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Araştırma
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Nonsynonymous Substitution Detected In Heat Stress-Associated HSPA8 gene in Artlı Sheep

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Abstract

Objective: Climate change lies at the core of the multifaceted global crises confronting humanity today. Sheep breeding plays a crucial role in ensuring the sustainability of global food security. Heat stress resulting from the increasing rise in global temperatures necessitates the genetic improvement of sheep breeds in terms of the genes that determine their resistance to this stress. The present study aimed to screen single-nucleotide polymorphisms (SNPs) located in the eighth exon of the HSPA8 gene, which is one of the important heat shock proteins, in Artlı and Çepni sheep populations using the Sanger sequencing method.

Material and Methods: The eighth exon region of the HSPA8 gene was sequenced, and SNPs within the obtained sequences were identified using the MEGA X software. The potential effects of the detected mutations on the protein structure were modeled using the HOPE software. In addition, to assess the accuracy of the predicted protein structure, confidence scores (pLDDT) were obtained using the AlphaFold2 software.

Results: Artlı sheep are traditionally known for their high resistance to low-temperature stress and harsh environmental conditions. In this study, a C>T substitution at the 210th nucleotide position of the 8th exon of the HSPA8 gene was detected in 50% of the analyzed Artlı sheep samples, resulting in the replacement of proline with leucine at amino acid position 605. Furthermore, 40% of the Artlı genotypes exhibited a G>A substitution at the 46th nucleotide position of the same exon, which was found to be heterozygous and caused a silent mutation at amino acid position 550. In contrast, all analyzed samples of the Çepni breed were found to be monomorphic with respect to the examined exon region.

Conclusion: The genetic basis of adaptation to climate change is shaped not only by mutations but also by other genetic processes, such as epistatic interactions between genes and epigenetic regulatory mechanisms. Rising temperatures resulting from global climate change have led to a decline in livestock productivity. Sustainable sheep breeding depends on evaluating local breeds, such as Artlı and Çepni, as potential breeding materials. Therefore, there is a need to conduct comprehensive genetic studies on these unique genotypes and to strengthen efforts aimed at conserving local breeds.

Keywords: Adaptation, climate change, genetic improvement, sheep, SNP.

Artlı koyununda sıcaklık stresiyle ilişkili HSPA8 geninde eşanlamlı olmayan baz değişimi tespit edildi

Öz

Amaç: İklim değişikliği, günümüzde insanlığın karşı karşıya olduğu çok boyutlu küresel krizlerin temelinde yer almaktadır. Koyun yetiştiriciliği, küresel gıda güvenliğinin sürdürülebilirliğinde kilit bir rol oynamaktadır. Artan küresel sıcaklık artışına bağlı oluşan sıcaklık stresi, koyun ırklarının bu strese karşı dayanıklılığını belirleyen genler açısından genetik olarak iyileştirilmesini zorunlu kılmaktadır. Bu çalışmada, Artlı ve Çepni koyun popülasyonlarında önemli ısı şoku proteinlerinden biri olan HSPA8 geninin sekizinci ekzonundaki tek nükleotid polimorfizmlerinin (SNP), Sanger dizileme yöntemi kullanılarak taranması amaçlanmıştır.

Materyal ve Yöntem: HSPA8 geninin sekizinci ekzon bölgesi dizilenmiş ve elde edilen dizilerdeki SNP'ler, MEGA X yazılımı kullanılarak belirlenmiştir. Tespit edilen mutasyonların protein yapısı üzerindeki potansiyel etkileri HOPE yazılımı ile modellenmiştir. Ayrıca, protein yapısının tahmin doğruluğunu değerlendirmek amacıyla AlphaFold2 yazılımı kullanılarak güvenilirlik değerleri (pLDDT) elde edilmiştir.

Araştırma Bulguları: Artlı koyunlarının, geleneksel olarak düşük sıcaklık stresine ve zorlu çevresel koşullara karşı yüksek direnç gösterdiği bilinmektedir. Analiz edilen Artlı koyun örneklerinin %50'sinde HSPA8 geninin 8. ekzonun 210. nükleotid pozisyonunda gerçekleşen C>T değişimi sonucunda 605. amino asit olan prolinin, lösin ile yer değiştirdiği tespit edilmiştir. Ayrıca Artlı genotiplerinin %40'ında, 8. ekzonun 46. nükleotid pozisyonunda heterozigot durumda bulunan ve 550. amino asit pozisyonunda sessiz mutasyona neden olan G>A değişimi gözlenmiştir. Buna karşın, Çepni ırkına ait örneklerin tamamının incelenen ekzon bölgesi bakımından monomorfik yapıda olduğu belirlenmiştir.

Sonuç: İklim değişikliğine adaptasyonun genetik temelini; mutasyonlarla birlikte genler arası epistatik etkileşimler ve epigenetik düzenleyici mekanizmalar gibi diğer genetik süreçler oluşturmaktadır. Küresel iklim değişikliğinin bir sonucu olarak ortaya çıkan sıcaklık artışları çiftlik hayvanlarında verimliliğin azalmasına neden olmaktadır. Sürdürülebilir koyun yetiştiriciliği, Artlı ve Çepni gibi yerel koyun ırklarının potansiyel ıslah materyali olarak değerlendirilmesine bağlıdır. Bu nedenle bu özgün genotipler üzerinde kapsamlı genetik araştırmaların yürütülmesine, yerel ırkların korunmasına yönelik çabaların da artırılmasına gereksinim bulunmaktadır.

Anahtar Kelimeler: Adaptasyon, genetik ıslah, iklim değişikliği, koyun, SNP

Introduction

The global food access crisis is one of the significant consequences of climate change, and it is known to cause many other economic and sociological effects, including irregular migration. This situation requires the characterisation and positioning of genetic variants adapted to this change, or at least have the potential for genetic adaptation, among farm animal species regarding sustainable agricultural production and food security.

Due to the relative change of climatic zones, the flexibility of genetic improvement programs requires high genetic diversity in adaptation-related genes. However, integrating variations in genes that are not fully defined, unrelated or have controversial associations with yield traits into genetic improvement programs is tricky. In this respect, functional gene variations should be examined in detail individually instead of using consensus reference genomes, and animals with the detected variant should be positioned wherever they are in the world for global livestock breeders.

Sheep have been a critical domestic animal in human life's economic, cultural and social aspects for thousands of years. Since their domestication, sheep have been intensively reared for meat, milk, wool, fur, leather and many by-products. Sheep are also raised in many parts of the world, especially in rural areas, as a primary source of income, affecting national and global economies. Sheep, which have very high adaptability, are also well known to be important in humanity's coping with the problems related to global climate change (FAO, 2024).

Due to the husbandry practices, sheep depend more on ecological conditions than many other farm animal species. Therefore, conserving adaptation to climate change, maintaining and increasing survivability and other productivity performances despite changing ecologic conditions are more critical for sheep farming. Thus, local breeds in each microclimate zone consist of genotypes that are well adapted to their environment despite their relatively low yield performances.

Global climate change will pose a significant adaptation problem for livestock, leading to low productivity and severe losses in national economies (Gujar, Tiwari, & Yadav, 2023; Lang et al., 2024). It is reported that heat stress negatively affects nutritional intake, growth and fertility in sheep also, and heat stress exposure during pregnancy causes deaths and diseases in newborn lambs (Luna-Nevarez et al., 2020).

Sheep breeding has a nomadic structure in the mountainous areas of the Black Sea region of Türkiye (Tozlu Çelik & Tüfekci, 2024). Artlı and Çepni sheep breeds, registered by the Turkish Ministry of Agriculture and Forestry in 2020, are well adapted to the ecological and breeding practices of the region (Mercan et al., 2022). Because the Artlı and Çepni are newly recognised sheep breeds by researchers, so

few molecular studies have been conducted on these breeds.

Heat shock proteins (HSP) are highly conserved proteins found in all organisms, and one of their functions is to protect the organism against extreme heat stress (Gujar et al., 2023). The *HSPA8* gene comprises nine exons and is located on chromosome 15 of the sheep. Exon 1 is non-coding, but the remaining eight exons comprise the entire HSPA8 protein, comprising 650 amino acids with a molecular weight of 71 kDa, and the expression of the *HSPA8* gene has increased in response to heat stress in sheep (Sunil Kumar, Magotra, Kumar, & Bangar, 2024).

The possible beneficial variants in adaptation genes of local breeds adapted to different environmental conditions may give livestock breeders an advantage in managing the climate change crisis. In this study, we aimed to investigate whether the HSPA8 gene contains a possible non-synonymous amino acid sequence variant in the Artlı and Çepni sheep breeds, which are among the sheep genetic resources registered newly, and if so, how the protein conformation may change.

Material and Methods

The genetic material used in this study consisted of previously isolated 23 DNA samples from the blood of registered Artlı (11 samples) and Çepni (12 samples) sheep breeder's flocks that were genetically unrelated (Miller, Dykes, & Polesky, 1988). The Artlı samples were coded as HA1 to HA11, while the Çepni samples were HÇ1 to HÇ12.

Primer3 software was used to design primers that amplify the eighth exon of the HSPA8 gene in PCR processes (Untergasser et al., 2012). Primer sequences, Tm temperature and proximate PCR product size, are given in Table 1.

Table 1 Primers used in the study

Primer	Primer sequence	T_m (℃)	Product size
8th Exon of *HSPA8*	F: CCCGCACCCTCAAGTTTCTA	57.81	336 bp
	R: TGGAGTTGCAGGGTAGTTGT	57.29	

For the PCR reaction, a total of 20 µL mixture was prepared to contain 4 µL of PCR Ready Mix (2X) (final concentration 0.4X), 0.5 µL each of F and R primers (10 µM) (final concentration 0.25 µM), 4 µL of template DNA (20 ng/µL) (final concentration 4 ng/µL), and 11 µL of nuclease-free water. PCR conditions were as follows: one initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec and extension at 72°C for 45 sec. The final elongation step was performed at 72°C for 10 min. PCR amplifications were checked and confirmed in horizontal electrophoresis at 80 V for 60 minutes, using 1% agarose gel and a UV transilluminator.

Sanger sequencing automation generally enables the formation of nucleic acid sequences with sizes between 800 and 1,000 bp (Crossley et al., 2020). Despite more advanced methods, due to its reliability, Sanger sequencing remains the gold standard in various applications (Al-Shuhaib & Hashim, 2023; Lehner & Miller, 2016). DNA sequencing was performed using ABI 3130 Genetic Analyzer (Applied Biosystems, USA) in the Black Sea Advanced Technology Research and Application Centre. The *HSPA8* gene sequence was retrieved from the NCBI database with the accession number XM_012095633.3.

The obtained DNA sequences were examined, and SNPs were detected with the MEGA X program. The SNP content of the DNA fragments was determined by alignment analysis using the Mega X program. Mega X has an extensive program memory to assemble sequence alignments, build evolutionary trees, estimate genetic distances and diversities, reveal ancestral sequences, calculate temporal relationships between sequences, and test genomic selections. Multiple sequence alignments can be generated with the obtained DNA sequence data using MEGA's native ClustalW application or the integrated MUSCLE alignment program (Sudhir Kumar, Stecher, Li, Knyaz, & Tamura, 2018). ClustalW uses a stepwise algorithm for multiple sequence alignment. This process begins by pairing the sequences to create a similarity score matrix. The algorithm gradually creates a multiple sequence alignment by combining increasingly distant relatives, starting with the most closely related sequences. This approach allows Clustal W to efficiently align multiple sequences (Jafari, Javidi, & Kuchaki Rafsanjani, 2019). HOPE program gathers data from various sources, including predictions made by DAS services, sequence annotations from the UniProt database, and computations on the protein's 3D coordinates using WHAT IF Web services. With YASARA, homology models are constructed

(Venselaar, Te Beek, Kuipers, Hekkelman, & Vriend, 2010). Data is kept in a database and utilised in a decision-making method to determine how a mutation affects a protein's three-dimensional structure and function. HOPE creates a report that is simple to use and comprehend for biomedical researchers, complete with text, figures, and animations (Venselaar et al., 2010). AlphaFold also provides a confidence score for each residue, known as pLDDT, ranging from 0 to 100, indicating the model's predicted accuracy (Jumper et al., 2021; Varadi et al., 2024).

These results are kept as mmCIF and PDB files. High accuracy is indicated by scores over 90, making them appropriate for binding site characterisation. Predictions with scores between 70 and 90 are considered reliable, while those between 50 and 70 suggest lower confidence and should be used cautiously. A score of less than 50 frequently denotes disorganised areas. In general, isolated helices or extended linkers are less dependable than structured domains. The pLDDT scores and the possibility of errors should be carefully considered when users interpret the model (Jumper et al., 2021; Varadi et al., 2024).

Results

PCR procedures were successfully performed on samples of both populations, except the HA4 sample from the Artlı population. PCR failed for this sample despite being repeated more than once. Therefore, this sample was not included in further analysis.

Agarose gel images of the amplified exon in both populations were shown in Figs. 1 and 2.

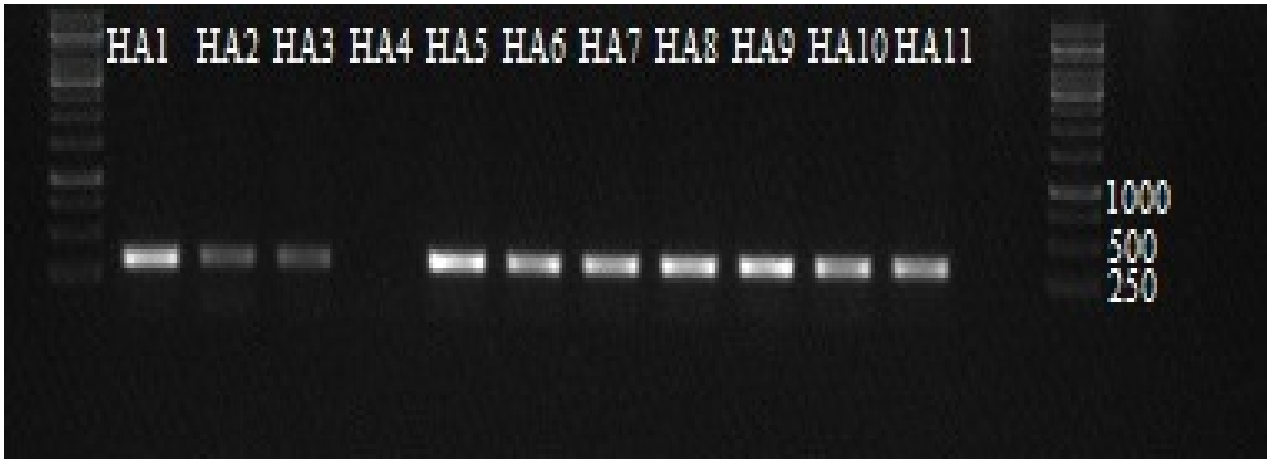

Fig. 1 HSPA8/Exon 8 PCR amplicons of the Artlı samples

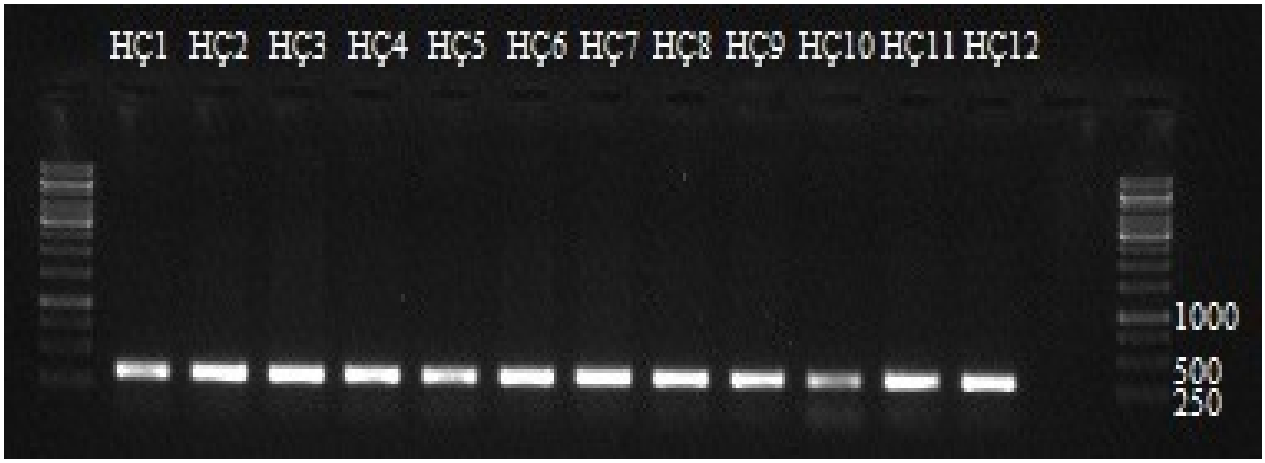

Fig. 2 HSPA8/Exon 8 PCR amplicons of the Çepni samples

	T	G	C	A	A	T	C	T	T	T	T	A	T	G	C	A	A	G	A	A	T	A	A	A	-	C	A	A	C	T	A	C	C	T
HA1HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	•
HA2HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	-	.	.	.	.	.	.	.	.	•
HA3HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HA5HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	•
HA6HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HA7HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HA8HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HA9HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	•
HA10HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	•
HA11HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC1HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC2HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC3HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC4HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C
HC5HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC6HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC7HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC8HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC9HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC10HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
HC11HF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C
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Fig. 3 Exon 8 of *HSPA8*'s comparison for the sheep samples (C > T substitutions marked with a blue dot)

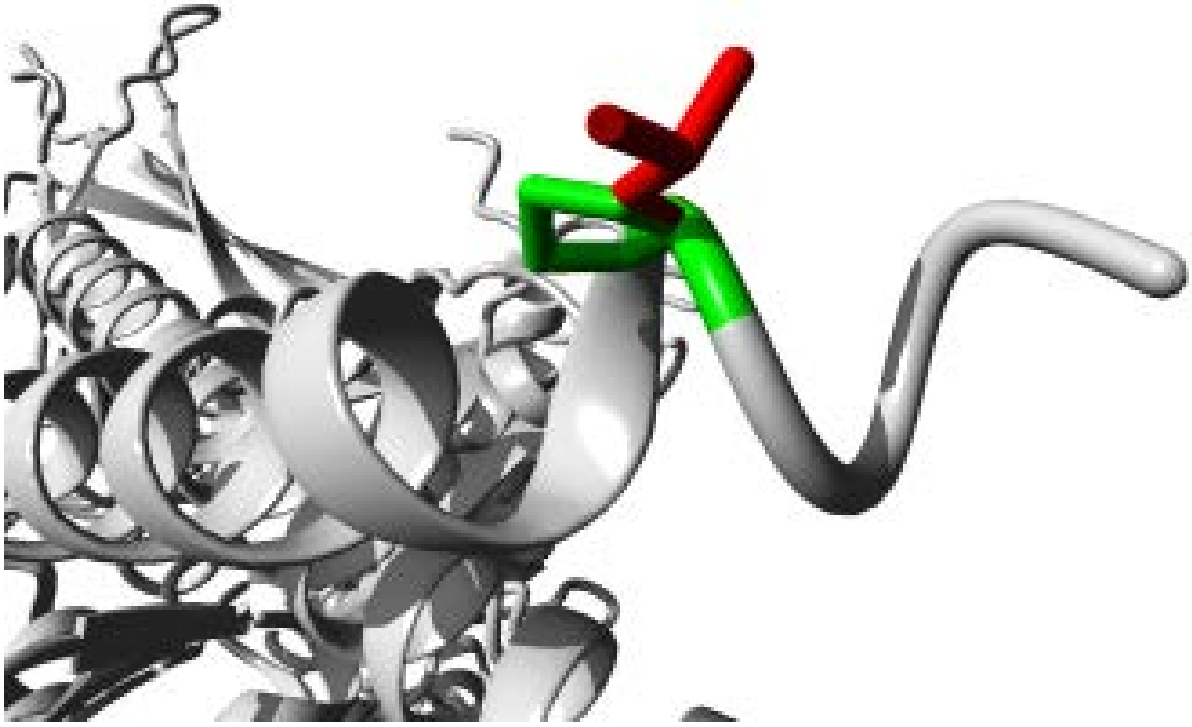

Fig. 4 HSPA8 protein's three-dimensional structure formed due to the non-synonym substitution amino acid change. The protein is grey, and the side chains of both substitutions were shown in green (wild) and red (mutant) colours.

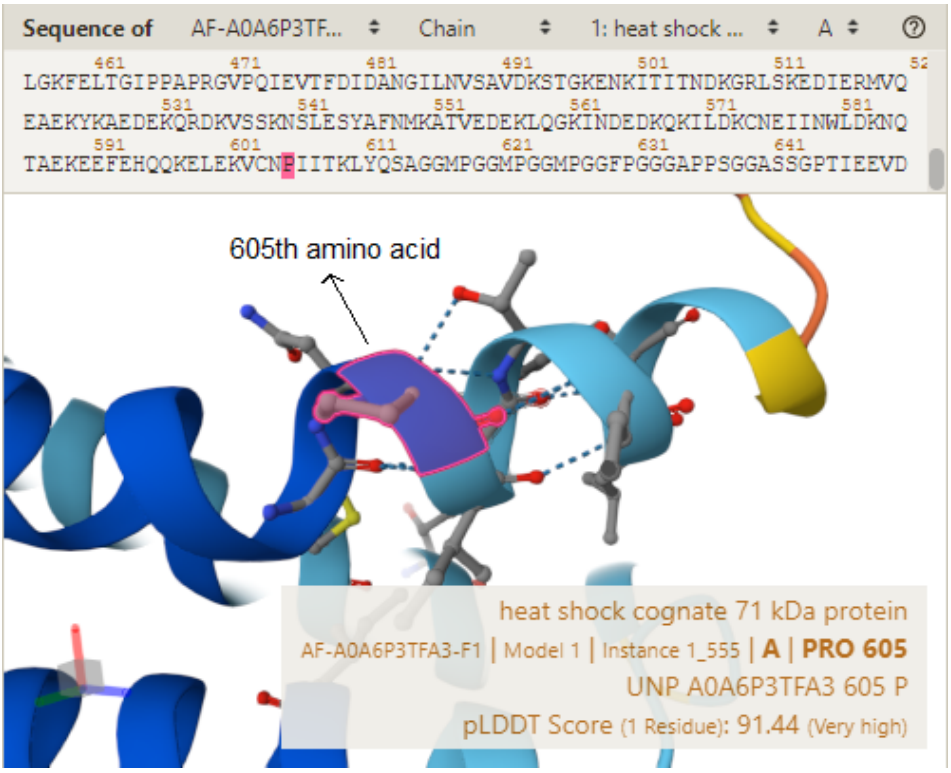

Fig. 5 HSPA8 protein structure variant prediction using AlphaFold2 (The red-marked site is the non-synonymous amino acid variant)

Samples selected for DNA sequence analysis were recoded as HA1HF-HA11HF for Artlı samples and HÇ1HF-HÇ12HF for Çepni samples. Several point mutations detected in the alignment analysis using ClustalW in the MEGA software were shown in Fig. 3.

DNA sequencing chromatogram was carefully examined to avoid reading errors. Samples HA2HF (T > C) and HC4HF (N > A) were considered misreads because there were non-acceptable noises in the sequence chromatogram checking.

A C > T substitution was detected at the nucleotide position 210th in HSPA8 gene exon 8 in HA1HF, HA2HF, HA5HF, HA9HF and HA10HF coded samples. This is a possible non-synonymous substitution that proline to leucine in the 605th amino acid of HSPA8. Additionally, HA1HF, HA2HF, HA3HF, and HA5HF coded samples were heterozygous at the nucleotide position 46th with G > A substitutions. Nevertheless, this mutation is synonymous translationally in the 550th amino acid position.

Both predictions of the HSPA8 protein's three-dimensional structures using HOPE and AlphaFold2 programmes were shown in Figs. 4 and 5.

Amino acid sequences analysed by both programs, HOPE and AlphaFold2, were compatible, and the protein in the mutated samples transformed into similar three-dimensional structures. However, a possible non-synonymous variation detected in some samples was in a critical amino acid for binding, folding or affinity to other molecules and a functionally important subunit.

Discussion

In the Artlı samples, we identified potential non-synonymous variations occurring at critical amino acid positions essential for molecular binding and affinity. These variations were also observed in a functionally significant subunit of the protein. An amino acid within the protein structure interacts with amino acids in a distinct subunit of the same protein or with those in a larger protein complex (Yan et al., 2014). Complicated interactions between different functional units of a protein and protein-protein interactions are inherently dynamic processes. Non-synonymous amino acid alterations can potentially disrupt the intricate balance of these interactions, both within individual subunits and between subunits that constitute the protein or larger protein complexes. Given that the amino acid sequence is crucial in defining a protein's structural and functional architecture, even minor alterations can lead to significant functional consequences.

Understanding these changes is essential for elucidating the broader implications of protein dynamics and stability (Yasmin, 2022).

Furthermore, some Artlı genotypes were heterozygous in another nucleotide position, but this variant is translationally synonymous. Recent reports concluded that synonymous mutation could also affect protein conformation and function by affecting post-transcriptional processing and RNA regulation, altering the mRNA's local and global structure and influencing the translation kinetics (Sauna & Kimchi-Sarfaty, 2011).

Astuti et al. (2022) reported that any SNP allele in the HSPA8 gene was absent in sheep breeds tolerant to high temperatures but present in breeds tolerant to low temperatures. Their study confirmed that SNPs in the HSPA12A, HSPA8, HSP90AA1 and IL33 genes were potential markers for heat tolerance adaptation in sheep.

Al-Thuwaini, Al-Shuhaib, and Hussein (2020) studied the relationship between coding region variations in the HSPA8 gene and heat stress in Awasi and Arabi sheep breeds. While both breeds were monomorphic in most coding regions, heterogeneity was observed in exon 4, revealing two SSCP patterns (TT and TG). The TG genotype, characterised by the missense variant, showed a frequency of 77% in Awasi and 54% in Arabi. In our study, mutations are detected only in Artlı sheep; Çepni sheep samples are all monomorphic in the analysed region. Their study showed that TT-genotype sheep had a significantly lower rectal temperature, respiratory rate, and heat tolerance coefficient than TG-genotype sheep. The study proved that the HSPA8 gene affected the ability to change various sheep breeds' heat tolerance mechanisms.

Singh et al. (2017) conducted a study investigating the relationship between HSP90 and HSP70 gene polymorphisms and their association with hemato-physio-biochemical parameters concerning thermo-tolerance in sheep. The study analysed expression profiles in four breeds: Chokla, Magra, Marwari, and Madras Red, suggesting that less adapted genotypes exhibited higher expression levels of HSPA8. This suggestion may prove that thermotolerance-related gene interactions are very complex.

Peng et al. (2019) investigated the effects of acute cold stress on immune function and the expression of HSP70 family genes in sheep. The study exposed eight female sheep to cold stress for 12 hours, with blood and tissue samples collected before and after exposure. The findings indicated that cold stress increased pro-inflammatory cytokines, including TNF-α, IL-1β, IL-2, and IL-6, while levels of IL-4 and IgG were reduced. Additionally, the expression of HSP70 family genes varied among different tissues, with HSPA1A exhibiting the most pronounced response to cold exposure. Based on these results, the study concluded that acute cold stress negatively impacts immune function and modulates gene expression, highlighting HSPA1A as a potential biomarker for cold stress in sheep.

Wen et al. (2021) identified two key mechanisms underlying the response to altitude-related hypoxia in Tibetan sheep. One of the mechanisms highlighted the differential expression patterns of heat shock proteins (HSPs) under hypoxic conditions, with HSP27 upregulated to maintain intracellular proteostasis. At the same time, HSP60 was downregulated to conserve energy for essential cellular processes under extreme hypoxia. These findings provide strong evidence of the physiological adaptations that contribute to the high-altitude resilience of Tibetan sheep. Changes in the expression of genes belonging to the HSP protein family under heat stress indicate that this family is actively involved in adaptation to heat stress. Türkiye's Artlı sheep breed is known to resist harsh environmental conditions, especially low-temperature stress. Raza et al. (2021) suggested that variations in the HSP70 protein may be associated with the distribution of certain animal species across different climatic and geographical regions. They may confer an adaptive advantage in response to selective pressures imposed by specific environmental conditions. Thus, one of the reasons for the resistance of the Artlı sheep to low-temperature stress may be a nonsynonymous mutation in the HSPA8 protein as a member of the HSP protein family. Sunil Kumar et al. (2024) investigated the polymorphism and expression of the heat shock protein 70 (HSP70) gene in Munjal sheep to assess its potential role in thermotolerance. They determined a SNP, A-to-G mutation at position c.459. AG and AA genotypes were detected; but no significant association was observed between these genotypes and growth or thermotolerance traits. Their inability to find a relationship between SNPs in the HSP70 gene and heat stress in their study can be explained by the fact that the annual average temperature of the location where the animals were kept is 25.5 ℃. As understood from thestudies, it is suggested that the HSP70 protein and, more

generally, the HSP protein family are primarily associated with low-temperature stress. The Artlı sheep used in our study are distributed in the high-altitude and mountainous areas of Türkiye's Eastern Black Sea region. They are a sheep breed highly adapted to low-temperature stress. Therefore, the possibility of a relationship between the SNPs identified in the study, which cause a significant mutation in the HSPA8 protein, and low temperature should not be overlooked.

Conclusion

This is one of the first molecular reports about the HSPA8 gene investigated in Artlı and Çepni breeds. Although some of the studied samples showed variation and some did not in the coding region of HSPA8, all Artlı and Çepni sheep flocks, the populations from which the samples came, were well adapted to the shared environmental conditions and regional rearing practices. This adaptation could be due to other variations in the studied gene or epistatic interaction in different genes involved in temperature regulation. It is also possible that the shared environmental conditions created this typical adaptation response through epigenetic regulators and many more genetic mechanisms.

Sheep populations worldwide possibly have richer genetic diversity than the Rambouillet sheep used as the reference genome in the GenBank database. The study results provided additional comparable data for determining the polymorphism values of genes related to thermal regulations and adaptations of the two native sheep breeds.

There is a need for strategies to ensure the sustainable conservation of all autochthonous breeds like Artlı and Çepni as genetic resources on which few studies have been conducted, in case the global climate change crisis we face worsens. Thus, comprehensive studies and conservation efforts on livestock genotypes and genes are still needed to avoid the extinction or genetic erosion of these unique breeds, which have economic, cultural, and genetic resource value.

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Conflict of Interest

There is no conflict of interest among the authors.

Data availability

All the data is provided within the article.

Author's Contribution

Levent Mercan designed the experiment. Mehmet Akif Cam supplied the sheep materials. Cihat Erdem Bulbul and Fatih Bilgi conducted the experiment. Cihat Erdem Bulbul performed and visualized the bioinformatics analyses. All authors contributed to writing and editing the manuscript.

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