TÜBİTAK

Turkish Journal of Zoology
http://journals.tubitak.gov.tr/zoology

Turk J Zool
(2021) 45: 455-463

Research Article

doi: 10.3906/zoo-2104-3

Effect of breeds on heat-evoked gene responses in sheep adipose tissue

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Received: 02.04.2021 **Accepted/Published Online:** 21.07.2021 **Final Version:** 14.09.2021

Abstract: Sheep is one of the ungulates and critical for animal husbandry development like on grassland, whereas there are few pieces of research about their heat responses to date. In the present study, we selected two sheep breeds consisting of Mongolian sheep with evolving cold resistibility and Dorper sheep specifically bred for heat tolerance to explore how the different breeds of sheep positively respond to heat conditions. Three Mongolian sheep and 3 Dorper sheep underwent 37 °C stress (2 h per day) during 4 weeks. The energy metabolism-associated adipose tissues of the experimental subjects were sampled, prepared and performed to RNA-seq following heat condition. Through the analysis and comparison of sequencing outcomes on such aspects as the quality control, sequence alignment and quantification, we obtained 236 annotated protein-coding genes as well as 98 long noncoding RNA (lncRNA) genes with expression differences. Following Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis measures, ten protein-coding genes were screened as active genes triggered by heat stress in adipose tissue in which candidates referred to *DNAJA1*, *FABP1*, *HMOX1*, *NABP1*, *PNP*, *FNDC5*, and *PTGS2* of Mongolian sheep and *PPP5C*, *PARP4*, and *SOD3* of Dorper sheep. The biological functions of the candidate genes were severally targeted to heat shock protein binding, oxidation resistance, DNA repair, and brown adipocyte differentiation. Taking these findings as a basis, we hope to promote comprehending on heat-tolerance of sheep and provide some inspiration to molecular stockbreeding.

Key words: Sheep, heat response, adipose tissue, RNA-seq, lncRNA

1. Introduction

Sheep (*Ovis aries*) belong to ruminants, and their diets primarily include grasses, plant leaves, and lichens (Clutton-Brock, 1999). Mongolian sheep originated from the Mongolian plateau have a strong physique and good cold-tolerance and, as an ancient sheep breed in China, are widely distributed in Inner Mongolia with long and severe winters and short warm summers, while Mongolian sheep also have an excellent antioxidant capacity with prolificacy (Hou, 2014). In South African, Dorper sheep are cross-bred by Dorset Horn sheep and Blackheaded Persian sheep since the 1930s, because of the initial development in the arid areas they can positively respond to dehydration and rapidly supplement weight loss through available water (Degen and Kam, 1992; Cloete et al., 2000). As one of the most prolific sheep breeds, Dorper sheep characterized with resistance to extremely high summer temperature and radiation as the main breeding direction can adapt to various climate and grazing conditions and, is widely located in South Africa with abundant sunshine and high temperature in summer (Cloete et al., 2000; Milne, 2000; Briggs, 2018). There is report indicating that the modifications in different pathways like body size, physiological responses, and energy metabolism achieve heat-resistant capacity of sheep, but not via the coat type (McManus et al., 2020). Additionally, heat stress negatively influences the physiological responses and oxidative status of sheep, while high dietary antioxidants supplementation possibly contributes to protecting sheep from heat stress through regulating skeletal muscle expression of NF-κB transcription, proinflammatory cytokine, and heat shock proteins (Chauhan et al., 2014a,b). In accordance with some polymorphisms in the gene encoding the inducible form of the cytoplasmic Hsp90 (HSP90AA1), sheep of bearing the CC(-660) genotype than that of presenting the CG(-660) or GG(-660) exhibit higher expression levels in summer (Marcos-Carcavilla et al., 2010). Two SNPs including G/C-660 and A/G-444 located at the *HSP90AA1* promoter are coupled with heat stress-induced gene overexpression (Salces-Ortiz et al., 2013). Research on *HSP90* and *HSP70* gene polymorphism demonstrates that TACCA haplotype combination of multi-SNPs possesses underlying selection advantage for the identification of sheep more adaptable to heat stress (Singh et al., 2017). So far, the researches on Mongolian sheep and Dorper sheep mainly focus on growth, reproduction rate and adaptability, but the study of gene expression and posttranscriptional regulation coupled to heat response is still inadequate.

Ribonucleic acids, a class of polymer molecules in the organism, play essential biological roles in gene coding, gene decoding and gene expression regulation. It is well known that messenger RNA (mRNA) is used to transmit genetic information and guide protein synthesis in cells. In addition to the mRNA transcribed by protein-coding gene, long noncoding RNA (lncRNA) is also very pivotal for animal life activities and is generally defined as a nonprotein-coding transcript with a length of more than 200 nucleotides (Perkel, 2013). At posttranscriptional level, *cis*-acting lncRNAs (*cis*-lncRNAs) regulate the expression of genes in close genomic proximity via transcriptional interference or chromatin modification, while *trans*-acting lncRNAs (*trans*-lncRNAs)

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control the expression of distant genes by recruiting chromatin-modifying complexes and binding to transcription elongation factors or RNA polymerases (Ma et al., 2013).

Adipose tissue maintains energy balance via storing and expending energy, moreover, adipocytes secrete free fatty acids and lipids to other tissues like muscle and liver (Leiria and Tseng, 2020). Using the RNA-seq method (Wang et al., 2009) and statistical strategy, the present work is conducive to investigate mRNAs and lncRNAs induced by heat stress in fat depot-associated white adipose tissues, through comparing Mongolian sheep with Dorper sheep. Following gene annotation and enrichment, and analyses of mRNA-lncRNA colocation and coexpression, we expect to reveal the effect of sheep breeds on molecular actions, further interpreting how sheep adipose tissue respond to heat condition.

2. Materials and methods

2.1. Animal groups distribution and treatment

Procedures involving animals, their care, and humane kill were conducted in conformity with Guidelines on the Humane Treatment of Laboratory Animals (HTLA Pub. Chapter 2–6, revised 2006 in China) and were approved by the Animal Care and Use Committee of the Inner Mongolia Agricultural University (IMAU-IACUC-2019-31360271). The written informed consent of experimental research achieved by the animal owner was obtained. The adult female Mongolian sheep and Dorper sheep were individually signed as test groups and baseline group with 3 subjects per groups (Appendix). The six sheep were incubated at 37 ℃ stress and underwent 2 h per day over a 4 week period. All of the sheep were sacrificed through bleeding of the carotid artery following intravenous injection of 20 mg/kg pelltobarbitalum natricum. Then we collected the white adipose tissues and kept the tubes loaded with samples in liquid nitrogen (–196 °C).

2.2. RNA extraction, quantification, and qualification

RNeasy Mini Kit (QIAGEN, Germany) worked for isolating total RNA from adipose tissue. RNA purity was assessed by the NanoPhotometer spectrophotometer (IMPLEN, USA) after the detection of degradation and contamination using agarose gels. The Qubit 2.0 Fluorometer with Qubit RNA Assay Kit (Life Technologies, USA) and the Bioanalyzer 2100 system with RNA Nano 6000 Assay Kit (Agilent Technologies, USA) individually monitored RNA concentration and integrity.

2.3. Library construction and sequencing

Taking total RNA as input stuff, the amount of 3 μg each sample served to establish the lncRNA library. Epicentre Ribo-zero rRNA Removal Kit (Epicentre, USA) fulfilled ribosomal RNA removing, then rRNA free residue was cleaned up by ethanol precipitation. Through using rRNA-depleted RNA by NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB, USA), the library generation went on according to the following programs: first-strand cDNA synthesis, second-strand cDNA synthesis, end repair, adenylation of 3' ends of DNA fragments, adaptor ligation, the selection of cDNA fragment size, U-excision, PCR and product purification, quality identification of library. The cBot Cluster Generation System involving TruSeq PE Cluster Kit v3-cBot-HS (Illumina, USA) and Illumina HiSeq 4000 platform was exerted for index-coded samples clustering and library construction via RNA-seq (Konczal et al., 2014) in the light of the manufacturer's instructions.

2.4. Quality control, sequence alignment, and transcriptome assembly

The approaches removing reads with adapter, ploy-N, and low quality converted raw data into clean data which Q20, Q30, and GC content were further computed. Reference genome file was downloaded from *Ovis aries* genome Ensembl Release-81 (ftp://ftp. ensembl.org/pub/release-81/fasta/ovis_aries/) (Oliveira et al., 2020). Index of the reference genome was created by Bowtie (v2.0.6) (Langmead et al., 2009) while paired-end clean reads were mapped to the reference genome by TopHat (v2.0.9) (Kim et al., 2013). Cufflinks (v2.1.1) (Trapnell et al., 2010) was implemented in the aligned reads assembly for each sample.

2.5. Terminal data analysis

CNCI (v2) (Sun et al., 2013), CPC (0.9-r2) (Kong et al., 2007), Pfam Scan (v1.3) (Bateman et al., 2002; Punta et al., 2012), PhyloCSF (v20121028) (Lin et al., 2011) were applied to distinguish protein-coding and noncoding sequences through adjoining nucleotide triplets, the extent and quality of the open reading frame in the transcript, corresponding known protein family domains, and evolutionary signatures characteristic to alignments of conserved coding regions in turn. The protein-coding and lncRNA genes were annotated by *Ovis aries* databases, and the quantification and differential analysis linked with gene expression was performed by using Cuffdiff (v2.1.1) (Trapnell et al., 2012). According to cis and trans roles, the lncRNA targeting genes were envisioned as colocated characteristic since upstream/downstream 100 kb localized on lncRNA gene, and coexpressed feature owing to Pearson correlation coefficient in line with the absolute value of Pearson correlation higher than 0.95 (Ma et al., 2013). Metascape (https://metascape.org) (Zhou et al., 2019) was invoked to build protein-protein interaction (PPI) network, and analyze Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

3. Results

3.1. Differential gene expression and target prediction

As shown in statistic results of RNA-seq data, 236 of 495 protein-coding genes identified as the significant difference ($p < 0.05$) are completely annotated, comprising 160 upregulated genes (e.g., *AACS* and *TGM5*) and 76 downregulated genes (e.g., *QPCT* and *NDNF*) in Mongolian sheep compared to Dorper sheep (Figures 1A and 1B). There were palpably ($p < 0.05$) upregulated 50 (e.g., XLOC_4890562 and XLOC_816003) and downregulated 58 lncRNA genes (e.g., XLOC_3472117 and XLOC_5086154) detected in the adipose tissue (Figure 1C). Along with 2 active lncRNA transcripts originated from XLOC_1801419 and XLOC_5119031, XLOC_1443013 and XLOC_1801452 generated 3 lncRNA transcripts of higher expression, respectively. XLOC_4747998

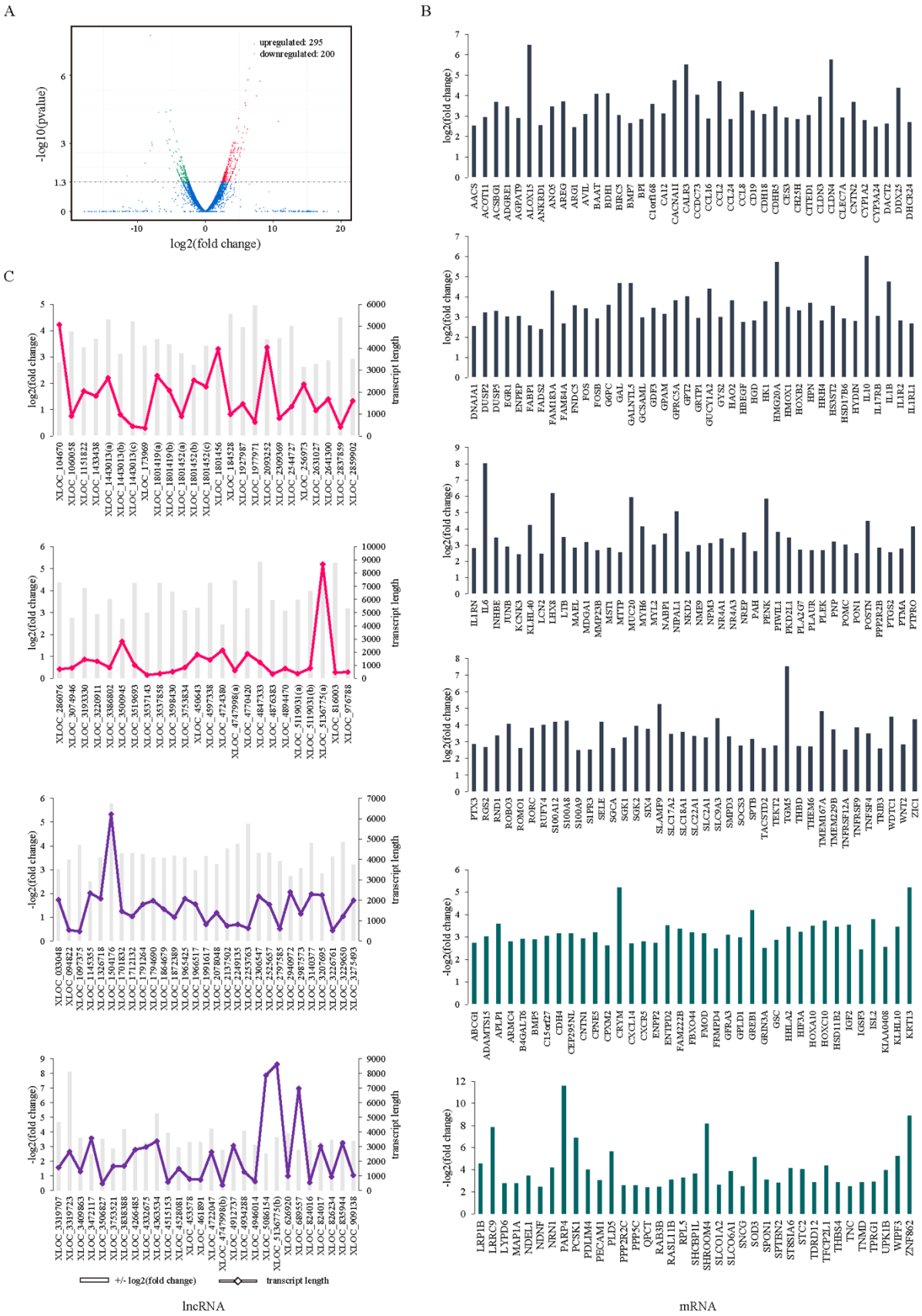


Figure 1. Differential genes in adipose tissue. (A) Number of protein-coding genes of differential expression. Log2(fold change) value of differential transcripts of protein-coding genes (B) and lncRNA genes (C). a or b or c indicates different transcripts from same lncRNA gene.

as well as XLOC_5136775 presented 1 upward and 1 downward regulation of transcripts. Notably, *IL-8* and XLOC_4890562 had specific expression in the adipose tissue of Mongolian sheep while *DGKG*, *CCDC170*, and XLOC_819851 were uniquely invoked in the fat tissue of Dorper sheep (Figure 2A). The transcentric role was exposed between differentially expressed lncRNA and their 2454 potential targets.

3.2. Establishment of proteins interaction network

The PPI network was built and ranged over 76 participants based on the proteins corresponded by 236 protein-coding genes of differential expression. DNAJA1 had more partners (DEGREE = 10) with the interactive relations, followed by POMC (DEGREE = 8) (Figure 2B). Three clusters were MCODE1, MCODE2, and MCODE3, while here were their independent members: MCODE1 referring to CCL16, CXCR5, POMC, HRH4, PENK, GAL and S1PR3; MCODE2 including PPP2R2C, RPL5, HK1, DNAJA1 and PPP2R2B; MCODE3 consisting of FOS, JUNB and FOSB (Figure 2B).

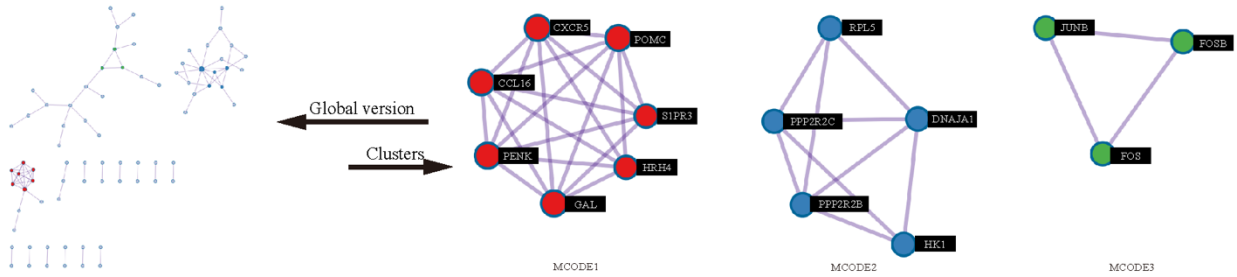
3.3. Enrichment analyses coupled with GO terms and KEGG pathways

The 160 upregulated and 76 downregulated protein-coding genes were recruited significantly ($p < 0.05$) to 72 (e.g., calcium ion binding and growth factor receptor binding) and 16 (e.g., heparin-binding and lipid binding) molecular functions, 996 (e.g., cell chemotaxis and leukocyte migration) and 141 (e.g., cell part morphogenesis and exocrine system development) biological processes, 49 (e.g., cytoplasmic vesicle lumen and specific granule lumen) and 20 (e.g., collagen-containing extracellular matrix and basement membrane) cellular components, 49 (e.g., cytokine-cytokine receptor interaction and adipocytokine signaling pathway) and 1 [Cell adhesion molecules (CAMs)] pathways (Figures 2C and 3A). In accord with statistical significance ($p < 0.05$), the underlying targets aimed by differential lncRNAs were enriched to 448 (e.g., kinase binding and transcription factor binding) molecular functions, 2769 (e.g., actin cytoskeleton organization and plasma membrane-bounded cell projection morphogenesis) biological processes, 373 (e.g., adherens junction and focal adhesion) cellular components, and 141

A

	mRNA	lncRNA
Mongolian sheep	IL-8	XLOC_4890562
Dorper sheep	DGKG CCDC170	XLOC_819851

B



C

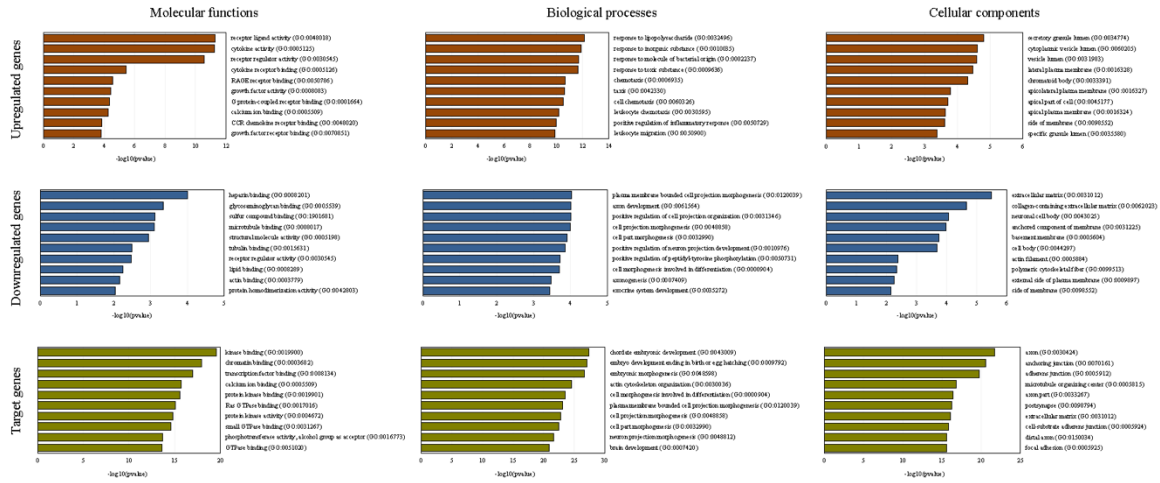


Figure 2. Specific genes and machine learning. (A) Breed-specific expression of genes in adipose tissue under heat stress. (B) Differential protein-coding genes corresponding PPI network. (C) Top 10 of Gene Ontology terms enriched by differential protein-coding genes and potential targets of differential lncRNAs, respectively.

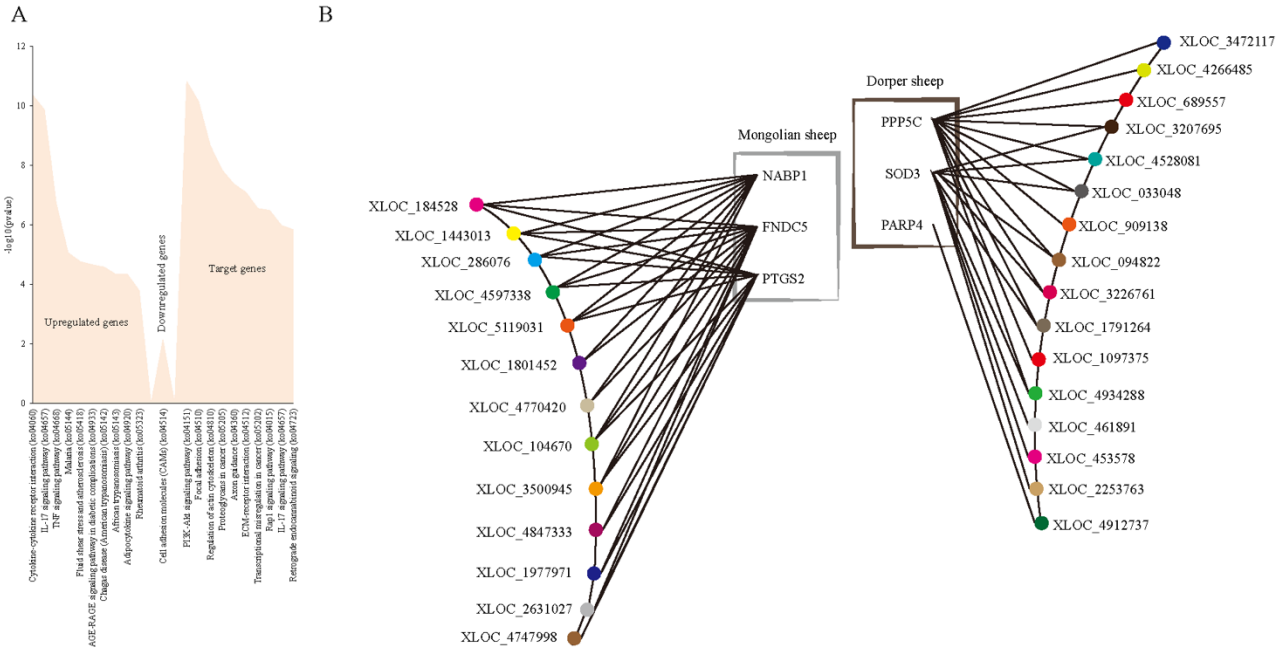


Figure 3. Pathway analysis and lncRNA-target relations. (A) Top 10 of KEGG pathways recruited individually by differentiated protein-coding genes and underlying targets of differentiated lncRNAs. (B) Screening of potential lncRNA-target relations in response to heat.

(e.g., ECM-receptor interaction and retrograde endocannabinoid signaling) pathways (Figures 2C and 3A).

3.4. Screening of protein-coding genes and lncRNA genes in response to heat stress

Based on enrichment analysis, the heat-responsive protein-coding genes were screened. Seven upregulated protein-coding genes including *DNAJA1*, *FABP1*, *HMOX1*, *NABP1*, *PNP*, *FNDC5* and *PTGS2* were identified as positive heat-response genes in adipose tissue of Mongolian sheep while the biological effect of the 7 genes was severally focused on positive regulation of HSP70 binding, peroxidase activity, mitochondrial DNA repair and brown adipocyte differentiation. The 3 downregulated genes containing *PPP5C*, *PARP4* and *SOD3* were selected as positive regulated genes for heat stress in adipose of Dorper sheep with HSP90 binding, superoxide dismutase activity and DNA repair, respectively.

Taking *cis*- and *trans*-acting of lncRNA as selection criteria, 101 differentially expressed protein-coding genes were screened as potential genes targeted by differential lncRNA genes. Subsequently, XLOC_4847333, XLOC_1977971, XLOC_184528, XLOC_1443013, XLOC_286076, XLOC_4597338, XLOC_5119031, XLOC_1801452, XLOC_4770420, XLOC_104670, XLOC_2631027, XLOC_3500945 and XLOC_4747998 were predicted to mediate gene expression of *NABP1*, *FNDC5* and *PTGS2* potentially related to positive heat response by *trans*-acting in adipose tissue of Mongolian sheep, while XLOC_3472117, XLOC_4266485, XLOC_689557, XLOC_3207695, XLOC_4528081, XLOC_033048, XLOC_909138, XLOC_094822, XLOC_3226761, XLOC_1791264, XLOC_1097375, XLOC_4934288, XLOC_461891, XLOC_453578, XLOC_2253763 and XLOC_4912737 were performed regulation to *PPP5C*, *SOD3* and *PARP4* (Figure 3B).

4. Discussion

Mongolian sheep and Dorper sheep are developed from two different environment. Evidence shows that, in new environments, change in gene expression serves to ecological speciation via enhancing population persistence (Rajkov et al., 2021). For rapid evolution, one putative route is through variation in the expression of genes affecting traits under selection (Hamann et al., 2021). Some changes in expression of metabolism- and physiology-related genes also signify that natural environment can alter gene expression (Krishnan et al., 2020). In terms of livestock breeding, the heat-tolerance is generally considered as one of the important objectives, but the class, quantity and characterization of genes coupled to heat response are still vague in sheep. Our study presented 236 annotated protein-coding genes and 108 lncRNA genes with appreciably differential expression. By analyzing the top ten GO terms and KEGG pathways based on statistical significance, we found that calcium ion binding (GO:0005509) in molecular functions, plasma membrane-bounded cell projection morphogenesis (GO:0120039), cell projection morphogenesis (GO:0048858), cell part morphogenesis (GO:0032990), cell morphogenesis involved in differentiation (GO:0000904) in biological processes, extracellular matrix (GO:0031012) in cellular components, IL-17 signalling pathway (ko04657) in pathways as common were enriched by upregulated/downregulated protein-coding genes and lncRNA targeting genes. Certain of these terms also were tightly linked to thermoregulation, such as growth factor activity (GO:0008083) and positive regulation of inflammatory response (GO:0050729). The cold condition causes fibroblast growth factor 21 expression with increased

circulating levels, cooccurring with brown fat thermogenesis (Klein Hazebroek and Keipert, 2020). The increased levels of IL-4 and IL-10, the intensive thermogenic protein activity and browning in adipose tissues invest Fas-mutant mice with high-fat diet-elicited obesity resistance (Choi et al., 2020).

The previous extensive researches are focused on heat shock proteins (HSPs), produced by cells in response to stress, which is related to heat shock (Ritossa, 1962) and expressed under cold and ultraviolet exposure (Matz et al., 1995; Cao et al., 1999) and during wound recovery or tissue remodeling (Laplante et al., 1998). HSPs mainly induced by heat shock factor play essential roles in heat shock response, and the members of the HSP family usually perform molecular chaperone function by the folding and translocation of polypeptides across membranes and the resolubilization of denatured proteins under the stress (Wu, 1995; De Maio, 1999). In addition to HSP, we discovered that there is a positive correlation between antioxidant capacity and heat-tolerance based on the previous studies of fish (Lu et al., 2016), *Gracilaria lemaneiformis* (Lu et al., 2012) and wheat seedling (Kolupaev and Oboznyĭ, 2012). As one of the most heat-tolerance life forms, *Bacillus amylolyticus* can survive from DNA damage caused by exposure to 420 ℃ high temperature via an effective DNA repair mechanism (Beladjal et al., 2018).

Given the past studies, the enhanced heat shock protein binding, antioxidant capacity and DNA repair are closely associated with effective heat-tolerance. Besides Dorper sheep, the heat-responsive genes were also enriched in heat shock protein binding, antioxidant capacity and DNA repair in adipose tissue of Mongolian sheep. Concerning heat shock protein binding, *DNAJA1* exhibits upregulated expression in adipose tissue of Mongolian sheep with more interactive partners, while prior studies indicate that DnaJ heat shock protein family (HSP40) member A1 encoded by *DNAJA1* binds HSP70 with 70 kilodaltons and mediates thermal adaptation of organisms by protein folding and unfolding (Chellaiah et al., 1993; Terada and Mori, 2000; Gotoh et al., 2004; Ajayi et al., 2018). *PPP5C* was highly expressed in adipose tissue of Dorper sheep. As chaperone protein of HSP90 with 90 kilodaltons, protein phosphatase 5 catalytic subunit encoded by *PPP5C* is active responded to heat through maintenance of steroid receptors and transcription factors (Schlesinger, 1990). Compared with HSP90, HSP70 is more effective in capturing unfolded proteins (Wegele et al., 2006). In the aspect of antioxidation, the expression of *FABP1* and *HMOX1* is upregulated in the adipose tissue of Mongolian sheep. Fatty acid-binding protein 1 encoded by *FABP1* and heme oxygenase 1 encoded by *HMOX1* protect cells from the damage mediated by hydrogen peroxide (Wang et al., 2005; Lin et al., 2007), however, as part-time antioxidant protein, their functions mainly focus on long-chain fatty acids binding and heme decomposition (Nelson and Cox, 2008; Smathers and Petersen, 2011; Huang et al., 2016; Schroeder et al., 2016). The expression level of *SOD3* is high in the adipose tissue of Dorper sheep. Previous researches present that superoxide dismutase 3 encoded by *SOD3* catalyzes the disproportionation of superoxide radical (O_2^-) to an ordinary oxygen molecule (O_2) (Carlsson et al., 1996; Freiberger et al., 2004; Hayyan et al., 2016). On DNA repair, the expression of *NABP1* and *PNP* was upregulated in adipose tissue of Mongolian sheep. *NABP1* encoding nucleic acid-binding protein 1 is integral for a variety of DNA metabolism encompassing replication, recombination, damage detection and repair (Richard et al., 2008; Huang et al., 2009). *PNP* encoding purine nucleoside phosphorylase participates to accelerate the repair of mitochondrial DNA by preventing the accumulation of mitochondrial dGTP (Arpaia et al., 2000). A high expressed level of *PARP4* was detected in adipose tissue of Dorper sheep. Poly (ADP-ribose) polymerase family member 4 encoded by *PARP4* as a critical nick sensor is necessarily required in nicking and rejoining of DNA strands (Jean et al., 1999). As an exciting discovery, the expression levels of *FNDC5* and *PTGS2* related to positive regulation of the differentiation of brown adipocytes were upregulated in adipose tissue of Mongolian sheep (Vegiopoulos et al., 2010; Boström et al., 2012). Brown adipose tissue contains abundant small fat droplets and mitochondria (Enerbäck, 2009) while nonshivering heat production is mediated by uncoupling protein promotes cold-tolerance (Hayward and Lisson, 1992; Cannon and Nedergaard, 2004; Emmett et al., 2017). Moreover, according to the research of FOK rat adapting to the hot environment, brown adipocyte demonstrates low reactivity to weak sympathetic stimulation in thermogenesis while FOK rats achieve heat-tolerance through low heat production of brown adipocytes under 39.9 ℃ heat stress (Tanaka et al., 1997). In adipose tissue of Mongolian sheep and Dorper sheep, the above *NABP1*, *FNDC5*, *PTGS2*, *PPP5C*, *SOD3* and *PARP4* were predicted as targeted genes that regulated by 29 lncRNAs, such as XLOC_184528, XLOC_3207695 and XLOC_4912737.

In conclusion, RNA-seq data evidence that Mongolian sheep and Dorper sheep may positively respond to heat stress by triggering HSP binding, DNA repair and antioxygenation in white adipose tissue with lncRNA regulation. However, the molecular mass and function of HSPs aimed by the high expressed chaperone genes are different in Mongolian sheep and Dorper sheep. Mongolian sheep increased the gene expression of mitochondrial DNA repair but lacked the full-time antioxidant gene like *SOD3* of Dorper sheep adipose tissue. In addition, Mongolian sheep potentially improve heat adaptation by invoking genes coupled to positive regulation of brown adipocyte differentiation that Dorper sheep do not possess. Although Mongolian sheep were not bred specifically for heat tolerance, they show heat-tolerance actions at the molecular level. Focusing on the heat-tolerance genes in the future, samples increasement, quantitative detection, and further cellular study would be favorable for verifying these results. We hope that our findings can assist people to comprehend the heat response of sheep and inspire sheep's molecular breeding on heat endurance.

Acknowledgment
The study was funded by the National Natural Science Foundation of China (31360271).

Conflict of interest
The authors declare no conflicts of interest.

Abbreviations: *cis*-lncRNAs, *cis*-acting lncRNAs; HSP, heat shock protein; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long noncoding RNA; PPI, protein-protein interaction; *trans*-lncRNAs, *trans*-acting lncRNAs.

References

Ajayi OO, Peters SO, De Donato M, Mujibi FD, Khan WA et al. (2018). Genetic variation in N- and C-terminal regions of bovine DNAJA1 heat shock protein gene in African, Asian and American cattle. Journal of Genomics 6: 1-8. doi: 10.7150/jgen.23248

Arpaia E, Benveniste P, Di Cristofano A, Gu Y, Dalal I et al. (2000). Mitochondrial basis for immune deficiency. Evidence from purine nucleoside phosphorylase-deficient mice. Journal of Experimental Medicine 191 (12): 2197-2208. doi: 10.1084/jem.191.12.2197

Bateman A, Birney E, Cerruti L, Durbin R, Etwiller L et al. (2002). The Pfam protein families database. Nucleic Acids Research 30 (1): 276-280. doi: 10.1093/nar/30.1.276

Beladjal L, Gheysens T, Clegg JS, Amar M, Mertens J (2018). Life from the ashes: survival of dry bacterial spores after very high temperature exposure. Extremophiles 22 (5): 751-759. doi: 10.1007/s00792-018-1035-6

Boström P, Wu J, Jedrychowski MP, Korde A, Ye L et al. (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481 (7382): 463-468. doi: 10.1038/nature10777

Briggs P (2018). Weather and Climate - South Africa. SAFARIBOOKINGS.

Cannon B, Nedergaard J (2004). Brown adipose tissue: function and physiological significance. Physiological Reviews 84 (1): 277-359. doi: 10.1152/physrev.00015.2003

Cao Y, Ohwatari N, Matsumoto T, Kosaka M, Ohtsuru A et al. (1999). TGF-beta1 mediates 70-kDa heat shock protein induction due to ultraviolet irradiation in human skin fibroblasts. Pflugers Arch 438 (3): 239-244. doi: 10.1007/s004240050905

Carlsson LM, Marklund SL, Edlund T (1996). The rat extracellular superoxide dismutase dimer is converted to a tetramer by the exchange of a single amino acid. Proceedings of the National Academy of Sciences of the United States of America 93 (11): 5219-5222. doi: 10.1073/pnas.93.11.5219

Chauhan SS, Celi P, Fahri FT, Leury BJ, Dunshea FR (2014a). Dietary antioxidants at supranutritional doses modulate skeletal muscle heat shock protein and inflammatory gene expression in sheep exposed to heat stress. Journal of Animal Science 92 (11): 4897-4908. doi: 10.2527/jas.2014-8047

Chauhan SS, Celi P, Leury BJ, Clarke IJ, Dunshea FR (2014b). Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. Journal of Animal Science 92 (8): 3364-3374. doi: 10.2527/jas.2014-7714

Chellaiah A, Davis A, Mohanakumar T (1993). Cloning of a unique human homologue of the Escherichia coli DNAJ heat shock protein. Biochimica et Biophysica Acta 1174 (1): 111-113. doi: 10.1016/0167-4781(93)90103-k

Choi EW, Lee M, Song JW, Kim K, Lee J et al. (2020). Fas mutation reduces obesity by increasing IL-4 and IL-10 expression and promoting white adipose tissue browning. Scientific Reports 10 (1): 12001. doi: 10.1038/s41598-020-68971-7

Cloete SW, Snyman MA, Herselman MJ (2000). Productive performance of Dorper sheep. Small Ruminant Research 36 (2): 119-135. doi: 10.1016/s0921-4488(99)00156-x

Clutton-Brock J (1999). A Natural History of Domesticated Mammals. Cambridge, UK: Cambridge University Press.

De Maio A (1999). Heat shock proteins: facts, thoughts, and dreams. Shock 11 (1): 1-12. doi: 10.1097/00024382-199901000-00001

Degen AA, Kam M (1992). Body mass loss and body fluid shifts during dehydration in Dorper sheep. Journal of Agricultural Science 119 (3): 419-422. doi: 10.1017/S0021859600012260

Emmett MJ, Lim HW, Jager J, Richter HJ, Adlanmerini M et al. (2017). Histone deacetylase 3 prepares brown adipose tissue for acute thermogenic challenge. Nature 546 (7659): 544-548. doi: 10.1038/nature22819

Enerbäck S (2009). The origins of brown adipose tissue. New England Journal of Medicine 360 (19): 2021-2023. doi: 10.1056/NEJMcibr0809610

Freiberger J, Coulombe K, Suliman H, Carraway M, Piantadosi C (2004). Superoxide dismutase responds to hyperoxia in rat hippocampus. Undersea and Hyperbaric Medicine 31 (2): 227-232.

Gotoh T, Terada K, Oyadomari S, Mori M (2004). hsp70-DnaJ chaperone pair prevents nitric oxide- and CHOP-induced apoptosis by inhibiting translocation of Bax to mitochondria. Cell Death and Differentiation 11 (4): 390-402. doi: 10.1038/sj.cdd.4401369

Hamann E, Pauli CS, Joly-Lopez Z, Groen SC, Rest JS et al. (2021). Rapid evolutionary changes in gene expression in response to climate fluctuations. Molecular Ecology 30 (1): 193-206. doi: 10.1111/mec.15583

Hayward JS, Lisson PA (1992). Evolution of brown fat: its absence in marsupials and monotremes. Canadian Journal of Zoology 70 (1): 171-179. doi: 10.1139/z92-025

Hayyan M, Hashim MA, AlNashef IM (2016). Superoxide ion: generation and chemical implications. Chemical Reviews 116 (5): 3029-3085. doi: 10.1021/acs.chemrev.5b00407

Hou CL (2014). Genome sequencing of Mongolia sheep and genetic basis of cold resistance traits based on transcriptome analysis. PhD, Inner Mongolia Agricultural University, Hohhot, China.

Huang H, McIntosh AL, Martin GG, Landrock D, Chung S et al. (2016). FABP1: a novel hepatic endocannabinoid and cannabinoid binding protein. Biochemistry 55 (37): 5243-5255. doi: 10.1021/acs.biochem.6b00446

Huang J, Gong Z, Ghosal G, Chen J (2009). SOSS complexes participate in the maintenance of genomic stability. Molecular Cell 35 (3): 384-393. doi: 10.1016/j.molcel.2009.06.011

Jean L, Risler JL, Nagase T, Coulouarn C, Nomura N et al. (1999). The nuclear protein PH5P of the inter-alpha-inhibitor superfamily: a missing link between poly (ADP-ribose) polymerase and the inter-alpha-inhibitor family and a novel actor of DNA repair? FEBS Letters 446 (1): 6-8. doi: 10.1016/s0014-5793(99)00173-8

Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R et al. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biology 14 (4): R36. doi: 10.1186/gb-2013-14-4-r36

Klein Hazebroek M, Keipert S (2020). Adapting to the cold: a role for endogenous fibroblast growth factor 21 in thermoregulation? Front Endocrinol (Lausanne) 11: 389. doi: 10.3389/fendo.2020.00389

Kolupaev IuE, Oboznyĭ AI (2012). Participation of the active oxygen forms in the induction of ascorbate peroxidase and guaiacol peroxidase under heat hardening of wheat seedlings. Ukrains′kyi Biokhimichnyi Zhurnal 84 (6): 131-138.

Konczal M, Koteja P, Stuglik MT, Radwan J, Babik W (2014). Accuracy of allele frequency estimation using pooled RNA-Seq. Molecular Ecology Resources 14 (2): 381-392. doi: 10.1111/1755-0998.12186

Kong L, Zhang Y, Ye ZQ, Liu XQ, Zhao SQ et al. (2007). CPC: assess the protein-coding potential of transcripts using sequence features and support vector machine. Nucleic Acids Research 35 (Web Server issue): W345-W349. doi: 10.1093/nar/gkm391

Krishnan J, Persons JL, Peuß R, Hassan H, Kenzior A et al. (2020). Comparative transcriptome analysis of wild and lab populations of Astyanax mexicanus uncovers differential effects of environment and morphotype on gene expression. Journal of Experimental Zoology 334 (7-8): 530-539. doi: 10.1002/jez.b.22933

Langmead B, Trapnell C, Pop M, Salzberg SL (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biology 10 (3): R25. doi: 10.1186/gb-2009-10-3-r25

Laplante AF, Moulin V, Auger FA, Landry J, Li H et al. (1998). Expression of heat shock proteins in mouse skin during wound healing. Journal of Histochemistry and Cytochemistry 46 (11): 1291-1301. doi: 10.1177/002215549804601109

Leiria LO, Tseng YH (2020). Lipidomics of brown and white adipose tissue: Implications for energy metabolism. BBA Molecular and Cell Biology of Lipids 1865 (10): 158788. doi: 10.1016/j.bbalip.2020.158788

Lin MF, Jungreis I, Kellis M (2011). PhyloCSF: a comparative genomics method to distinguish protein coding and non-coding regions. Bioinformatics 27 (13): i275-i282. doi: 10.1093/bioinformatics/btr209

Lin Q, Weis S, Yang G, Weng YH, Helston R et al. (2007). Heme oxygenase-1 protein localizes to the nucleus and activates transcription factors important in oxidative stress. Journal of Biological Chemistry 282 (28): 20621-20633. doi: 10.1074/jbc.M607954200

Lu N, Zang X, Zhang X, Chen H, Feng X et al. (2012). Gene cloning, expression and activity analysis of manganese superoxide dismutase from two strains of *Gracilaria lemaneiformis* (Gracilariaceae, Rhodophyta) under heat stress. Molecules 17 (4): 4522-4532. doi: 10.3390/molecules17044522

Lu Y, Wu Z, Song Z, Xiao P, Liu Y et al. (2016). Insight into the heat resistance of fish via blood: Effects of heat stress on metabolism, oxidative stress and antioxidant response of olive flounder *Paralichthys olivaceus* and turbot *Scophthalmus maximus*. Fish and Shellfish Immunology 58: 125-135. doi: 10.1016/j.fsi.2016.09.008

Ma L, Bajic VB, Zhang Z (2013). On the classification of long non-coding RNAs. RNA Biology 10 (6): 924-933. doi: 10.4161/rna.24604

Marcos-Carcavilla A, Mutikainen M, González C, Calvo JH, Kantanen J et al. (2010). A SNP in the HSP90AA1 gene 5' flanking region is associated with the adaptation to differential thermal conditions in the ovine species. Cell Stress and Chaperones 15 (1): 67-81. doi: 10.1007/s12192-009-0123-z

Matz JM, Blake MJ, Tatelman HM, Lavoi KP, Holbrook NJ (1995). Characterization and regulation of cold-induced heat shock protein expression in mouse brown adipose tissue. American Journal of Physiology 269 (1 Pt 2): R38-R47. doi: 10.1152/ajpregu.1995.269.1.R38

McManus CM, Faria DA, Lucci CM, Louvandini H, Pereira SA et al. (2020). Heat stress effects on sheep: Are hair sheep more heat resistant? Theriogenology 155: 157-167. doi: 10.1016/j.theriogenology.2020.05.047

Milne C (2000). The history of the Dorper sheep. Small Ruminant Research 36 (2): 99-102. doi: 10.1016/s0921-4488(99)00154-6

Nelson DL, Cox MM (2008). Lehninger Principles of Biochemistry. New York, USA: W.H. Freeman and Company.

Oliveira JA, Egito AAD, Crispim BDA, Vargas Junior FM, Seno LO et al. (2020). Importance of naturalized breeds as a base for the formation of exotic sheep (*Ovis aries*) breeds in tropical altitude regions. Genetics and Molecular Biology 43 (2): e20190054. doi: 10.1590/1678-4685-GMB-2019-0054

Perkel JM (2013). Visiting "noncodarnia". BioTechniques 54 (6): 301, 303-304. doi: 10.2144/000114037

Punta M, Coggill PC, Eberhardt RY, Mistry J, Tate J et al. (2012). The Pfam protein families database. Nucleic Acids Research 40 (Database issue): D290-D301. doi: 10.1093/nar/gkr1065

Rajkov J, El Taher A, Böhne A, Salzburger W, Egger B (2021). Gene expression remodelling and immune response during adaptive divergence in an African cichlid fish. Molecular Ecology 30 (1): 274-296. doi: 10.1111/mec.15709

Richard DJ, Bolderson E, Cubeddu L, Wadsworth RI, Savage K et al. (2008). Single-stranded DNA-binding protein hSSB1 is critical for genomic stability. Nature 453 (7195): 677-681. doi: 10.1038/nature06883

Ritossa F (1962). A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia 18 (12): 571-573. doi: 10.1007/BF02172188

Salces-Ortiz J, González C, Moreno-Sánchez N, Calvo JH, Pérez-Guzmán MD et al. (2013). Ovine HSP90AA1 expression rate is affected by several SNPs at the promoter under both basal and heat stress conditions. PLoS One 8 (6): e66641. doi: 10.1371/journal.pone.0066641

Schlesinger MJ (1990). Heat shock proteins. Journal of Biological Chemistry 265 (21): 12111-12114.

Schroeder F, McIntosh AL, Martin GG, Huang H, Landrock D et al. (2016). Fatty acid binding protein-1 (FABP1) and the human FABP1 T94A variant: roles in the endocannabinoid system and dyslipidemias. Lipids 51 (6): 655-676. doi: 10.1007/s11745-016-4155-8

Singh KM, Singh S, Ganguly I, Nachiappan RK, Ganguly A et al. (2017). Association of heat stress protein 90 and 70 gene polymorphism with adaptability traits in Indian sheep (*Ovis aries*). Cell Stress and Chaperones 22 (5): 675-684. doi: 10.1007/s12192-017-0770-4

Smathers RL, Petersen DR (2011). The human fatty acid-binding protein family: evolutionary divergences and functions. Human Genomics 5 (3): 170-191. doi: 10.1186/1479-7364-5-3-170

Sun L, Luo H, Bu D, Zhao G, Yu K et al. (2013). Utilizing sequence intrinsic composition to classify protein-coding and long non-coding transcripts. Nucleic Acids Research 41 (17): e166. doi: 10.1093/nar/gkt646

Tanaka E, Yamakawa A, Ito K, Yamamura M, Furuyama F (1997). Heat production by brown adipocytes of FOK rats genetically resistant to a hot environment. Journal of Veterinary Medical Science 59 (12): 1157-1159. doi: 10.1292/jvms.59.1157

Terada K, Mori M (2000). Human DnaJ homologs dj2 and dj3, and bag-1 are positive cochaperones of hsc70. Journal of Biological Chemistry 275 (32): 24728-24734. doi: 10.1074/jbc.M002021200

Trapnell C, Roberts A, Goff L, Pertea G, Kim D et al. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature Protocols 7 (3): 562-578. doi: 10.1038/nprot.2012.016.

Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G et al. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nature Biotechnology 28 (5): 511-515. doi: 10.1038/nbt.1621

Vegiopoulos A, Müller-Decker K, Strzoda D, Schmitt I, Chichelnitskiy E et al. (2010). Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. Science 328 (5982): 1158-1161. doi: 10.1126/science.1186034

Wang G, Gong Y, Anderson J, Sun D, Minuk G et al. (2005). Antioxidative function of L-FABP in L-FABP stably transfected

Chang liver cells. Hepatology 42 (4): 871-879. doi: 10.1002/hep.20857

Wang Z, Gerstein M, Snyder M (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics 10 (1): 57-63. doi: 10.1038/nrg2484

Wegele H, Wandinger SK, Schmid AB, Reinstein J, Buchner J (2006). Substrate transfer from the chaperone Hsp70 to Hsp90. Journal of Molecular Biology 356 (3): 802-811. doi: 10.1016/j.jmb.2005.12.008

Wu C (1995). Heat shock transcription factors: structure and regulation. Annual Review of Cell and Developmental Biology 11: 441-469. doi: 10.1146/annurev.cb.11.110195.002301

Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH et al. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nature Communications 10 (1): 1523. doi: 10.1038/s41467-019-09234-6

Appendix. Details of six sheep.

Indicator	**Sheep**					
No.	1	2	3	4	5	6
Sheep breed	Mongolian sheep	Mongolian sheep	Mongolian sheep	Dorper sheep	Dorper sheep	Dorper sheep
Weight (kg)	60.5	61	59.8	85.9	85.1	84.8
Age (months old)	9	9	9	7	8	8
Physiological stage	Sexual maturation	Sexual maturation	Sexual maturation	Sexual maturation	Sexual maturation	Sexual maturation
Lactation number	Null	Null	Null	Null	Null	Null
Birth number	Null	Null	Null	Null	Null	Null
Body condition	Normal	Normal	Normal	Normal	Normal	Normal
Nutritional condition	Grass silage	Grass silage	Grass silage	Grass silage	Grass silage	Grass silage
Environmental condition	Naturally ventilated sheep house in Hohhot (Inner Mongolia) located in the sub temperate zone with continental monsoon climate					