAACAGAAACGGG	51	253	Grosz *et al*. (1994)
HSP70-5'UTR	F: GCCAGGAAACCAGAGACAGA R: CCTACGCAGGAGTAGGTGGT	55	539	Starkey *et al.* (2007)

The sequences were aligned with sequences through the National Centre for Biotechnology Information (NCBI) database (GenBank accession numbers AY626950.1 and M98823.1 for the 3' and 5' UTR, respectively). The sequences were arranged with the BioEdit program and aligned with CLUSTALW (http://ebi.ac.uk/clustalw) software. Potential promoter binding regions were identified with Proscan software (http://www-bimas.cit.nih.gov/molbio/proscan/).

Diversity parameters such as sequence variation index, nucleotide diversity, and linkage disequilibrium (LD) were calculated with DNAsp (DNA sequence polymorphism software) 5.1 (Librado & Rozas, 2009).

The chi-square test ($\chi 2$) was used to determine whether the populations were in Hardy-Weinberg equilibrium. The genotype and allele frequencies were calculated with the PopGene32 program (Yeh *et al*., 1997). All SNPs detected in the 5' UTR region in all the native breeds and the Holstein Friesian breed were used in estimating genetic diversity. A dendrogram was constructed with unweighted paired group cluster analysis

(UPGMA), a modified neighbour procedure implemented in PHYLIP version 3.5 software and PopGene32 (Nei, 1972).

Results

Sequencing of 253 bp and 539 bp fragments of the *HSP70.1*-3' UTR and *HSP70.1*-5' UTR (Figure 2) showed substantial variability in both regions. A total of 13 SNPs and one indel were identified in the *HSP70.1*-3' UTR (Table 3), while the Holstein Friesian breed was found to be monomorphic. Six of these SNPs (A101G, C176T, A202G, T209C, A210G, and A215G) were transitions and the others were transversions (G63T, T110A, T167A, T174A, T184G, and T226A). These SNPs and their minor allele frequencies were listed in Table 3. The indel was found only in one GAK animal. On the other hand, three alleles were detected at the 184th position. All of the detected mutations were observed at low frequencies (Table 3). Only A101G was observed in all of the breeds. Strong LD was observed between T110A and T174A (R = 0.813), T167A and T174A (R = 0.769), and T174A and T209C (R = 0.742).

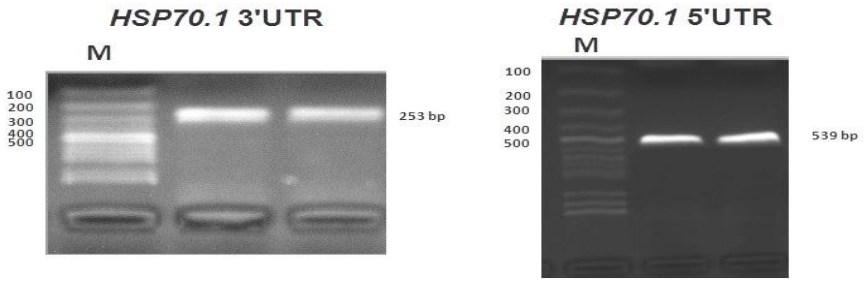

Figure 2 Images of Polymerase Chain Reaction products from the HSP70.1- 3' and 5' untranslated regions on an agarose gel. (M: 100 bp DNA ladder (NEB, N3231S))

Table 3 Distribution of minor allele frequencies within the investigated *HSP70.1*-3' untranslated regions according to breeds

SNPs	Minor allele frequencies					
	YGS	BI	DAK	GAK	YK	Whole native breeds
G63T	-	-	-	-	0.079	0.012
A101G	0.060	0.021	0.069	0.014	0.026	0.030
T110A	0.024	0.033	-	0.014	-	0.024
T167A	0.036	0.110	-	0.014	0.013	0.030
T174G	0.024	0.065	-	0.014	-	0.018
C176T	0.036	-	0.042	0.014	-	0.014
T184G	0.024	-	-	-	-	0.004
T184A	-	-	-	0.014	-	0.002
A202G	-	-	0.014	-	-	0.002
T209C	0.024	0.032	-	-	-	0.010
A210G	-	0.011	0.014	-		0.004
A215G	0.083	0.065	0.083	0.027		0.030
T226A	-	-	-	0.083	0.066	0.026

SNPs: Single nucleotide polymorphisms; YGS: Yerli Güney Sarısı; BI: Boz Irk; GAK: Güney Doğu Anadolu Kırmızısı; DAK: Doğu Anadolu Kırmızısı; YK: Yerli Kara; SA: Holstein Friesian (Siyah Alaca)

While two animals from the YGS breed carried the T→G base substitution at this position, a T→A transversion was observed only in one animal from the YK breed (Table 3). The nucleotide diversity (Pi) and the haplotype diversity were found to be 0.00207 and 0.345, respectively (Table 4). The highest nucleotide diversity (Pi) was observed in the YGS breed.

Table 4 Nucleotide diversity values of the *HSP70.1*-3' and 5'- untranslated regions for YGS, BI, GAK, DAK, YK and SA breeds.

Breeds	3' UTR	5' UTR
	Pi	
YGS	0.00254	0.00546
BI	0.00251	0.00500
GAK	0.00145	0.00691
DAK	0.00188	0.00640
YK	0.00159	0.00575
Native breeds	0.00207	0.00615
SA	0.00000	0.00528

UTR: Untranslated region; Pi: Nucleotide diversity; YGS: Yerli Güney Sarısı; BI: Boz Irk; GAK: Güney Doğu Anadolu Kırmızısı; DAK: Doğu Anadolu Kırmızısı; YK: Yerli Kara; SA: Holstein Friesian (Siyah Alaca)

The *HSP70.1*-5' UTR was found to be more variable than the *HSP70.1*-3' UTR; 43 SNPs and three indels were observed in this region (Table 11). The majority of the loci were not in Hardy-Weinberg equilibrium. Frequencies, expected (He) and observed heterozygosities (Ho), and $\chi 2$ values for SNPs observed among all native breeds are provided in Tables 5, 6, 7, 8, 9, and 10. Of the loci, only A949C and C983G in the YGS, BI, DAK and SA breeds, A949C and A1117C in YGS, and A870C, C983G and G1045A in the YK were at Hardy-Weinberg equilibrium (Tables 5, 6, 7, 8, 9, 10). The highest Pi was found in the GAK (Table 4). The C852T and G1107A nucleotide substitutions were observed only in the Holstein Friesian (Table 11).

Table 5 Expected and observed heterozygosities, allele and genotype frequencies and Chi square values for Single Nucleotide Polymorphisms detected in all native breeds for the Yerli Güney Sarısı population

Locı	Yerli Güney Sarısı (YGS)																		
	Allele frequencies (%)					Genotype frequencies (%)											Ho	He	χ^2
	A	C	G	-	T	AA	AC	CC	C-	--	GG	GC	GA	GT	TT	TC			
A870C	0.9559	0.0441				94.2	2.94	2.94									0.0294	0.0856	21.661538**
Indel895		0.8382		0.1618				79.41	8.82	11.77							0.0882	0.2752	17.056015**
A949C	0.9118	0.0882				82.35	17.65										0.1765	0.1633	0.261766^{ns}
C983G		0.9706	0.0294					94.2				5.8					0.0588	0.0579	0.015385^{ns}
G1045A	0.2059		0.7941			14.71					73.53		11.76				0.1176	0.3319	15.087336**
G1117A	0.1912		0.8088			17.65					79.41		2.94				0.0294	0.3139	29.907692**
A1125C	0.8382	0.1618				82.35	2.86	14.71									0.0294	0.2752	29.473684**
G1128T			0.8676		0.1324						85.29			2.94	11.76		0.0294	0.2331	28.836158**
T1134C		0.1618			0.8382			14.71							85.29	2.94	0.0294	0.2752	29.473684**
T1204C		0.4118			0.5882			41.18							58.82		0.0000	0.4917	35.099715**

- C deletion at position 895
- - Homozygote phenotype for C deletion at position 895
Ho: Observed heterozygosities; He: Expected heterozygosities

Table 6 Expected and observed heterozygosities, allele and genotype frequencies and Chi square values for Single Nucleotide Polymorphisms detected in all native breeds for the Boz Irk population

Locı	Boz Irk (BI)																	
	Allele frequencies (%)					Genotype frequencies (%)										Ho	He	χ^2
		C	G	-	T	AA	AC	CC	C-	--	GG	GC	GA	GT	TT	TC			
A870C	0.9688	0.0312				96.88		3.12									0.0000	0.0615	63.016393**
indel895		0.7812		0.2188				68.75	18.75	1.25							0.1875	0.3472	7.208352**
A949C	0.2188	0.0312				93.75	6.25										0.0625	0.0615	0.01639^{ns}
C983G		0.9531	0.0469					90.63				9.37					0.0938	0.0908	0.050820^{ns}
G1045A	0.2500		0.7500			21.88					71.87		6.25				0.0625	0.3810	23.598338**
G1117A	0.2500		0.7500			18.75					68.75		1.25				0.1250	0.3810	15.244415**
A1125C	0.8906	0.1094				87.5	3.13	9.37									0.0312	0.1979	26.105263**
G1128T			0.8750		0.1250						84.38			6.25	9.37		0.0625	0.2222	18.635227**
T1134C		0.2344			0.7656			18.75							71.87	9.38	0.0938	0.3646	18.710714**
T1204C		0.3594			0.6406			34.37							3.13	62.5	0.0312	0.4678	28.928950**

- C deletion at position 895
- -Homozygote phenotype for C deletion at position 895
Ho: Observed heterozygosities; He: Expected heterozygosities

Table 7 Expected and observed heterozygosities, allele and genotype frequencies and Chi square values for Single Nucleotide Polymorphisms detected in all native breeds for the Güney Doğu Anadolu Kırmızısı population

LOCI	Güneydoğu Anadolu Kırmızısı (GAK)																		
	Allele frequencies (%)					Genotype frequencies (%)											Ho	He	χ^2
	A	C	G	-	T	AA	AC	CC	C-	--	GG	GC	GA	GT	TT	TC			
A870C	0.9054	0.0946				86.49	8.11	5.40									0.0811	0.1736	12.114751**
indel895		0.7838		0.2162				72.97	10.81	16.22							0.1081	0.3436	18.352813
A949C	0.9595	0.0405				92.90	8.10										0.0811	0.0789	0.043461^{ns}
C983G		0.7432	0.2568					48.65				51.35					0.5135	0.3869	4.145455**
G1045A	0.1351		0.8649			5.40					78.38		16.22				0.1622	0.2369	4.048016**
G1117A	0.0946		0.9054			2.70					83.79		13.51				0.1351	0.1736	2.096530^{ns}
A1125C	0.7162	0.2838				70.27	2.70	27.03									0.0270	0.4121	33.638814
G1128T			0.6892		0.3108						64.86			8.11	27.03		0.0811	0.4343	25.392681**
T1134C		0.1216			0.8784			5.40							81.09	13.51	0.1351	0.2166	5.817308**
T1204C		0.4189			0.5811			40.54							56.76	2.70	0.0270	0.4935	34.028507**

- C deletion at position 895
- -Homozygote phenotype for C deletion at position 895
Ho: Observed heterozygosities; He: Expected heterozygosities

Table 8 Expected and observed heterozygosities, allele and genotype frequencies and Chi square values for Single Nucleotide Polymorphisms detected in all native breeds for the Doğu Anadolu Kırmızısı population

LOCI	Doğu Anadolu Kırmızısı(DAK)																	
	Allele frequencies (%)					Genotype frequencies (%)										Ho	He	χ^2
	A	C	G	-	T	AA	AC	CC	C-	--	GG	GC	GA	GT	TT	TC			
A870C	0.9730	0.0270				97.30		2.70									0.0000	0.0533	73.014085**
indel895		0.8243		0.1757				78.38	8.11	13.51							0.0811	0.2936	20.774023**
A949C	0.8243	0.1757				64.86	35.14										0.3514	0.2936	1.534426^{ns}
C983G		0.9730	0.0270					94.60				5.40					0.0541	0.0533	0.014085^{ns}
G1045A	0.0946		0.9054			8.11					2.70		89.19				0.0270	0.1736	30.396588**
G1117A	0.0811		0.9189			8.11					91.9						0.0000	0.1511	43.844766**
A1125C	0.6622	0.3378				59.46	13.51	27.03									0.1351	0.4535	18.867347**
G1128T			0.6216		0.3784						51.40			21.60	27.00		0.2162	0.4769	11.401334**
T1134C		0.0270			0.9730			2.70							97.30		0.0000	0.0533	73.014085**
T1204C		0.4054			0.5946			40.54							60.46		0.0000	0.4887	38.107458**

- C deletion at position 895
- -Homozygote phenotype for C deletion at position 895
Ho: Observed heterozygosities; He: Expected heterozygosities

Table 9 Expected and observed heterozygosities, allele and genotype frequencies and Chi square values for Single Nucleotide Polymorphisms detected in all native breeds for the Yerli Kara population

LOCI	Yerli Kara (YK)																		
	Allele frequencies (%)					Genotype frequencies (%)											Ho	He	χ^2
	A	C	G	-	T	AA	AC	CC	C-	--	GG	GC	GA	GT	TT	TC			
A870C	0.9861	0.0139				97.22	2.78										0.0278	0.0278	0.000000^{ns}
indel895		0.8472		0.1528				80.56	8.33	11.11							0.0833	0.2625	18.235817**
A949C	0.6250	0.3750				0.25	0.75										0.7500	0.4754	12.409091**
C983G		0.9861	0.0139					97.22				2.78					0.0278	0.0278	0.000000^{ns}
G1045A	0.0972		0.9028								80.56		19.44				0.1944	0.1780	0.353365^{ns}
G1117A	0.0972		0.9028			2.78					83.33		13.89				0.1389	0.1780	2.003205^{ns}
A1125C	0.8611	0.1389				86.11		13.89									0.0000	0.2426	39.526412**
G1128T			0.7917		0.2083						75.00			8.33	16.67		0.0833	0.3345	21.520677**
T1134C		0.0694			0.9306										86.11	13.89	0.1389	0.1311	0.158299^{ns}
T1204C		0.2917			0.7083			27.78							69.44	2.78	0.0278	0.4190	32.679739**

- C deletion at position 895
- -Homozygote phenotype for C deletion at position 895
Ho: Observed heterozygosities; He: Expected heterozygosities

Table 10 Expected and observed heterozygosities, allele and genotype frequencies and Chi square values for Single Nucleotide Polymorphisms detected in all native breeds for the Siyah Alaca population

LOCI	Siyah Alaca (SA)																		
	Allele frequencies (%)					Genotype frequencies (%)											Ho	He	χ^2
	A	C	G	-	T	AA	AC	CC	C-	--	GG	GC	GA	GT	TT	TC			
A870C																			
indel895		0.5167		0.4833				46.67	0.10	43.33							0.1000	0.5079	20.018587**
A949C	0.9000	0.1000				0.80	0.20										0.2000	0.1831	0.303983^{ns}
C983G		0.8000	0.2000					0.60				0.40					0.4000	0.3254	1.696809^{ns}
G1045A																			
G1117A																			
A1125C	0.4500	0.5500				36.67	16.66	46.67									0.1667	0.5034	13.895990**
G1128T			0.4500		0.5500						36.67			16.66	46.67		0.1667	0.5034	13.895990**
T1134C																			
T1204C		0.4833			0.5167			46.67							0.50	3.33	0.0333	0.5079	27.096774**

- C deletion at position 895
- -Homozygote phenotype for C deletion at position 895
Ho: Observed heterozygosities; He: Expected heterozygosities

Table 11 Distribution of minor allele frequencies within the *HSP70.1*-5' untranslated region according to breed investigated

	Minor allele frequencies						
SNPs	**YGS**	**BI**	**DAK**	**GAK**	**YK**	**SA**	**Whole native breeds**
C794T	0.0294	-	-	-	0.0277	-	0.0101
C852T	-	-	-	-	-	0.0500	-
A866C	-	-	0.0270	-	-	-	0.0050
A870C	0.0441	0.0313	0.0946	0.0270	0.0139	-	0.0370
A888C	-	0.0156	-	0.0135	-	0.0333	0.0060
DEL(C)895	0.1618	0.2031	0.1892	0.1757	0.1528	0.4833	0.1407
A896C	0.0147	-	0.0270	-	-	-	0.0050
G915C	-	0.0156	0.0811	-	-	0.0333	0.0060
A949C	0.0882	0.0313	0.4054	0.1891	0.3750	0.1000	0.1457
G954A	-	-	-	0.0135	-	-	0.0030
C983G	0.0294	0.0469	0.2568	0.0270	0.0139	0.2000	0.6784
G997A	-	-	-		0.0277	-	0.0050
A1008C	0.0441	-	-	0.1757	0.0972	-	0.0578
G1013A	-	0.0156	-	0.0270	0.0139	-	0.0101
G1016C	0.0441	-	-	0.1757	0.0972	0.4333	0.0578
T1021G	0.0294	-	-	-	0.0417	-	0.0126
G1041A	-	0.0313	-	-	-	-	0.0050
G1043A	-		-	-	0.0139	-	0.0025
G1045A	0.2059	0.2344	0.1351	0.0946	0.0972	-	0.1307
T1047G	-	-	-	0.1081	-	-	0.0201
A1051G	-	-	-	0.1081	-	-	0.0201
A1058G	0.0147	-	-	-	-	-	0.0025
G1060A	0.0441	-	-	-	-		0.0075
G1066A	-	0.0938	-	0.0270	-	-	0.0351
G1076A	-	0.0313	0.0270	0.0405	0.0417	-	0.0251
C1086A	0.0294	-	-	-	0.0277		0.0101
G1089A	-	-	-	0.0270	-	-	0.0050
INS(C)1090	0.0294	-	-	-	0.0277	-	0.0101
C1092T	0.0294	-	-	-	-	-	0.0050
A1096G	-	-	0.0405	0.0135	0.0139	0.1000	0.0126
G1107A	-	-	-	-	-	0.0333	-
G1117A	0.1912	0.2500	0.1081	0.0811	0.0972	-	0.1256
A1125C	0.1618	0.1094	0.2838	0.3378	0.1389	0.5500	0.2688
G1128T	0.1324	0.1094	0.2973	0.3784	0.1944	0.5500	0.2010
A1132G	-	0.0156	-	-	-	-	0.0025
T1134C	0.1618	0.2343	0.1216	0.0270	0.1389	-	0.1181
C1152T	0.0441	-	-	-	-	-	0.0075
T1140G	-	-	-		0.0277	-	0.0050
C1154G	-	-	0.0270	0.0135	0.0139	-	0.0100
G1164T	0.0294	-	-		0.4167	-	0.0126
C1166G	-	-	0.0270	0.1351	-	-	0.0075
T1193C	-	-	-	0.0270	-	-	0.0050
DEL(T)1194	0.0294	-	-	0.0541	-	-	0.0101
C1204T	0.4118	0.3594	0.4324	0.4054	0.3387	0.4833	0.3367
A1246G	-	-	0.0811		0.0556	-	0.0251
C1255T	-	-	-	-	0.0277	-	0.0050

The database results showed that one of the newly identified SNPs (C983G) was located within the SpI-hsp70 (1) promoter binding region (Figure 3).

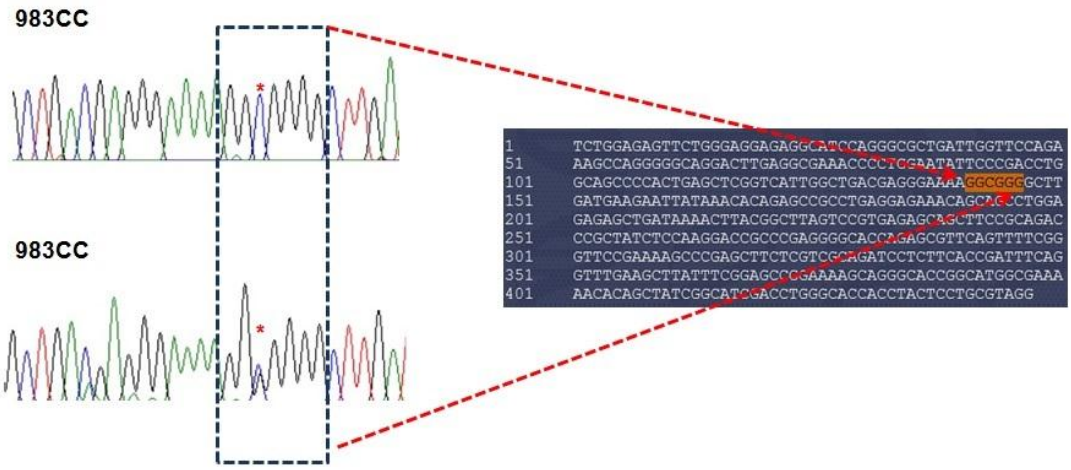

Figure 3 Putative SpI-HSP70 (1) promoter binding region

According to the test, strong LD was observed between the G1045A and G1117A (R = 0.784), A1125C and G1128T (R = 0.861) loci in all of the investigated breeds.

The constructed UPGMA cluster could not be distinguished in the breeds according to geographical region, whereas the Holstein Friesian breed was clustered separately from all the native breeds (Figure 4).

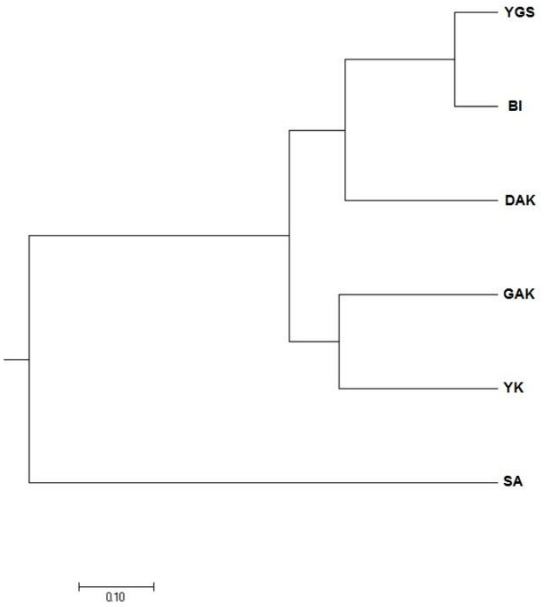

Figure 4 UPGMA (unweighted pair group method with arithmetic mean) dendrogram constructed by using Single Nucleotide Polymorphisms detected at the *HSP70.1*-5' untranslated region.

Discussion

Sequencing of the two fragments of the *HSP70.1*-3' UTR and *HSP70.1*-5' UTR revealed that these regions are more variable among Turkish native cattle breeds than other cattle breeds that have previously been investigated (Grosz *et al*., 1994; Adamowicz *et al*., 2005; Basirió *et al*., 2011; Sodhi *et al*., 2013). In the less polymorphic region *HSP70.1*-3'UTR, 13 SNPs and one indel were detected (Table 3). Previously, only two SNPs had been reported in European cattle breeds at a low frequency (Grosz *et al*., 1994; Adamowicz *et al*., 2005), while the Holstein Friesian population investigated here was found to be monomorphic. The G63T

base substitution reported previously (Adamowicz *et al*., 2005) was detected only in three animals from the YK breed in a homozygous fashion (Table 3). The additional SNPs and the indel are reported in this work for first time. Although more SNPs were found in the present study, all of them were at a low frequency. Only the SNP at the 110th nucleotide was detected in all of the native cattle breeds (Table 3). Two SNPs were reported at nucleotides 63 and 2154 of the *HSP70.1*-3' UTR in the European origin cattle breeds at low frequencies by Grosz *et al*., (1994) and Adamowicz *et al*., (2005). On the other hand, Basirió *et al*., (2011) and Sodhi *et al*., (2013) found that the 253 bp region of the *HSP70.1*-3' UTR was monomorphic in both the *Bos taurus* and *Bos indicus* cattle breeds. These results are concordant with findings in the current study. It is clear that the *HSP70.1*-3' UTR was less variable than the *HSP70.1*-5' UTR.

Xiong *et al*. (2013) investigated a different region of the *HSP70.1*-3' UTR and reported SNPs at base positions 3494, 6400, 6600, and 6601. Of these SNPs, three were found to be ligated and to influence thermal tolerance in the Chinese Holstein-Friesian breed.

The results of the current study show that the *HSP70.1*-3' UTR and *HSP70.1*-5' UTR are both more polymorphic in Turkish native cattle breeds than the Holstein Friesian. According to the sequence analysis, SNPs previously detected in a 539 bp region of the *HSP70.1*-5' UTR, including G1117A, A1125C, T1134C, G1045A, C1154G, and T1204C, and the indels C895 and G1128T (Banks, 2007a; Rosenkrans *et al*., 2010; Basiricó *et al*., 2011; Deb *et a*l., 2013), were observed in the Turkish native cattle breed except for the A1069G base substitution and the insertion at nucleotide 1112 (Banks, 2007b). Together with these previously reported SNPs, a total of 43 SNPs and 3 indels were found in the Turkish native cattle breeds in the *HSP 70.1*- 5' UTR. Thirty-three SNPs and two indels were reported for the first time in the present study.

The most thoroughly investigated mutation was the C deletion at base 895 of the detected region (Schwerin *et al*., 2003, Banks, 2007a; Rosenkrans *et al*., 2010; Basiricó *et al*., 2011; Deb *et al*., 2013). As previously reported (Schiwerin *et al*., 2003), the C deletion had possible negative effects on pregnancy and calving rate. Thermotolerance and expression levels were also detected in all of the breeds. This deletion is located within AP2 box transcription binding site and may result in decreased transcription binding capacity (Schwerin *et al*., 2002). It was found to affect pregnancy and calving rates negatively, as well as thermal tolerance parameters that depend on the mRNA level (Banks, 2007a; Rosenkrans *et al*., 2010; Deb *et al*., 2013). Only Basiricó *et al*. (2011) reported high cell survival and thermaltolerance level in animals carrying the C deletion. In this study, this deletion was found in all of the breeds. Surprisingly, the deletion frequency in the native breeds was half of that found in the Holstein Friesian.

Accordingly, the LD between A1125C and G1128T was found to be as reported by Rosenkrans *et al*. (2010). The positive effects of the A and G alleles on the calving and pregnancy rates for 1125 and 1128, respectively, were reported (Banks, 2007a).

The SNPs T1134C, G1045A, C1154G, and T1204C are related to the serum concentration of T3 and *IGF-I* and body condition (Banks, 2007b; Rosenkrans *et al*., 2010). Of these mutations, only T1204C was observed in all the breeds investigated in the current study.

One of the newly identified SNPs, a C→G transversion at nucleotide 983, was located in the putative SpI-*hsp70* (1) promoter binding site (Figure 3). It is known that the expression of the *HSP70.1* gene begins in an early embryonic stage. A previous study in mice showed that *HSP70.1* gene expression at this stage is dependent on the SpI transcription factor (Fiorenza *et al*., 2004). Although this new SNP was detected in all of the breeds, it occurred in the Holstein Friesian and DAK breeds at a relatively higher frequency. This frequency and the absence of homozygote carrier animals of the DAK breed, which is reared in the coldest climate, suggest the functional importance of this SNP.

Although the UPGMA dendrogram, which was constructed with SNPs for all the breeds, did not distinguish the native breeds according to geographical location, the Holstein Friesian breed was clustered separately from the native breeds.

Conclusion and Recommendation

This study shows that the 3' and 5' UTRs of *HSP70.1* among Turkish native breeds differ genetically from the majority of investigated cattle breeds worldwide. These regions were found to be much more variable than previously reported. Owing to the lack of phenotypic data, the functional importance of the mutation could not be evaluated deeply. The relationship between SNPs that occur at a moderate frequency and stress tolerance parameters should be investigated. The functional role of the newly identified SNP (C983G), located at the putative SpI-*hsp70* (1) promoter binding region, needs to be determined to produce biological proof of embryonic survival. Additionally, genomic characterization and biological proof of function of variable regions in the other HSP genes should be performed.

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Authors' Contributions

YÖ was the principal investigator of the project and conducted all steps of the study, including study design, laboratory processes, and statistical analysis. She was also responsible for drafting and submitting the manuscript. AK, HÜ, DS and VK carried out field studies to obtain blood samples from these cattle breeds.

Conflict of Interest Declaration

We certify that there is no actual or potential conflict of interest in relation to this article.

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