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## Unraveling candidate genes related to heat tolerance and immune response traits in some native sheep using whole genome sequencing data

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## ABSTRACT

**Background:** In the sheep breeding industry, heat stress caused by the general increase in global temperature has become a significant issue, having both direct and indirect effects on animal health and productivity. The identification of effective pathways for responding to heat stress and immune responses, as well as the discovery of related genes, can improve the production and sustainability of genetic diversity in this area. In this study, we used nucleotide diversity ( $\theta\pi$ ) and FST statistical measurements to analyze the genomic data of the native Iranian sheep in order to find potential genes related to heat adaptation and immune response. We also compared the whole genome sequencing data of 29 indigenous Iranian sheep (the Afshari, Ghezel, Makuei, Moghani, Shal, Zel, Karakul, Grey-Shiraz, Baluchi, and Kermani breeds) with a number of other sheep breeds from Asia ( $n = 28$ ), Europe ( $n = 28$ ), and Africa ( $n = 25$ ) to evaluate the genetic structure of the Iranian sheep population.

**The results:** The results from the sheep population genetic analysis showed a clear separation between different populations that corresponds well with their geographic origins. Iranian sheep were further divided into a southern and a northern group, which coincided with the most prominent climatic division in Iran. Our search to identify potential genomic regions under selection showed several candidate genes involved in (1) response to heat stress (*SIK2*, *FER*, *ATP1A1*, *CDK5RAP3*, and *TLR4*), (2) immune response in hot and dry environments (*CD109*, *CR2*, *EOMES*, and *MARCHF1*), (3) response to drought stress and adaptation to desert areas (*ZFP1*, *PLCB1*, and *PDGFD*), and also (4) response to heat stress by controlling digestive metabolism (*HTR4*, *TRHDE*, and *ALDH1A3*).

**Conclusions:** The findings of this study may aid in our understanding of the molecular processes by which tiny ruminants adapt to hot, dry environments. In addition to the results of earlier studies, our findings also revealed a number of novel candidate genes related to heat adaptation. These genes will be valuable for future studies to choose livestock breeds that live in desert regions.

**Abbreviations:** AMSL, above mean sea level; BAM, Binary alignment map; Breed n, The number of breeds BWA: Burrows wheeler aligner; FST, Fixation index; GATK, Genome analysis toolkit;  $\theta\pi$ , Nucleotide diversity; GCTA, Genome-wide complex trait analysis; NCBI, National Center for Biotechnology Information; PCA, Principal component analysis; SNP, Single-nucleotide polymorphism; SRA, Sequence Read Archive.

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## 1. Introduction

Farm animals provide unique models for genetic investigations of phenotypic evolution, as they contain useful alleles acquired by vigorous selection over thousands of generations (Andersson, 2016). Local livestock breeds have been adapted to diverse environmental climates as the results of thousands natural selection. Sheep were among the earliest animals that were likely domesticated from Asian Mouflons (*Ovis Orientalis*) in the Fertile Crescent, presumably in southeast Anatolia and/or the Zagros area, some 11,000 years before the present (BP) (Zeder, 2008). Following that, this animal's phenotypic features showed a substantial amount of change as a result of artificial selection and geographic isolation.

Sheep provide the main animal-based proteins for human consumption. As a result, they have a significant impact on the agricultural economy. Asia is home to over 44 % of the world's sheep (Skapetas and Kalaitzidou, 2013), and Iran has the greatest population of sheep in the Middle East with 52 million sheep of various genetic potentials (Mohammadabadi, 2016). These breeds' names typically reflect their physical traits and place of origin. Since they have not yet been subjected to specific artificial breeding programs, indigenous Iranian sheep may have unique gene pools as a result of long-term adaptability to their habitat (Mohammadabadi et al. 2018).

Numerous studies and pieces of evidence support the idea that future ruminant product availability will be impacted by climatic changes. Among the environmental variables affecting animals, heat stress is one of the factors that has an effect on animal production in many parts of the world (Sejian et al. 2018). The susceptibility of livestock to heat stress differs by species, genetic potential, life stage, method of production, and also nutritional state (Das et al. 2016). According to previous studies, heat stress has an impact on the majority of livestock's production processes, including growth performance (Baumgard et al. 2012), the production of milk (Das et al. 2016), reproductive efficiency (Rhoads et al. 2009), meat production (Archana et al. 2018), and disease occurrences (Rojas-Downing et al. 2017). Sheep in hot, semi-arid climates are usually reared using extensive systems. In these geographical regions, the extreme environmental conditions impact the ability of animals to survive and production. For example, in these regions, the quantity and quality of water and the availability of feed resources greatly vary in different seasons of a year. During the summer, the animals frequently have to travel vast distances to pursue these limited resources. Therefore, grazing animals may be subjected to a variety of stressors, including heat stress, a lack of food and water, and physical stress from moving through hot, dry conditions (Sejian et al., 2013). Effective strategies are needed to reduce the negative effects of the climate changes (Henry et al. 2018). Identification of specific genes that are related to the thermo-tolerance may assist in the selection of superior adapted breeds, which can withstand the heat stress adversities effectively. Finding the genes responsible for important ecological and economic traits can be aided by examining the genomes of locally adapted animals (de Simoni Gouveia et al., 2017).

Iran, which is located in the Africa-Asia belt and has 90 % dry regions, are regarded as a hot and arid nation in terms of climate globally (Nouri and Homae, 2020). There are more than 27 different breeds and ecotypes of sheep in Iran that have adapted to various climatic conditions (Mohammadabadi, 2016). For example, medium-sized Moghani (fat-tailed) and Zel sheep (tailed and without fat-tailed) are raised in Iran's cold, mountainous regions, which have a diverse range of vegetation and rainfall. While Kermani, Baluchi, and Karakul sheep (fat-tailed, carpet-wool) live in desert regions with high solar radiation, low rainfall and high daytime temperatures. This results in a lack of forage and a lack of species diversity in rangeland plants. Although native Iranian sheep breeds have significant genetic potential for production, less study has been done on them than that on other sheep breeds to boost their productivity. In Iranian sheep breeds, only a few studies on genome-wide associations (Abdoli et al. 2019; Gholizadeh et al. 2015;

Almasi et al. 2020; Ghasemi et al. 2019) and signature selection mapping (Manzari et al. 2019) have been conducted. In recent research on native Iranian chickens, several genes have been identified as candidates for desert climate tolerance (Asadollahpour Nanaei et al. 2022).

In this study, whole-genome sequencing (WGS) data from indigenous Iranian breeds of sheep (ten different populations) (Fig. 1), together with breeds from Asia, Europe, and Africa, were carried out to characterize the genetic structure of the Iranian sheep population. Additionally, nucleotide diversity ( $\theta\pi$ ) and *F*<sub>ST</sub> statistical methods were performed to compare the genomic data of the native sheep population from cold and hot parts of Iran in order to find potential candidates for genes involved in heat tolerance and immune response. Our findings can be used for the genetic improvement of thermal tolerance in harsh environments, especially for small ruminants.

## 2. Material and methods

### 2.1. Genomic sequence data

In the present study, we collected blood samples (5 ML from the jugular vein) from three indigenous breeds of Iran, including Zel (*n* = 4), Karakul (*n* = 4), and Kermani (*n* = 2), that live in the Mazandaran (AMSL: 2 m, temperate and mild mountainous climate), North Khorasan (AMSL: 300 m, hot and dry climate), and Kerman (AMSL: 1755 m, hot and dry climate) provinces, respectively. Using the salting out approach, total genomic DNA was extracted from whole blood samples. Genome sequencing was done in China using the Illumina NovaSeq 6000 platform. We also used SRA data of 109 sheep from different breeds worldwide, including Iran (*n* = 19, breed *n* = 7), China (*n* = 11, breed *n* = 1), Turkey (*n* = 8, breed *n* = 5), Iraq (*n* = 3, breed *n* = 2), Pakistan (*n* = 6, breed *n* = 3), Africa (*n* = 25 of Libya *n* = 8, breed *n* = 1, Egypt *n* = 7, breed *n* = 3, South-Africa *n* = 3, breed *n* = 2, Ethiopia *n* = 7, breed *n* = 3), Europe (*n* = 28 of Germany *n* = 3, breed *n* = 1, Italy *n* = 3, breed *n* = 2, Switzerland *n* = 6, breed *n* = 2, Spain *n* = 9, breed *n* = 4, United Kingdom *n* = 7, breed *n* = 6), and Wild (*n* = 9), which was downloaded from the NCBI database (<https://trace.ncbi.nlm.nih.gov/Traces/sra/?run>) (Additional file 1: Table 1) (Fig. 2 A).

### 2.2. Quality checking, and SNP calling

The quality of all the genome data was checked by the FastQC software. Then, using the Burrows-Wheeler Aligner (BWA mem Version 0.7.10), all the examined samples were aligned to the sheep genome reference ([https://www.ncbi.nlm.nih.gov/assembly/GCF\\_00274215.1/](https://www.ncbi.nlm.nih.gov/assembly/GCF_00274215.1/)) (Li and Durbin, 2009). SAM (.sam) and BAM (.bam) files were created using the SAMtools program, which was also used to read, sort, and index the files (Li et al. 2009). Potential PCR duplicates were eliminated using the Picard toolkit (<http://broadinstitute.github.io/picard>) to reduce the possibility of false-positive variant calling. Using tools from the Genome Analysis Toolkit (GATK), base quality score recalibration (BQSR) and local realignment around indels were carried out to improve the alignment accuracy (McKenna et al. 2010). The GATK tool was used to call and filter final variations (SNPs, single nucleotide polymorphism).

### 2.3. Population structure and admixture analyses

The phylogenetic tree was generated in this study using the maximum likelihood (ML) method. First, we used Samtools' vcfutils.pl file to convert the filtered VCF file into consensus FASTA files, and then we utilized FastTree 2 software (Price et al. 2010) to generate the tree. Visualizing the topological structure was done using the web application iTOL (<https://itol.embl.de/>). SNP datasets in PLINK were first pruned for LD (using the PLINK option in pairs 50 50 0.1) (Purcell et al. 2007) because linkage disequilibrium (LD) can affect genetic structure analysis (Calus and Vandenplas, 2018). Then, using SNPs

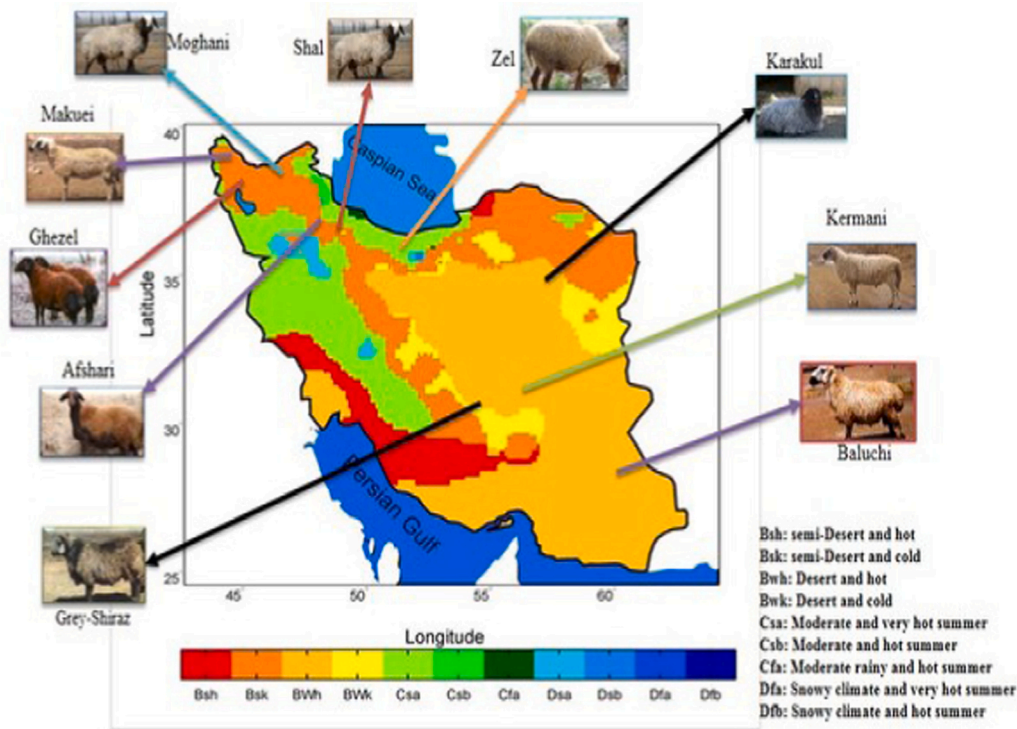


Fig. 1. Geographic locations of indigenous Iranian sheep breeds (Raziei, 2017).

pruned for LD, principal component analysis (PCA) and ADMIXTURE were carried out. To examine genetic variances among all sheep groups, we employed genome-wide complex trait analysis (GCTA) based on SNP genotypes (Yang and Lee, 2011). We utilized the admixture model implemented in the ADMIXTURE program, with an ancestral population (K) size ranging from 2–6, and 10,000 iterations for each run, to investigate the potential genetic admixture between sheep populations (Alexander et al. 2009).

#### 2.4. Statistics to explore selective sweep region

To find selective sweeps in the current work, nucleotide diversity ( $\pi$ ) and  $F_{ST}$  approaches were applied. One of the most frequently used methods for studying genome-wide variance is the  $F_{ST}$  statistic. We calculated the weighted genome-wide  $F_{ST}$  (Weir and Cockerham, 1984), which is a more accurate indicator of the mean genetic distance between groups with different sample sizes (Spolaore and Wacziarg, 2009). Then, the software VCFtools was used to assess the nucleotide diversity ( $\theta\pi$ ) (window- $\pi$  50,000 —window- $\pi$ -step 25,000) (Danecek et al. 2011) and the top 1 % was detached as positively selected regions. Sliding window analyses with a window size of 50 kb and a step size of 25 kb were conducted for the entire genome. SNPs in each window were averaged for  $F_{ST}$  and  $\log_2(\theta\pi \text{ Iran's desert sheep population}/\theta\pi \text{ Iran's cold sheep population})$ .

### 3. Results

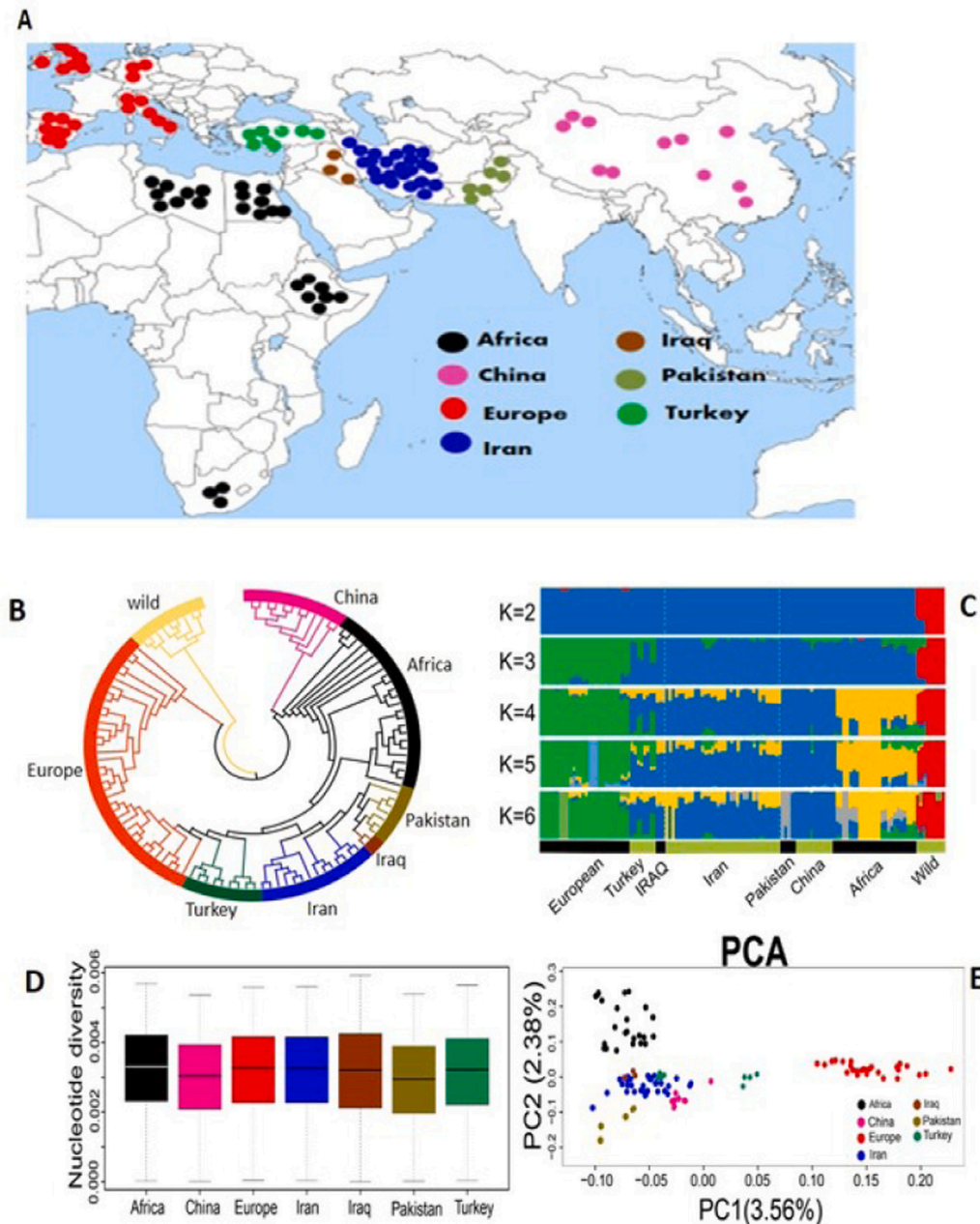
#### 3.1. Population structure

The results of the phylogenetic tree evaluation clearly showed the differentiation of various populations. The wild breed of sheep with a distinct node and branch was the most recognizable breed in the phylogenetic tree. Additionally, European breeds were placed next to Turkish sheep and were divided into two separate clusters (Fig. 2 B). In an admixture analysis with  $K = 2$ , wild sheep were separated from domesticated sheep breeds. While at  $K = 3$ , European sheep breeds were

separated from the other domesticated breeds. By running the admixture from  $K = 4-5$ , the domestic breeds were separated based on the geographical region (Asia, Europe, and Africa). From  $K = 4-6$ , Iranian breeds showed some admixture with European and African breeds (Fig. 2 C). Also, the topological patterns were found in the phylogenetic tree and the admixture supported by PCA analysis. In the level genome, PC1 and PC2 accounted for 3.56 % and 2.38 % of the genetic variation, respectively. PCA analysis divided all individuals into three general categories. The first cluster included all individuals from Asian countries (Iran, Iraq, Pakistan, and Turkey); the second cluster is related to European breeds; and the third cluster included all individuals from Africa (Fig. 2 E). The population of indigenous Iraqi sheep had the highest levels of nucleotide variation, while samples of sheep from China and Pakistan had the lowest levels, according to the box plot of nucleotide diversity ( $\pi$ ). Other sheep populations, including those from Africa, Europe, Iran, and Turkey, had similar levels of nucleotide diversity (Fig. 2 D).

#### 3.2. Scanning of highly differentiated genomic regions

In total, 742 and 798 protein-coding genes were found in windows with high  $F_{ST}$  (5 % cutoff) and low  $\log_2 \theta\pi$  ratio values (1 % cutoff), respectively, in the genomics data comparison between the indigenous sheep populations from cold climates and the desert and hot areas of Iran (Additional file 2: Table 2 and Fig. 3 and Additional file 3: Table 3). We identified a number of genes associated with environmental adaptation to heat (Additional file 4 Table 4). The findings demonstrated that a number of genes, including *SIK2*, *FER*, *TLR4* (top 1 % for  $\pi$ ), and *ATP1A1*, *CDK5RAP3* (top 5 % for  $F_{ST}$ ), play a significant role in the pathways related to heat stress responses, including the activation of the oxidative stress system, cell protection from heat damage, and tissue repair. We identified several genes, including *CD109*, *CR2* (top 5 % for  $F_{ST}$  and 1 % for  $\pi$ ), *EOMES*, and *MARCHF1* (top 1 % for  $\pi$ ), that are significant for the signals relating to immune system activity and heat resistance. The *ZFP1*, *PLCB1* (top 5 % for  $F_{ST}$ ), and *PDGFD* genes (top 1 % for  $\pi$ ) were further identified as potential candidates for adaptation



**Fig. 2.** A) Geographic locations of sheep populations. B) A phylogenetic tree was constructed based on the maximum likelihood (ML) approach. C) The clustering analysis based on the ADMIXTURE theory, a distinct number of ancestors ( $K = 2-6$ ) is assigned to each individual. D) Boxplot of nucleotide diversity ( $\pi$ ) for the seven sheep populations. E) Principal Component (PC) analysis, PC1 against PC2.

to arid environments. The results also showed that the *HTR4*, *ALDH1A3* (top 1 % for  $P_i$ ), and *TRHDE* genes (top 5 % for  $F_{ST}$ ) are resistant to heat shock because they function in the pathways that regulate digestion and energy.

#### 4. Discussion

##### 4.1. Genetic diversity and population structure

The analysis of the genetic structure of sheep breeds reflects their domestication and subsequent organization into separate breeds. Genetic variety conservation results in the preservation of evolutionary potential and adaptive capacity.

In the present study, using complete genome sequencing data, we investigated the genetic diversity and population structure of indigenous

Iranian sheep and several worldwide sheep breeds. Our results from the genetic divergence between wild and domestic sheep breeds are in accordance with the previous studies that showed significant divergence between these two species (Cheng et al., 2023). After domestication in the Fertile Crescent, sheep were introduced to other geographical regions (Peters et al. 1999; Rowley-Conwy et al. 2016). It has been suggested that about 4000 Before the Common Era (BCE), the replacement of the initial domesticated sheep population with woolly sheep started in Europe (Rowley-Conwy et al. 2016; Mason, 1984). Also, it has been proved that about 3000 BCE, fat-tailed and fat-rumped sheep in different parts of the central and western Asia and also in East Africa became predominant (Ryder, 1964), which may explain the reason for the genetic differentiation of European sheep with other studied populations in this study (e.g. Asian and African breeds). Furthermore, according to earlier research (Ryder, 1983) the majority of the sheep breeds in

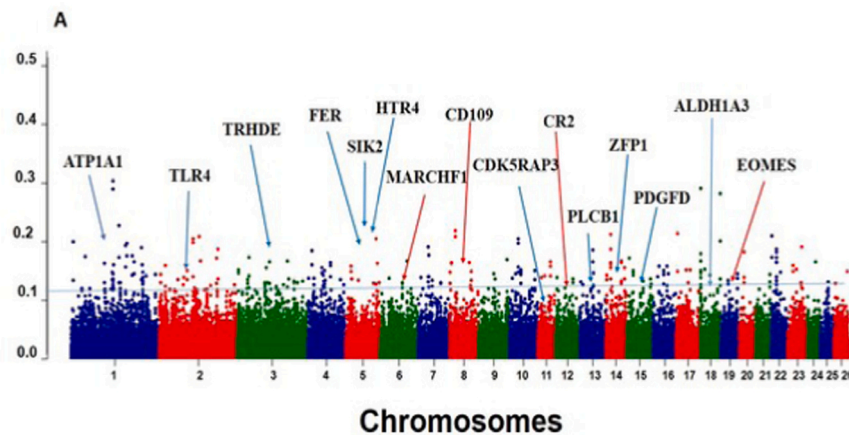


Fig. 3. Manhattan design of genome-wide  $F_{st}$  levels among the sheep population of cold and hot regions of Iran.

Turkey and Southeast Europe are distinct from other European breeds. These findings confirmed our results from phylogenetic tree and PCA that showed the separation of European individuals' sheep breeds and their affinity to Turkish sheep populations. Collectively, our findings from population genetic analysis, including admixture analysis, suggested that sheep populations from west Asia, including native sheep from Iran, Iraq and Turkey may have been originated from common ancestor.

#### 4.2. Scanning of highly differentiated genomic regions

Ecological change or human intervention can lead to adaptation in an agricultural setting. Natural adaptation in animals is the result of various mechanisms (Gaughan et al. 2019). Animal adaptation is influenced by physiological, biochemical, immunological, anatomical, and behavioral responses (Sejian et al. 2018). Breeding techniques and breed type selection can potentially be adaptation strategies because the future climate is expected to be hotter with more frequent extreme heat (Daghir, 2008; Zumbach et al. 2008).

Due to the changing global environment, the impact of heat stress is particularly important. In livestock species, the impact of heat stress is reduced productivity and welfare (Sejian et al. 2018). Immune responses that are cell-mediated and humoral might be adversely affected by heat stress (Bagath et al. 2019). Thus, hot weather can make animals more susceptible to disease and increased the prevalence of certain diseases (Chirico et al., (1997); Mashaly et al. 2004; Dahl et al. 2020). Some breeds, such as smaller, lighter-colored animals, are less susceptible to heat stress (Hoffmann, 2013), compared to dark-colored animals, which reflect 50–60 % of the direct sun's energy (McManus et al. 2009). If these characteristics are inherited, selective breeding for heat tolerance may be utilized to enhance an animal's capacity to adapt to environmental stress (Hayes et al. 2013; Renaudeau et al. 2012). Indigenous sheep breeds that are well adapted to dry and semi-arid regions show some specific morphological traits (such as carpet-type wool), which may act as a protective barrier against direct sun radiation and allow for the efficient cutaneous evaporative dissipation of heat (Mahgoub et al. 2010). The tail fat deposition is another obvious morphological trait in these animals for better heat transfer (Gootwine, 2011). Reduced feed intake which helps to decrease heat production in warm environments is another well-documented behavioral response to heat stress. According to previous studies, sheep and goats naturally consume less feed in the summer (Spiers et al. 2004; Shilja et al. 2016). It is also reported that, one of the important physiological mechanisms relating to hot adaptation is accelerating breath (Naga et al. 2021). Breeds adapted to desert areas compensate for the higher water loss during high heat loads by condensing the urine (Chedid et al. 2014). For example, Kim et al. (2016) identified several candidate regions in Barki Sheep, potentially

associated with adaptation to hot dry conditions. The majority of the identified genes are those involved in multiple signal pathways in a wide variety of cellular and biochemical processes, including adaptation to the hot, dry environment, thermo-tolerance melanogenesis, body size and development, energy and digestive metabolism (Kim et al. 2016). A number of genes related to heat stress adaptation and immunity were identified by comparing the whole genome data of native sheep populations from Iran's hot, dry desert and cold regions. The most significant of these genes are discussed below. Several candidate genes were identified as being involved in the following processes: (1) responding to heat stress (*SIK2*, *FER*, *ATP1A1*, *CDK5RAP3*, and *TLR4*); (2) immune response in hot and dry conditions (*CD109*, *CR2*, *EOMES*, and *MARCHF1*); (3) response to drought stress and adaptation to desert locations (*ZFP1*, *PLCB1*, and *PDGFD*); and (4) response to heat stress via regulating digestive metabolism (*HTR4*, *TRHDE*, and *ALDH1A3*).

#### 4.3. Candidate genes responding to heat stress

The *SIK2* gene (salt inducible kinase 2, Chr. 5 sheep, top 5 % for FST) is known to have a significant role in the response to heat stress and has been shown to enhance the capacity of Antarctic fish to adapt to rising temperatures, according to previous studies (Bilyk et al. 2018). The *FER* (*FER* tyrosine kinase, Chr. 5 sheep, top 5 % for FST) gene is a cellular antioxidant that controls the amount of iron ions available in the Fenton reaction to prevent the production of reactive oxygen species (Orino et al., 2001). It was reported with increasing the expression of antioxidant genes, included *FER* in the intestine of red swamp crayfish that had been exposed to heat stress. This shows that acute heat stress negatively affects the immune system and the antioxidant status in the intestine (Guo et al. 2020; Guo et al., (2022)). The *ATP1A1* (ATPase  $\text{Na}^+/\text{K}^+$  transporting subunit alpha 1, Chr. 1, top 1 % for Pi) gene encodes the subunit of the  $\text{Na}^+/\text{K}^+$  ATPase enzyme. Heat stress results in oxidative stress and has an impact on the plasma's ATPase enzyme-generated alternation of ion gradients, which are suitable candidates for heat tolerance characteristics. This gene is significant for the heat tolerance coefficient, according to an analysis of the association between the *ATP1A1* gene and rectal temperature in Jersey cows under heat stress (Das et al. 2015). One of the main defense mechanisms in cells is the heat shock response. As a new nucleoplasmic shuttle or molecular chaperone, the *CDK5RAP3* gene (*CDK5* regulatory subunit associated with protein 3, Chr. 11, top 1 % for Pi) regulates the cellular stress response. The findings indicate that *CDK5RAP3* gene actively contributes to the heat stress response and shields cells from heat damage (Shen et al. 2020). On sheep's chromosome 2 there is a known gene (toll-like receptor 4 (*TLR4*), top 5 % for FST), which is a member of the Toll-like receptor family (*TLRs*). Toll-like receptors are pattern-recognition cells that detect molecular patterns associated with tissue damage and activate

signaling cascades to cause inflammatory responses. According to analysis of tissue samples from Indian Catla fish that had been subjected to thermal stress (Basu et al. 2015), the *TLR4* gene is implicated in the signaling pathway during thermal stress.

#### 4.4. Genes related to immune response in hot and dry conditions

The high-temperature surroundings makes problems for animals' health through the inhibition of the immune system, which results in an increased disease incidence. The *CD109* gene, is identified in this study, belongs to the complement gene family (*CD109* molecule, Chr. 8, top 5 % for FST and top 1 % for Pi). Its differential expression in various human carcinomas has been documented (Hashimoto et al. 2004; Zhang et al. 2005). Moiola et al. (2015) introduced the *CD109* gene as a paratuberculosis disease resistant gene (Moioli et al., 2015). In addition, the *CR2* (complement C3d receptor 2, Chr. 12 sheep, top 5 % for FST and top 1 % for Pi) gene, involved in early complement components for normal antibody responses, is mostly expressed on B cells. The findings suggest that the immune system utilizes three effective functions mediated by *CR2* to create optimal antibody responses (Rutemark et al. 2012). Another identified gene in this study is the *EOMES* (eomesodermin, Chr. 19, top 1 % for Pi). The *EOMES* is one of the two T-box proteins that are expressed in the immune system and has a role in driving the differentiation and activity of natural killer (NK) cells. NK cells contribute to the primary defense against pathogens and tumors (Zhang et al. 2018). The *EOMES* gene has been discovered in chicken selection signatures from arid and tropical environments (Walugembe et al. 2019). Also, the *MARCHF1* gene, which participates in molecular-cellular signaling and transport activities, has been related to the development of thermal resistance in a heat-exposed pigs by stimulating the immune system's activity (He et al. 2020).

#### 4.5. Genes associated with response to drought stress and adaptation to desert locations

Water availability and its utilization in animal production are predicted to decrease as a result of climate change (Field and Barros, 2014; Masson-Delmotte et al. 2021). In the coming decades, there will be increasing competition among livestock for water, and to address the problem of water shortages, more effective production methods are needed (Reynolds et al. 2010). In this study, the *ZFP1* gene (zinc finger protein 1, Chr. 14, top 5 % for FST) was identified as a novel candidate gene for drought and water scarcity stresses. According to a study, zinc finger domains are crucial for governing processes like cell development and programming as well as adaptive responses to environmental stress in eukaryotic cells. The results of the study of the genetic diversity of cotton plant species have shown that the expression of the *ZFP1* gene increases under the conditions of drought stress and water scarcity (Ciftci-Yilmaz and Mittler, 2008). *PLCB1* (phospholipase C beta 1, Chr. 13, top 5 % for FST), which hydrolyzes phospholipids into fatty acids and other lipophilic molecules, is another gene that was founded in this investigation. Previous studies show that the *PLCB1* gene has undergone positive selection in sheep and goats as a result of adaptation to dry environments (Kim et al. 2016), as well as in African cattle for heat tolerance (Taye et al. 2017). Sheep's fat tail may be a crucial energy source for dealing with upcoming weather changes. The *PDGFD* (platelet-derived growth factor D, Chr. 15, top 1 % for Pi) gene is involved in the fat-tailed phenotype through the differentiation of preadipocytes. This gene supports the adaptation of Iranian fat-tailed sheep to live in the dry, desert environment, as it has been reported as a signature of adaptation to desert environments by selective sweeps analyses in Chinese sheep (Mastrangelo et al. 2018).

#### 4.6. Genes associated with response to heat stress via regulating digestive metabolism

Livestock will consume less food and engage in fewer rumination activities under heat stress. As a result, less heat is generated by the metabolism, and the body temperature will stay constant. In response to heat stress, the *HTR4*, *ALDH1A3*, and *TRHDE* genes regulate digestive metabolism. Expressed protein with 5-Hydroxytryptamine receptor 4 (*HTR4*, Chr. 5 sheep, top 1 % of Pi) gene has important regulatory effects on digestive sensitivity and food intake. Through a transcriptome investigation of liver tissue in sheep, Lu et al. (2019) demonstrated that heat stress dramatically elevated the expression of the *HTR4* gene (Lu et al. 2019). The *TRHDE* (thyrotropin-releasing hormone degrading enzyme, Chr. 3 sheep, top 5 % of FST) gene encodes an extracellular peptidase that cleaves and inactivates thyrotropin-releasing hormone, thereby reducing appetite and metabolism. Thyrotropin-releasing hormone plays a key role in controlling metabolism and appetite (Friedman et al. 1995). The aldehyde dehydrogenase enzyme is encoded by the *ALDH1A3* (aldehyde dehydrogenase 1 family member A3, Chr. 18, top 1 % of Pi) gene. Studies on Barki sheep in the Egyptian desert have shown that the *ALDH1A3* and *TRHDE* genes indirectly influence the adaptability to hot and dry conditions through the energy metabolism and digestive system (Kim et al. 2016).

### 5. Conclusions

This work is the first attempt to identify candidate genes relevant to adaptation to Iran's predominant climate (hot and desert) using whole-genome sequencing data from a significant number of native Iranian breeds (ten different breeds), and it also examined the population structure of native Iranian sheep breeds in comparison to various breeds from Europe, Asia, and Africa. The *SIK2*, *FER*, *CDK5RAP3*, *TLR4*, and *ZFP1* genes were discovered novel candidate genes involved in heat stress adaptation and more research are needed to determine their phenotype-genotype relationships. Our findings may advance our knowledge of desert adaptation mechanisms and can also help with focused selection. A caveat of our study is that although a group of genes related to climate changes were identified, the relationship of the genotype and phenotype still needs additional functional evidence. Whole-genome sequences from multiple individuals from different native breeds would be necessary for validating the potential selective genomic regions, which should also lead to the determination of additional important mutations responsible for the adaptation to climate seen in the native Iranian sheep breeds.

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### Ethics approval and consent to participate

The ARRIVE guidelines 2.0 (<https://arriveguidelines.org/>) were followed in conducting of this study. The Shahid Bahonar University's animal science ethics committee in Kerman, Iran, gave its approval to all experimental protocols and blood sampling techniques. There were no killed or injured sheep. The Karakul Sarakhs Sheep Breeding Station, the Babol Livestock Gene Bank, and the Shahid Bahonar University of Kerman in Kerman, Iran have relevant rules and regulations that were followed in this investigation.

**Consent for publication**

Not applicable.

**CRedit authorship contribution statement**

**MRM, HAN and DMK** led, proposed and innovated the study. **LM** collected samples. **HAN, OAK and RVS** analyzed the data. **LM, OK and ZAG** explained the data and prepared the manuscript. All authors revised and certified the last version of manuscript.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Appendix A. Supporting information**

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.smallrumres.2023.107018](https://doi.org/10.1016/j.smallrumres.2023.107018).

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