Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2022, Vol. 6 (4), 401-409
https://doi.org/10.29329/ijiaar.2022.506.10

Original article

Determination of ATP1A1 Gene Polymorphism in the Turkish Holstein Cattle

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Abstract

Heat stress is an important factor negatively affecting the productive characteristics, immune response and reproductive performance of livestock. Sustainable livestock systems that can tolerate the impact of increasing environmental temperature are very important to ensure global food security. Oxidative stress triggered by heat stress influences plasma Na and K levels in cattle. The ATP1A1 gene encodes the α1 isoform that forms the transmembrane subunit of the NA,K ATPase enzyme. The α subunit plays a major role in maintaining sodium-potassium homeostasis in all animal cells. The aim of the study was to determine ATP1A1 gene polymorphisms in Turkish Holstein cattle. The target regions (intron 17 and exon 18) were amplified and sequenced in 50 Turkish Holstein cattle. Multiple alignments revealed three SNP. rs109703332 A>G and rs110455455 C>T were detected in intron 17 and a synonymous SNP rs110256520 C>A in exon 18. It was observed that the three SNPs were in strong linkage disequilibrium (LD) with each other and therefore had the same genotype and allele frequencies. The three SNPs were found to be highly linked in one haplotype block. This haplotype block consisted of 2 haplotypes (CCA and ATG). The frequency of the CCA haplotype was 0.860 and the ATG was 0.140. Individuals of Holstein cattle tolerate heat stress to different levels. This difference between individuals may be due to variations in the genes involved in the adaptation mechanism. Therefore, it is important to identify polymorphisms in genes involved in the heat stress tolerance mechanism. In conclusion, in this study, the three SNPs and the two haplotypes were determined on the ATP1A1 gene in Turkish Holsteins cattle.

Keywords: ATP1A1, Heat Stress, Cattle, Polymorphism.

Received: 01 September 2022 * **Accepted:** 02 December 2022 * **DOI:** https://doi.org/10.29329/ijiaar.2022.506.10

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INTRODUCTION

Multiple biotic and abiotic factors can cause stress by disturbing animal homeostasis (Sejian, Maurya, & Naqvi, 2010). Heat stress is the most harmful abiotic stress factor for livestock and causes significant economic losses (Wankar, Singh, & Yadav, 2014). The gene expression profile changes when the cell is exposed to a stress factor such as oxidative stress, changes in external pH or elevated temperature. The expression of stress response genes associated with the maintenance of cell viability is upregulated, while genes associated with protein synthesis and cellular metabolism are down-regulated (Collier et al., 2006; De Nadal, Ammerer, & Posas, 2011; Rivera et al., 2021). Genes whose transcription is upregulated in stressed cells can be used as stress markers, and mutations in these genes can be associated with stress sensitivity (AL-Luhaibe & Al-Azzawi, 2020; Hooper et al., 2019; Ramendra et al., 2018).

Heat stress causes oxidative stress and changes sodium (NA) and potassium (K) concentrations (Altan, Pabuçcuoğlu, Altan, Konyalioğlu, & Bayraktar, 2003; Banerjee & Ashutosh, 2011; Bernabucci, Ronchi, Lacetera, & Nardone, 2002). NA, K ATPase (NKA) is an integral membrane enzyme that plays a major role in maintaining sodium-potassium homeostasis in all animal cells (Lingrel, Orlowski, Shull, & Price, 1990). It hydrolyzes ATP to pump K into the cell and Na out of the cell (Pierre & Xie, 2006). The NKA enzyme is functional as a heterotrimer composed of 3 different subunits (α, β, γ) (Blanco & Mercer, 1998). Among them, the α subunit is the largest and takes part in ATP hydrolysis (Elayadeth-Meethal et al., 2021). The α subunit has four isoforms (α1, α2, α3, α4), with the main isoform α1 (Geering, 2008). The ATP1A1 gene encodes the ATPase Na+/K+ Transport Subunit Alpha 1 (subunit α1) protein, which plays a major role in maintaining sodium-potassium homeostasis in all animal cells (Ramendra et al., 2018).

The cattle ATP1A1 gene is located on chromosome 3. The gene with 23 exons encodes the 1,021 amino acid length ATP1A1 protein. There are 8379 SNPs in the gene according to Ensembl (Chen et al., 2010). The ATP1A1 gene is associated with temperature tolerance, and the SNPs in the gene are genetic marker candidates for breeding programs (Elayadeth-Meethal et al., 2021; Imran, Khan, & Qureshi, 2021; Kashyap et al., 2015; Ramendra et al., 2018; Ramendra et al., 2017).

Holstein cattle known for their high milk yield are very sensitive to heat stress (Correa-Calderón, Avendaño-Reyes, López-Baca, & Macías-Cruz, 2022; Gantner et al., 2017). Intensive selection to increase the milk yield has given Holstein cattle significant genetic gains associated with milk production (Gara et al., 2012). However, the reduction of genetic diversity in Holsteins as a result of intensive selection and inbreeding (Stachowicz, Sargolzaei, Miglior, & Schenkel, 2011) may have made them more vulnerable to harsh environmental conditions. The identification of genetic variations associated with thermotolerance in Holsteins is important for marker assisted breeding programs. The aim of this study is to determine the ATP1A1 gene variations in Turkish Holsteins.

MATERIALS and METHODS

Holstein cattle tissue samples were collected from carcasses after slaughter. Approximately 10 g of tissue samples were taken from the neck of 50 randomly selected Turkish Holstein cattle in the Thrace region of Turkey. Tissues were stored at -18 □C until molecular genetic studies. The DNA extraction was carried out by the phenol:chloroform:isoamyl alcohol method as described by Sambrook et al. (2006).

The primer pair (Table 1) that amplifications the entire exon 18, intron 17 and part of exon 17, intron 18 were designed using the NCBI primer blast tool. PCR reactions were performed on Proflex thermal cycler (Applied Biosystem) using the PCR master mix (K0171, Thermo Scientific). The PCR protocol and PCR mix content were shown in Table 2. After the PCR products were visualized in agarose gel electrophoresis, they were purified by the PEG precipitation method. The sequence reaction was carried out with the DTCS Quick Start kit (Beckman Coulter). The fragments were sequenced on the GenomeLab GeXP genetic analysis system (Beckman Coulter).

Table 3. The detailed information of primers

Primer sequence	Primer binding region	Amplification product
F: TCAGAAACCCTGTCTGAGGC	26891654 - 26891673	804 bp
R: GTGAGAGTGCTGCGTGAAAC	26892477 - 26892458	

Table 4. The PCR mix contain and PCR temperature protocol

PCR mix contain	PCR Protocol		
12.5 µL PCR master mix	95℃	3 s	
1 µL (10 pmol) each primer	95 ℃	30 s	35 cycle
5 µL template DNA	60 ℃	40 s	
5.5 µL nuclease free water	72 ℃	60 s	
25 µL total volume	72 ℃	5 mn	

Hardy Weinberg equilibrium (HWE) was calculated using the HardyWeinberg v.1.7.5 package (Graffelman & Graffelman, 2022) in R software. Linkage disequilibrium (LD) and haplotype analysis were carried out using Haploview software (Barrett, Fry, Maller, & Daly, 2005).

RESULTS

The targeted gene region was successfully PCR amplified (Figure 1) and sequenced for each sample (n=50). Multiple alignments using BioEdit revealed three SNP (rs109703332 A>G, rs110455455 C>T and rs110256520 C>A). Two SNPs (rs109703332 A>G and rs110455455 C>T) were detected in

intron 17 and a synonymous SNP (rs110256520 C>A) in exon 18 (Figure 2). The synonymous SNP causes the ATC>ATA substitution at codon 849, which encodes the isoleucine amino acid.

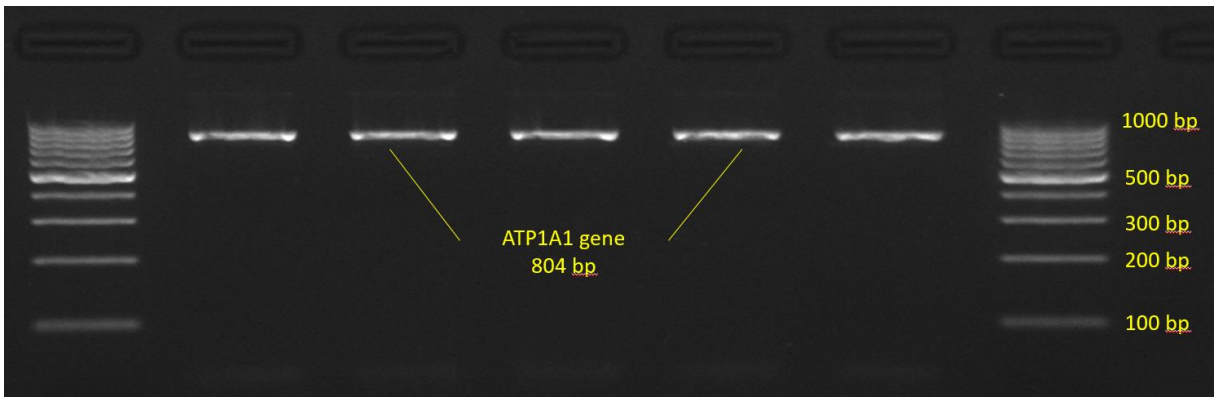

Figure 5. Agarose gel electrophoresis for amplified ATP1A1 gene

Genotype, allele frequency and HWE chi-square test were calculated for each SNP (Table 3). For the rs110256520, genotypes AA, AC and CC were detected with frequencies of 0.040, 0.200 and 0.760 respectively. The C allele frequency was found to be 0.860. Three genotypes were observed AA, AG and GG for rs109703332 with frequencies of 0,760, 0,200 and 0.040 respectively. The A allele frequency was found to be 0.860. For the rs110455455, genotypes CC, CT and TT were detected with frequencies of 0,760, 0,200 and 0.040 respectively. The C allele frequency was found to be 0.860.

It was observed that the three SNPs were in strong LD with each other and therefore had the same genotype and allele frequencies. The three SNPs were found to be highly linked in one haplotype block with D′= 1 using the Haploview (Barrett et al., 2005) (Figure 3). This haplotype block consisted of 2 haplotypes (CCA and ATG). The frequency of the CCA haplotype was 0.860 and the ATG was 0.140 (Table 3).

Table 5. The genotype, allele, and haplotype frequencies of the polymorphisms in the ATP1A1 gene

Polymorphisms	**Genotype frequencies**			**Allele frequencies**		**HW p-value**
rs110256520	CC 0.760 n=38	AC 0.200 n=10	AA 0.040 n=2	C 0.860	A 0.140	0.230
rs110455455	CC 0.760 n=38	CT 0.200 n=10	TT 0.040 n=2	C 0.860	T 0.140	0.230
rs109703332	AA 0.760 n=38	AG 0.200 n=10	GG 0.040 n=2	A 0.860	G 0.140	0.230
Haplotypes	Haplotype frequencies					
CCA	0.860					
ATG	0.140					

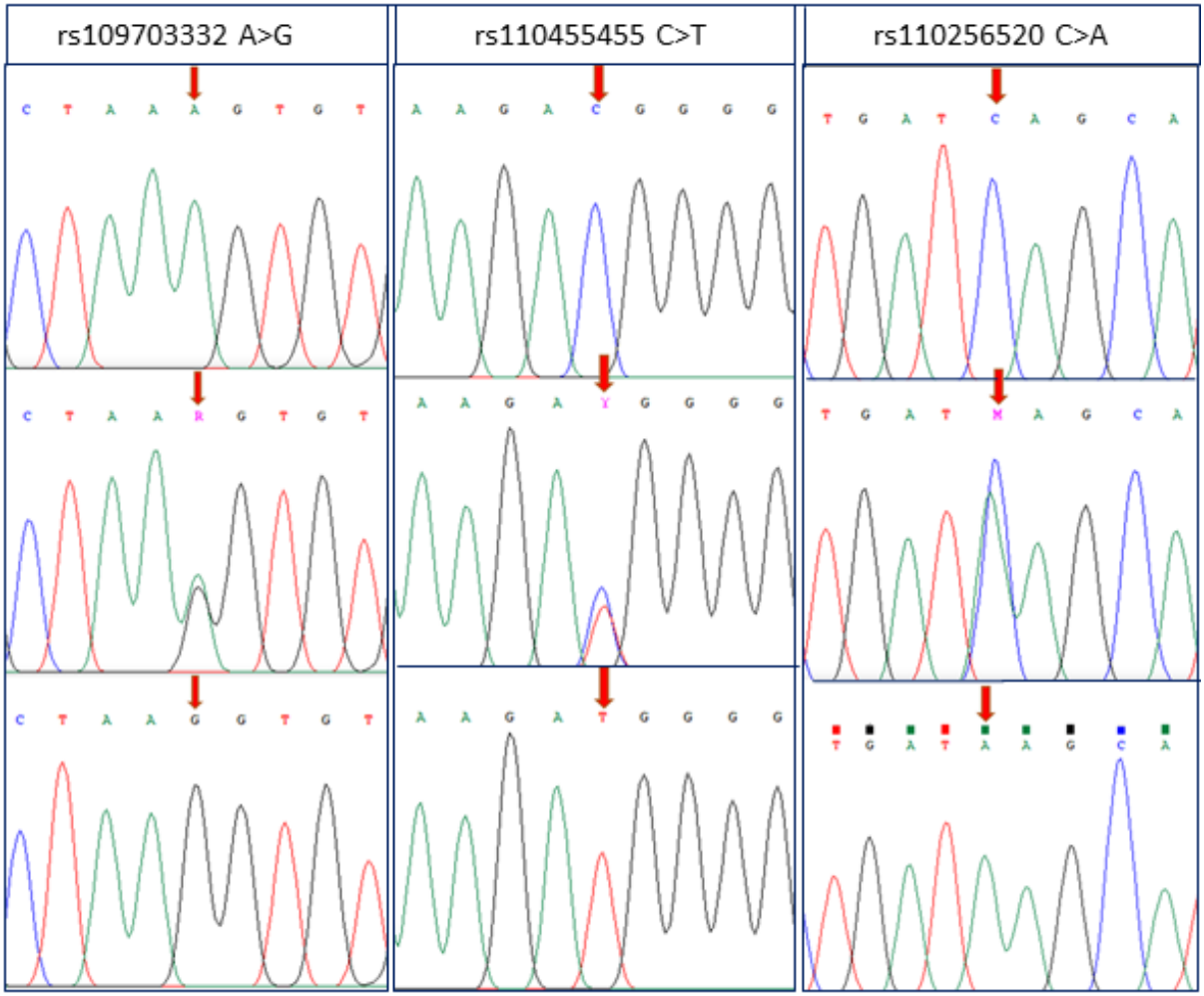

Figure 2. The sequencing chromatograms show that three genotypes for rs109703332, rs110455455 and rs110256520

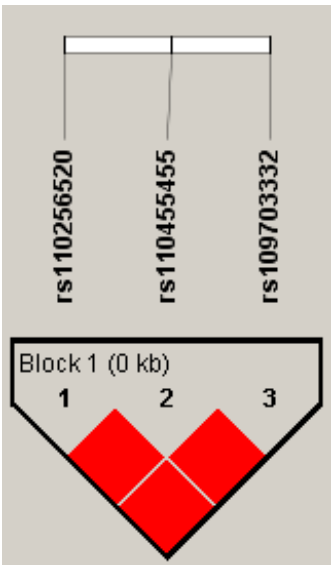

Figure 3. Linkage disequilibrium graphic of three single nucleotide polymorphisms in ATP1A1 gene.

DISCUSSION

The P-type ATPase family consists of integral membrane transport enzymes that use the energy of ATP hydrolysis to transport ions across the membrane (Bublitz, Morth, & Nissen, 2011). Despite having a simple structure with only one catalytic subunit, they are of vital importance in all kingdoms of life (Bublitz et al., 2011; Palmgren & Nissen, 2011). The NKA enzyme, the member of the p-type ATPase family, is responsible for establishing the electrochemical gradient of Na+ and K+ ions across the plasma membrane in animals. The ATP1A1 subunit of the NKA enzyme harbours the binding and catalytic site for Na+, K+ and ATP hydrolysis (da Silva, Gonçalves-de-Albuquerque, de Moraes, Garcia, & Burth, 2021).

Global warming is a major threat to livestock production (Palmgren & Nissen, 2011). Heat stress produces oxidative stress that results in plasma electrolyte imbalance and reduced productivity in cattle. Heat shock proteins (HSP), also known as stress proteins, are primarily responsible for the heat stress response. However, the expression of ATP1A1, which is not considered HSP, also increases under stress. ATP1A1 gene expression is altered in cattle under heat stress conditions (Kashyap et al., 2015). It has been reported in previous studies that ATP1A1 gene expression is affected in livestock by environmental temperatures (Kaushik, Goel, & Rout, 2019; Pires et al., 2021). Moreover, Pires et al. (2021) determined that ATP1A1 expression was influenced by environmental climatic in Bos taurus and Bos inducus cattle.

Yang et al. (2021) found that two SNPs located in the exon 15 (rs110420888) and exon 18 (rs110256520 C>A) regions of the ATP1A1 gene were associated with the percentage of milk protein in Chinese Holstein. They reported that the rs110256520 AC genotype was associated with a higher protein percentage than the AA genotype. rs110256520 was also associated with heat stress in Holstein (Yanxin Liu, Li, Li, Zhou, & Wang, 2011) and Tharparker and Vrindavani cattle (Kashyap et al., 2015). In these studies, it was determined that animals with CC genotype tolerated significantly high levels of heat stress compared to AC and AA genotypes. In another study (YX Liu, Xu, Gao, & Sun, 2012), the rs110256520 CC genotype was associated with a significantly lower somatic cell score, and researchers suggested that the C allele could be used as a marker for mastitis resistance. On the other hand, Ramendra et al. (2015) reported that the rs110256520 AA genotype was superior for heat tolerance in Jersey crossbreds relative to other genotypes. In the current study, the frequency of the C allele (0.86) for rs110256520 was higher than that of the A allele in Turkish Holstein. This result is consistent with the majority of previous studies (Kashyap et al., 2015; Yanxin Liu et al., 2011; YX Liu et al., 2012; Ramendra et al., 2015; Yang et al., 2021).

It has been suggested that 3 SNPs (T27008243C, A27008223G and T27008097A) in intron 17 of the ATP1A1 gene are associated with heat stress tolerance (Ramendra et al., 2018; Ramendra et al., 2017). Ramendra et al. (2018) reported that T27008097A (rs524366864) is significantly associated with

thermo tolerance traits in Sahiwal and Karan Fries cattle. They suggested that TA and AA genotypes enhanced heat tolerance. In another study, Ramendra et al. (2017) suggested that the TT genotype for T27008243C in Sahiwal cattle and the AA genotype for A27008223G (rs109703332) in Karan Fries cattle was associated with resistance to heat stress.

In this study, three SNPs were determined in the exon 18 and intron 17 regions of the ATP1A1 gene in Turkish Holstein cattle. It has been reported in previous studies that rs110256520 and rs10970332 are associated with tolerance to heat stress (Kashyap et al., 2015; Yanxin Liu et al., 2011; YX Liu et al., 2012; Ramendra et al., 2015; Yang et al., 2021(Ramendra et al., 2018; Ramendra et al., 2017). Allele and genotype frequencies determined in Turkish Holsteins for the two SNPs (rs110256520 and rs10970332) overlap with these studies. However, it has been reported that rs110455455 is not associated with heat stress in Sahiwal, Karan Fries and Jersey crossbred cattle. The CC genotype frequency was found to be 0.760 in Turkish Holsteins for rs110455455 (Table 2). However, Ramendra et al. (2018) reported that the CT genotype frequency was higher than other genotypes in Sahiwal (0.430), Karan Fires (0.480) and Jersey crossbreds (0.460).

In this study, three SNPs were observed to be in strong linkage disequilibrium (LD) with each other in Turkish Holsteins (Figure 3) and two haplotypes were determined (Table 2). The frequency of the CCA haplotype was 0.860 and the frequency of the ATG haplotype was 0.140. The common haplotype has two alleles associated with resistance to heat stress. These are the C allele from the rs110256520 and the A allele from the rs109703332. Individuals of Holstein cattle are known to tolerate heat stress at different levels (Vasconcelos et al., 2006). This difference between individuals may be due to variations in the genes involved in the adaptation mechanism. In conclusion, the ATP1A1 gene is polymorphic in Turkish Holsteins. Genetic variations play an important role in the adaptation of populations to different environments. It is thought that the ACC haplotype can be associated with resistance to heat stress.

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