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Research Article ***Araştırma Makalesi***

Genetic Variations in *HSP90AA1* Gene Region in Pırlak Sheep Breed

Pırlak Koyunlarında *HSP90AA1* Gen Bölgesindeki Genetik Varyasyonlar

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ABSTRACT

Objective: This study aimed to reveal variations in *HSP90AA1* gene region in Pırlak sheep breed.

Material and Methods: A total of 100 unrelated animals randomly chosen from representative herds raised in Antalya province were genotyped by Allele-Specific Polymerase Chain Reaction (AS-PCR) technique and genotypes were validated via agarose gel electrophoresis.

Results: *HSP90AA1* region was found to be polymorphic in which two alleles (C and G) and three possible genotypes (CC, CG and GG) were detected. The frequencies of C and G alleles were 0.39 and 0.61, respectively, whereas genotype frequency ranged from 0.183 (CC) to 0.413 (GG). The frequency of CG genotype was calculated as 0.404. Conservation of the genetic variations at *HSP90AA1* region will be required for planning selection programs against heat stress in the future.

Conclusion: *HSP90AA1* and similar genes may offer new opportunities to reduce heat stress caused by global climate change in the near future. Additionally, more genetic studies for the other genomic regions related to environmental stressors should be conducted in Pırlak breed in order to shape conservation studies.

Keywords: Pırlak sheep, AS-PCR, characterization, heat stress, heat tolerance, *HSP90AA1*, polymorphism.

ÖZET

Amaç: Bu çalışmanın amacı Pırlak koyun ırkında *HSP90AA1* geni varyasyonlarının incelenmesidir.

Materyal ve Metot: Antalya ilinde ırkı temsil eden sürülerden rastgele seçilen toplam 100 akraba olmayan hayvan Allel-Spesifik Polimeraz Zincir Reaksiyonu (AS-PZR) tekniğiyle genotiplendirilmiş ve genotipler agaroz jel elektroforeziyle tespit edilmiştir.

Bulgular: İki allel (C ve G) ve olası üç genotipin (CC, CG ve GG) tespit edildiği *HSP90AA1* bölgesinin polimorfik olduğu belirlenmiştir. C ve G allel frekansı sırasıyla 0.39 ve 0.61 idi, genotip frekansı ise 0.183 (CC) ve 0.413 (GG) aralığında değişmiştir. CG genotip frekansı 0.404 olarak hesaplanmıştır. *HSP90AA1* bölgesindeki varyasyonların korunması gelecekte ısı stresine karşı yapılacak olan seleksiyon programlarının planlanması için gerekli olacaktır.

Sonuç: *HSP90AA1* ve benzeri genler yakın gelecekte iklim değişikliğinden kaynaklanan ısı stresinin azaltılması için yeni fırsatlar sunabilmektedir. Ayrıca, Pırlak koyunlarında koruma çalışmalarının şekillendirilmesi için çevresel stresörlerle ilişkili diğer genomik bölgeler temelinde daha fazla çalışmanın yapılması gerekmektedir.

Anahtar sözcükler: Pırlak koyunu, AS-PZR, karakterizasyon, ısı stresi, ısı toleransı, *HSP90AA1*, polimorfizm.

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INTRODUCTION

In Türkiye, sheep rearing plays an important role in supplying society with animal-derived products such as meat and milk. Sheep not only make a contribution to effective use of the lands which are not suitable for crop production but also give rise to a lifestyle for farmers known as "nomadic breeding" in which sheep keepers migrate to other geographic areas according to the availability of grasslands (Karsli et al. 2020). This kind of rearing allows sheep to be exposed to different environmental conditions by which animals may develop adaptations against several stressors through generations. Although environmental stressors are many in numbers varying from management practices to climatic parameters (Demir et al. 2021), heat stress caused by climate change has negative effects on the growth, development, and productivity of sheep (Demir et al. 2022). Heat stress could be observed via numerous indicators such as physiological (rectum temperature, respiration rate, heart rate, etc.), behavioral (shade seeking, aggression, and feed preference), and performance (yield and content of the milk) (Hoffmann et al. 2020). On the other hand, there are several adaptation mechanisms (morphological, anatomical, physiological, behavioral, and genetic) to eliminate the negative effects of heat stress allowing for maintaining performance traits such as milk and meat yield (Niyas et al. 2015). Among these, genetic based adaptation mechanism occurs by variations in the related genomic regions and is passed to the next generations leading to the presence of thermo-tolerant animals. It is known that Heat Shock Proteins (HSPs) classified as *HSP47*, *HSP60*, *HSP70*, *HSP90,* and small proteins based on their molecular weight are of vital role in maintaining cellular homeostasis during heat stress (Park et al. 2007; Singh et al. 2017). During extreme temperatures, the expression level of HSPs significantly increases in heat-susceptible animals, whereas lower expression levels are expected in heat-tolerant animals (Kumar et al. 2018).

Developing molecular genotyping methods enable scientist to detect heat-tolerant animals at DNA level by screening genomic variations in the related genes. A previous study regarding HSPs variations in some Indian sheep breeds (Chokla, Magra, Marwari, and Madras Red) revealed five Single Nucleotide Polymorphisms (SNPs) of which SNP4 in *HSP90AA1* gene was reported to be directly associated with thermo-tolerance parameters in which C allele and CC genotype turned out to be advantageous in terms of rectal temperature, pulse rate, and neutrophil/lymphocyte ratio (Singh et al. 2017). Moreover, the authors reported an AS-PCR protocol to genotype animals in a cost-effective manner. Indeed, compared to several molecular genotyping methods, AS-PCR is cost-effective, since it requires amplification of wild and mutant alleles with specific primers and visualization of the alleles on agarose gel electrophoresis.

Being one of the native sheep breeds in Türkiye, Pırlak is reported to be obtained from crossbreeding studies between fat-tailed Dağlıç and thin-tailed Kıvırcık breeds (Çelikeloğlu et al. 2018). It was also highlighted that Pırlak sheep are more tolerant to environmental stressors than Kıvırvık breed as well as being advantageous over Dağlıç breed in terms of body weight and litter size (Çelikeloğlu et al. 2018). Unfortunately, no studies are available to investigate HSPs polymorphisms in Pırlak sheep breed. Therefore, this study aims to screen the *HSP90AA1* gene in Pırlak sheep in order to detect genetic variations in terms of SNP4 by AS-PCR protocol.

MATERIAL AND METHOD

Sample Collection and DNA Extraction

Located between 29°20'-32°35' east longitudes and 36°07'-37°29' north latitudes in the south of Türkiye in the Mediterranean Region, Antalya, in which the average summer temperature and the relative humidity are 32 °C and 62%, respectively, ranks amongst the hottest climatic conditions across the country (Sancar ve Güngör, 2022). A total of 100 female animals of Pırlak breed were randomly sampled from five representative herds reared in Antalya province. Healthy animals were chosen based on pedigree information in order to minimise kinship. Blood samples were collected from the jugular vein into vacutainer tubes containing EDTA solution as an anticoagulant and stored at -20 °C till DNA extraction was performed. DNA was extracted from whole blood samples via a salting-out method reported by Miller et al. (1988). DNA quality and quantity were checked by both 1% agarose gel electrophoresis and spectrophotometer (NanoDrop-SD 1000). DNA concentration was optimized at 50 ng/µL for AS-PCR amplification.

AS-PCR Amplification and Genotyping

AS-PCR protocol in order to amplify C and G alleles in *HSP90AA1* promoter region of the *Ovis aries* genome was summarised in Singh et al. (2017). Briefly, in this study, two recommended primer sets were used to amplify 254 base pairs (bp) length of C and G alleles in the PCR process. Gradient PCR was applied for different annealing temperatures (from 55 to 65 °C) in order to optimize PCR conditions and to avoid non-specific amplification in which the expected fragments were clearly detected at 60 °C. PCR was performed in

50 µL reaction volume with 50 ng template DNA, 5 µL 10X reaction buffer, 0.6 mM dNTPs, 2.5 mM $MgCl_2$, 10 pM of each primer, 1 U of Taq DNA polymerase (GeNet Bio, Korea) and 31.25 µL nuclease-free water. PCR amplification was carried out in initial denaturation at 94 °C for 10 min, followed by 31 cycles at 94 °C for 40 s, at 60 °C for 40 s and at 72 °C for 40 s. The final extension was applied at 72 °C for 10 min. Amplified C and G alleles were visualised by agarose gel electrophoresis in order to genotype animals. Individuals with both amplifications were considered heterozygous (CG), while single amplifications allowed to genotype individuals as homozygous (CC or GG) based on the type of amplified nucleotide.

Data Analyses

GenAlEx 6.5 software (Peakall and Smouse 2012) was used to calculate allele and genotype frequencies and to test Hardy-Weinberg equilibrium via the chi-square approach. Allele and genotype frequencies were calculated simply by direct count of the proportion of different alleles and genotypes, respectively, while chi-square value was calculated via the equation:

$$X^2 = \sum_{i=1}^{k} \frac{(O-E)^2}{E}$$

where O and E represent the observed and expected number of individuals of the i-th genotype.

RESULTS

Both C and G alleles for each animal were visualised by agarose gel electrophoresis in which animals possessing both amplifications were recorded as heterozygous. Accordingly, animals with single amplification were recorded as homozygous CC or homozygous GG based on the amplificated allele in agarose gel electrophoresis. A total of two alleles (C and G) and three genotypes (CC, CG, and GG) were observed in Pırlak breed in terms of *HSP90AA1* (112G>C) polymorphism (Figure 1). G allele frequency (0.61) was higher than C allele frequency (0.39) whereas CC, CG, and GG frequencies were 0.183, 0.404, and 0.413, respectively. X^2 test indicated that the Pırlak population was in Hardy-Weinberg equilibrium in terms of the *HSP90AA1* region.

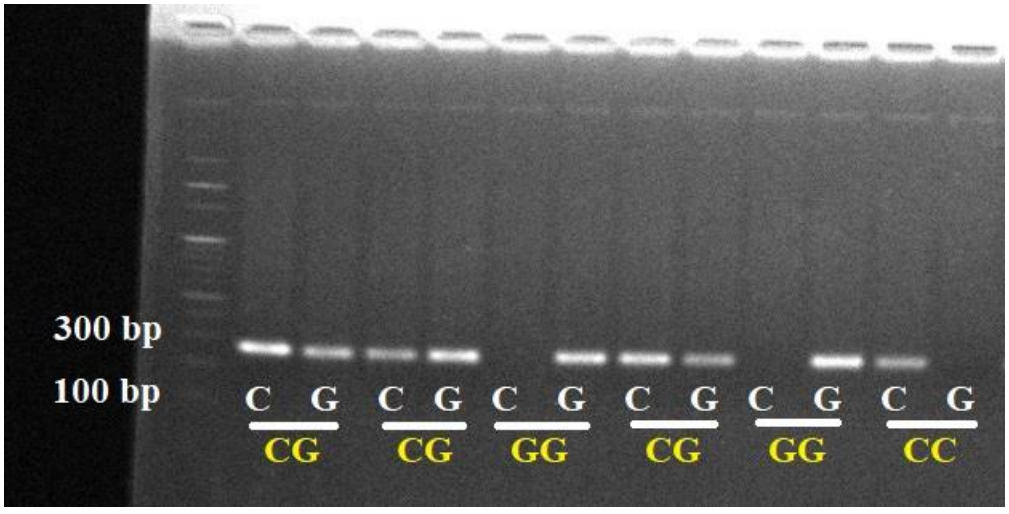

Figure 1. Agarose gel image of 254 bp length alleles amplified via AS-PCR

Şekil 1. *AS-PCR ile çoğaltılan 254 bç uzunluğundaki allellerin agaroz jel görüntüsü*

DISCUSSION AND CONCLUSION

As reported by McManus et al. (2020), climate change may affect environmental conditions such as alteration in the air temperature, precipitation, atmospheric carbon dioxide level as well as water availability which has negative effects on economically important traits and welfare in farm animals (Demir et al. 2020). Therefore, genomic regions related to heat stress should be analysed in order to obtain heat-tolerant animals with desired genotype combinations. Of these genomic regions, HSPs offer several desired genotypes to obtain heat-tolerant animals, since they play a vital role in adaptation to heat stress.

In this study, desired C allele and CC genotype in terms of heat tolerance were present in Pırlak breed at sufficient frequency. Moreover, the frequency of the C allele and CC genotype were higher in Pırlak breed than in Indian sheep breeds (Singh et al. 2017). It is not surprising since animals were randomly chosen from the five herds raised in the rangeland-based breeding

system in which animals have been grazed in meadows during the summer months for generations in Antalya province. As reported by Demir et al. (2022), the rangeland-based breeding system not only decreases feed costs for farmers but also exposes the animals to high temperatures and direct solar radiation by which animals may develop adaptation against heat stress through generations. Another study conducted by Oner et al. (2012) revealed new indel (AA) polymorphisms in the *HSP90AA1* gene region in ten Turkish sheep breeds (Akkaraman, Çine Çaparı, Dağlıç, Gökçeada, Hemşin, İvesi, Karayaka, Kıvırcık, Morkaraman, and Sakız). The authors revealed that although this new indel was not significantly associated with climatic parameters, the *HSP90AA1* gene region was polymorphic in all sheep breeds (Oner et al. 2012).

The *HSP90AA1* gene was also reported to be associated with thermotolerance in several farm animals such as cattle (Badri et al. 2018), and chicken (Chen et al. 2013). However, it is noteworthy that variations in the *HSP90AA1* gene are not only associated with heat stress but they may also affect several traits. For example, Marcos-Carcavilla et al. (2008) confirmed that polymorphisms in the *HSP90AA1* gene were also related to the scrapie incubation period, while they were reported to be associated with sperm DNA fragmentation in sheep (Salces-Ortiz et al. 2015).

In conclusion, genetic variations of the *HSP90AA1* gene region in Pırlak breed were assessed in a cost-effective manner by previously designed AS-PCR. The *HSP90AA1* gene region, which was reported to be directly associated with heat tolerance and susceptibility, was found polymorphic in Pırlak breed. Moreover, the frequency of the desired allele and genotype turned out to be sufficient in Pırlak. This polymorphism should be conserved in the population, since climate change is an ongoing phenomenon, and it is estimated that the temperature will increase gradually in the future. Polymorphisms in the genomic regions related to heat tolerance will play a vital role for farmers to conduct selection practices against environmental challenges in the future. However, compared to other native Turkish sheep breeds, molecular characterisation studies of Pırlak breed are scarce. Besides, phenotypic data related to heat stress (with rectal temperature, pulse rate, hemoglobin level, and neutrophil/lymphocyte ratio) are not recorded by the farmers. We highly recommend the subsequent studies to analyze the other genomic regions related to environmental stressors with phenotypic records in Pırlak breed.

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Ethical Statement

This study was approved by Akdeniz University Animal Experiments Local Ethic Committee (Protocol No: 1392/2022.01/004).

REFERENCES

Badri TM, Chen KL, Alsiddig MA, Li L, Cai Y, Wang GL. 2018. Genetic polymorphism in Hsp90AA1 gene is associated with the thermotolerance in Chinese Holstein cows. Cell Stress and Chaperones 23: 639-651.

Chen ZY, Gan JK, Xiao X, Jiang LY, Zhang XQ, Luo QB. 2013. The association of SNPs in Hsp90β gene 5′ flanking region with thermo tolerance traits and tissue mRNA expression in two chicken breeds. Molecular Biology Reports, 40: 5295-5306.

Çelikeloğlu K, Erdoğan M, Hacan Ö, Koçak S, Bozkurt Z, Tekerli M. 2018. Pırlak koyunlarında BMPR1B, BMP15 ve GDF9 genlerinde olası polimorfizmlerin araştırılması. Kocatepe Veterinary Journal 11(4): 356-362.

Demir E, Bilginer U, Balcioglu MS, Karsli T. 2021. Direct and indirect contributions of molecular genetics to farm animal welfare: a review. Animal Health Research Reviews 22(2): 177-186.

Demir E, Ceccobelli S, Bilginer U, Pasquini M, Attard G, Karsli T. 2022. Conservation and selection of genes related to environmental adaptation in native small ruminant breeds: a review. Ruminants 2(2): 255-270.

Hoffmann G, Herbut P, Pinto S, Heinicke J, Kuhla B, Amon T. 2020. Animal-related, non-invasive indicators for determining heat stress in dairy cows. Biosystems Engineering 199: 83-96.

Karsli BA, Demir E, Fidan HG, Karsli T. 2020. Assessment of genetic diversity and differentiation among four indigenous Turkish sheep breeds using microsatellites. Archives Animal Breeding 63(1): 165-172.

Kumar D, Yadav B, Choudhury S, Kumari P, Madan AK, Singh SP, Routh PK, Ramchandran N, Yadav S. 2018. Evaluation of adaptability to different seasons in goat breeds of semi-arid region in India through differential expression pattern of heat shock protein genes. Biological Rhythm Research 49(3): 466-478.

Marcos-Carcavilla A, Calvo JH, González C, Moazami-Goudarzi K, Laurent P, Bertaud M, Hayes H, Beattie AE, Serrano C, Lyahyai J, Martín-Burriel I, Serrano M. 2008. Structural and functional analysis of the HSP90AA1 gene: distribution of polymorphisms among sheep with different responses to scrapie. Cell Stress and Chaperones 13: 19-29.

Miller S, Dykes D, Polesky H. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16(3): 1215-1215.

Niyas PA, Chaidanya K, Shaji S, Sejian V, Bhatta R. 2015. Adaptation of livestock to environmental challenges.

Journal of Veterinary Science and Medical Diagnosis 4(3): 1-7.

Oner Y, Calvo JH, Serrano M, Elmaci, C. 2012. Polymorphisms at the 5′ flanking region of the HSP90AA1 gene in native Turkish sheep breeds. Livestock Science 150(1-3): 381-385.

Park H, Ahn IY, Lee HE 2007. Expression of heat shock protein 70 in the thermally stressed Antarctic clam Laternula elliptica. Cell Stress and Chaperones 12(3): 275-282.

Peakall R, Smouse PE 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformics 28: 2537-2539.

Salces-Ortiz J, Ramon M, Gonzalez C, Perez-Guzman MD, Garde JJ, Garcia-Alvarez O, Maroto-Morales A, Calvo JH, Serrano MM. 2015. Differences in the ovine HSP90AA1 gene expression rates caused by two linked polymorphisms at its promoter affect rams sperm DNA fragmentation under environmental heat stress conditions. PLoS One 10(2): e0116360.

Sancar MC, Güngör Ş. 2022. Analysis of Antalya province bioclimatic comfort features according to heat index. International Journal of Eurasia Social Sciences 13(48): 710-723.

Singh KM, Singh S, Ganguly I, Nachiappan RK, Ganguly A, Venkataramanan R, Chopra A, Narula HK. 2017. Association of heat stress protein 90 and 70 gene polymorphism with adaptability traits in Indian sheep (Ovis aries). Cell Stress and Chaperones 22: 675-684.