Transcriptome Profiles of the Liver in Two Cold-Exposed Sheep Breeds Revealed Different Mechanisms and Candidate Genes for Thermogenesis

Dan Jiao,1,2,3 Kaixi Ji,1,2,3 Wenqiang Wang,4 Hu Liu,4 Jianwei Zhou,4 A. Allan Degen,5 Yunsheng Zhang,6 Ping Zhou,7 and Guo Yang1,3

1Northwest Institute of Ecological Environment and Resources, Chinese Academy of Science, Lanzhou 730000, China
2University of Chinese Academy of Sciences, Beijing 100049, China
3Key Laboratory of Stress Physiology and Ecology, Northwest Institute of Ecological Environment and Resources, Chinese Academy of Science, Lanzhou 730000, Gansu, China
4School of Life Sciences, Lanzhou University, Lanzhou 730020, China
5Desert Animal Adaptations and Husbandry, Wyler Department of Dryland Agriculture, Blaustein Institutes for Desert Research, Ben-Gurion University of Negev, Beer Sheva 8410500, Israel
6Institute of Animal Husbandry, Xinjiang Academy of Animal Science, Urumqi 830000, Xinjiang, China
7State Key Laboratory of Sheep Genetic Improvement and Healthy Production, Xinjiang Academy of Agricultural and Reclamation Science, Shihezi 832000, China

Correspondence should be addressed to Guo Yang; yangguo@lzb.ac.cn

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Cold-induced thermogenesis plays an important role in the survival of lambs exposed to low air temperatures. The liver produces and mediates heat production in mammals; however, to date, little is known about the role of liver genes in cold-induced thermogenesis in lambs. In this study, the difference in the liver transcriptome between Altay and Hu ewe lambs was compared. Because of different backgrounds of the two breeds, we hypothesized that the transcriptome profiles of the liver would differ between breeds when exposed to cold. Cold-exposed Altay lambs activated 8 candidate genes (ACTA1, MYH1, MYH2, MYL1, MYL2, TNNC1, TNNC2, and TNNT3) involved in muscle shivering thermogenesis; 3 candidate genes (ATP2A1, SLN, and CKM) involved in muscle nonshivering thermogenesis related to the Ca2+ signal and creatine cycle; and 6 candidate genes (PFKM, ALDOC, PGAM2, ENO2, ENO3, and ENO4) involved in enhancing liver metabolism. In contrast, the liver may not act as the main tissue for thermogenesis in cold-exposed Hu lambs. We concluded that Altay lambs rely on liver-mediated shivering and nonshivering thermogenesis by muscle tissue to a greater extent than Hu lambs. Results from this study could provide a theoretical foundation for the breeding and production of cold-resistant lambs.

1. Introduction

Sheep are thermostable animals and rely on thermogenesis to maintain a relatively constant body temperature; however, exposure to extreme cold can cause hypothermia and, ultimately, high mortality in lambs. Prolonged exposure to cold results in the activation of hormonal and metabolic responses in different tissues [1], including nonshivering thermogenesis (NST) in brown adipose tissue (BAT) [2]. Adrenergic activated NST involves uncoupling protein 1 (UCP1) that facilitates proton leakage across the inner mitochondrial membrane, leading to futile cycling of protons and heat generation [3]. It has been estimated that NST contributes approximately 12–20 percent of the increase in total daily heat production, while NST in BAT contributes approximately 2–17 percent of the increase [4], which suggests that other tissues are also involved in the process. Recently, the liver has been reported to play an important role in thermogenesis.
role in providing “fuel” for NST by BAT, at least in cold-
exposed mice [5].

The liver produces approximately 10–13 percent of the
basal heat production of mammals and has been closely
linked with thermogenesis [6]. In rats, body temperature and
heat production from the liver decreased when NST was
inhibited [7]. Bond and Ntambi [8] reported that the liver
was related to an increase in adipose tissue and the synthesis
and transport of monounsaturated fatty acids in UCP1-
deficient mice. Some liver-derived factors, including fibro-
blast growth factor 21 (FGF21), have been reported to be
important in the regulation of thermogenesis during acute
cold exposure in mice [9]. However, the genetic mechanisms
of the liver during cold exposure in lambs are still unclear. To
fill this gap, at least in part, we examined the changes in the
expression of liver-derived genes in two sheep breeds ex-
posed to cold. Altay sheep are indigenous in the Altay
Prefecture of the northern Xinjiang province and are well
adapted to the harsh conditions. This region is extremely
cold in winter with an average January temperature of
−16.3°C (https://Climate-Data.org) and with a long period of
winter snow cover. In contrast, Hu sheep are typically raised
in the subtropical climate zone of China, a region charac-
terized by warm, humid conditions. In a companion study
on the Altay and Hu sheep, maintenance energy require-
ments were higher for Hu than for Altay sheep at −5°C, and
this temperature was below the thermoneutral zone for Hu
sheep but within the thermoneutral zone for Altay sheep
[10]. Because of different backgrounds and responses to cold
exposure between the two breeds, we hypothesized that the
transcriptome profiles of their livers would differ when exposed to cold. Results of this study provide (1) a better
understanding of the role of the liver in thermogenic
mechanisms employed by lambs and (2) a research base for
improving the cold tolerance in sheep.

2. Materials and Methods

2.1. Animals and Ethics. The study design and all procedures
on sheep were approved by the Academic Committee of
the Northwestern Institute of Eco-Environment Resources,
Chinese Academy of Sciences (protocol no. CAS201810082).
Twelve Altay (A) and 12 Hu (H) ewe lambs, all six months of
age and of similar body weight (29.3 ± 2.47 kg), were pur-
chased from a feedlot in the Altay region and were raised at
the Gansu Gaolan Field Scientific Observation and Research
Station for Agricultural Ecosystem (36°14′N, 103°47′E).
Wool length did not differ between the two breeds and averaged 8.87 ± 2.15 cm. The lambs were fed alfalfa pellets ad
libitum, with free access to water, prior to and during the
study. They were maintained in individual metabolic cages
(1.5 m × 1.0 m), and each breed was divided into two groups:
chronic cold-exposed (6 A * and 6 H *), which were main-
tained in a room at a constant temperature of −5°C for
25 days, after a gradual decrease of 2.5°C/day over 10 days, and
control (6 A w and 6 H w), which were maintained in a room
at a constant temperature of 20°C throughout this period.
The temperature and humidity of the cold room were
−5°C ± 0.03 and 88% ± 6.5, respectively, and of the
thermoneutral room were 20°C ± 0.42 and 87.5% ± 9.9, re-
spectively. The temperature humidity indices (THI), fol-
lowing Tucker et al. [11], were 26 ± 0.18 and 63 ± 0.62,
respectively. All lambs were slaughtered after 12 h of fasting
in the morning after 25 days of temperature exposure, and
their livers were excised and immediately frozen in liquid
nitrogen and then stored at −80°C.

2.2. RNA Extraction, Library Construction, and Sequencing.
Total RNA was extracted from the liver of 19 lambs (A c
(n = 5), A w (n = 4), H c (n = 6), and H w (n = 4)) using TRIzol
reagent (Invitrogen, Carlsbad, CA, USA) according to the
manufacturer’s protocol. The RNA quality and integrity
number (RIN) were measured using Agilent 2100 (Agilent
Technologies, Santa Clara, CA, USA). The concentration
and purity of the RNA samples were tested through the threshold
filter of RIN > 7.0 and 28S/18S rRNA ratio > 1.0 to ensure that
RNA quality meets sequencing standards. The RNA samples
from individual lambs in every group (independent bio-
logical replicates) were not pooled in order to exclude
samples with poor biological duplication to ensure the reli-
ability of all sequencing results and subsequent analyses.
Consequently, of 24 liver samples (12 Hu and 12 Altay
lambs) sequenced, 19 were used for analysis. Poly A mes-
senger RNA (mRNA) was isolated from total RNA by an
oligo dT extraction kit (NEBnext Poly(A) mRNA Magnetic
Isolation Module, NEB, USA) and then fragmented using
divalent cations under elevated temperature. First-strand
cDNA was synthesized from fragmented mRNA using
random oligonucleotide primers and reverse transcriptase
(SuperScript II Reverse Transcriptase, Invitrogen, Carlsbad,
CA, USA) and second strand from DNA polymerase I and
RNase H treatments. The cDNA fragments’ production had a
single “A” nucleotide base added, followed by ligation of an
adapter. The products were purified by AMPure XP beads
and then dissolved in EB solution and enriched with PCR
amplification to create the final cDNA library. The overall
quality of the PCR product was validated by the Agilent
Technologies 2100 bioanalyzer. The double-stranded PCR
products were heated, denatured, and circularized by the
spliced oligo sequence to obtain the final library. The cDNA
fragments in the library were sequenced using a BGISEQ-
500 platform (Beijing Genomics Institute (BGI), Beijing,
China) for producing raw reads.

2.3. RNA-Seq Data Analysis. The clean reads were filtered
out based on the raw reads, using quality control software
SOAPnuke (BGI), which were obtained by removing low-
quality reads (more than 20 percent of bases in the total
reads had quality scores lower than 15), adaptor reads (reads
with joint contamination), and unknown base N content
(reads which contain more than 5 percent undetermined
base information). The reads were aligned and annotated to
the reference genome of Ovis aries (Oar_rambouillet_v1.0;
https://www.ncbi.nlm.nih.gov/assembly/GCF_002742125.1)
using the HISAT alignment tool (Centre for Computational
Biology, Johns Hopkins University, MD, USA). HISAT is
based on the Burrows–Wheeler transform and
Ferragina–Manzini (FM) indexing methods [12]. We used Bowtie 2 for calculating the gene alignment rate [13] (Johns Hopkins University, MD, USA) and then calculated gene expression levels with RSEM (version 1.2.12, University of Wisconsin–Madison, USA), a software package for estimating gene and isoform expression levels from RNA-Seq data [14]. The gene expression levels were standardized by reads per kilobase per million (FPKM) mapped reads. The constrained principal coordinate analysis (cPCoA) was employed to visualize classical multidimensional scaling of Bray–Curtis distance matrices by using functions capscale and anova.cca of vegan package in R (version 4.0, Lucent Technologies, AZ, USA), and the P value was calculated by permutation tests [15, 16].

2.4. Differentially Expressed Gene Analysis. We compared differential gene expressions in the liver between breeds and between air temperatures using pairwise comparisons, as described by Wang et al. [17]. Differentially expressed genes (DEGs) were filtered by DESeq2 software and as fold changes (FC, |log2 FC| > 1) and q value (q < 0.05). The q value is based on the multiple hypothesis testing on the P value. Fold change (FC) was calculated as follows:

$$\text{FC} = \frac{\text{avg FPKM}(–5°C)}{\text{avg FPKM}(20°C)}$$

where DEGs meeting the above screening criteria were carried out by subsequent clustering analysis.

2.5. Function Enrichment and Analyses. Gene Ontology (GO) [18] and Kyoto Encyclopedia of Genes and Genomes (KEGG) [19] enrichment analyses were based on DEGs. GO terms were enriched by phyper functions in R software (version 4.0, Lucent Technologies, AZ, USA), and based on the GO annotation results, DEGs were mapped to the GO terms in the database (https://www.geneontology.org/). The KEGG pathway enrichment was used to identify genes and the metabolic pathways involved in the DEGs (https://www. kegg.jp/kegg/pathway.html/), and this method was consistent with the GO enrichment. In order to further study the effect of cold exposure on gene transcription regulation in sheep, we enriched the up- and downregulated genes into GO terms and KEGG pathways, respectively. A level of q value < 0.05 was accepted as a significant difference between means.

3. Results

3.1. Sequencing and Mapping. To examine the global difference between breeds and treatments in the transcriptome sequences, constrained principal coordinate analysis (cPCoA) by Bray–Curtis distances was employed for every biological replicate of each tissue and treatment (Figure S1). It emerged that the 19 samples explained 16.4% of the variance of the total sequencing data, and the two breeds clustered well (P = 0.43). The livers of −5°C and 20°C Hu lambs were distinct in CPCo 1 (explained 41.7% of 41.7% of the variance of the total sequencing data), whereas the liver tissues were distinct between breeds in CPCo 2 (explained 47.1% of the variance of the variance of the total sequencing data). The results indicated that the transcriptome sequences in liver tissues separated the two breeds and separated the two temperature treatments in the Hu lambs.

The alignments for the genome and gene sequences were all made with Oar_rambouillet_v1.0. The sequencing and mapping data are summarized in Table S1. The results indicated that sequencing quality met the requirements for subsequent analysis.

3.2. Gene Annotation. A total of 27,298 genes were annotated to the Ovis aries reference genome Oar_rambouillet_v1.0, including 24,244 previously identified genes and 3,054 potentially new genes. Moreover, the analyses revealed that 89.5% of the genes were common in the four lamb groups (Figure 1(a)). In addition, the common DEG number was greater in the compared groups of A-liver−2-A-liver−1 and A-liver−1-H-liver−2 than the compared groups of A-liver−1-H-liver−1 and H-liver−2-H-liver−1, which indicated that these two groups had more similar responsive DEGs (Figure 1(b)).
3.3. Analysis of the DEGs. After cold exposure, more DEGs were upregulated in the −5°C Altay lambs compared to −5°C Hu lambs, but more DEGs were downregulated in the −5°C Hu lambs compared to 20°C Hu lambs (Figure 1(c)).

According to the classification of GO terms, DEGs were clustered into molecular function (MF), cellular component (CC), and biological process (BP). The selected significant GO terms’ annotation is presented in Table S2, and
upregulated GO terms are presented in Figure 2. The CC GO category in the cold-exposed Altay lambs was related to muscle contraction, MF GO category was related to the regulation of the binding of muscle-related proteins, and BP GO category was related to muscle contraction, but GO terms in the cold-exposed Hu and Altay lambs differed and were related to the hemoglobin complex in the CC GO category, oxygen carrier activity in the MF GO category, and oxygen transport in the BP GO category. For the down-regulated GO terms, hormone activity and lipid binding were enriched in the −5°C Altay lambs compared to 20°C Altay lambs, but chemokine activity and Ca²⁺ binding were enriched in the −5°C Hu lambs compared to 20°C Hu lambs.

Upregulated KEGG pathways in the −5°C Altay lambs compared to 20°C Altay lambs were enriched in cardiac muscle contraction, methane metabolism, and adrenergic signaling in cardiomyocyte pathways. Cardiac muscle contraction and methane metabolism pathways were also upregulated in the −5°C Altay lambs compared to −5°C Hu lambs, but there was no difference in the metabolism pathway in the 20°C Altay lambs compared to 20°C Hu lambs. In contrast to upregulated KEGG pathways, the immune, disease, and metabolism-related pathways were downregulated in the 20°C Altay lambs compared to 20°C Hu lambs. The selected significant KEGG pathways are presented in Table S3.

The heatmap of the top 50 DEGs of the liver exhibited reversed expression trends in the two breeds under cold exposure (Figure 3(a)). The top 50 DEGs of the liver at different temperatures in Altay and Hu lambs are presented in Table S4. The DEGs in the −5°C Hu lambs compared to 20°C Hu lambs and the two breeds at 20°C were primarily downregulated, but DEGs in the −5°C Altay lambs compared to 20°C Altay lambs and compared to −5°C Hu lambs were primarily upregulated. These DEGs were related to energy metabolism, muscle development, and Ca²⁺ binding, whereas the downregulated DEGs in the −5°C Altay lambs compared to 20°C Altay lambs were related to lipid metabolism. We clustered the DEGs, which were enriched in the significant GO and KEGG pathways, including muscle contraction, methane metabolism, lipid metabolism, and oxygen transport (Figure 3(b)). The candidate genes related to muscle contraction, methane metabolism, and oxygen transport were upregulated significantly in the cold-exposed Altay lambs, and almost all of the candidate genes were downregulated in the cold-exposed Hu lambs. Lipid metabolism-related genes displayed large differences between breeds.

4. Discussion

A number of studies have reported that the liver has a key function in NST. For example, it was reported that the liver provided approximately 44% of the total metabolic energy with cold acclimation in short-tailed opossums [21], oxygenation capacity of the liver of ducks increased by 40% after cold adaptation, and with cold adaptation, the liver provided BAT with glucose and fatty acids from very-low-density lipoproteins (VLDLs), which contributed to heat production [22]. In a recent study on the transcriptome profiles of cold-adapted Mongolian sheep [23], cold exposure induced postponing cell senescence in the liver, but no direct evidence of liver involvement in thermogenesis was reported. Therefore, to date, little is known about transcriptome profiles of the liver of lambs when exposed to cold as most studies have been done on rodents and humans. Molecular genetic studies can provide new insights in understanding the mechanisms underlying the tolerance of lambs to cold exposure.

4.1. Altay and Hu Sheep Displayed Breed Differences. A large number of downregulated DEGs were enriched in the first level of KEGG pathways, including diseases, organismal systems, and metabolism pathways in Altay lambs compared to Hu lambs. These genetic differences between sheep breeds occurred, especially in immune responses. The DEGs CD40 and CD40 ligand (CD40L) were downregulated in Altay compared to Hu lambs and were enriched in all the top 5 KEGG pathways related to immune processes. CD40 and CD40L regulate inflammatory processes through secondary messengers [24]. By regulating the transcription of different downstream factors, they mediate the immune signal transduction due to Leishmania infection [25] and oxidation stress [26]. Enrichment of downregulated genes in immune-related pathways indicated that Altay lambs have stronger immune resistance than Hu lambs. This could be explained by their different backgrounds and adaptations as Altay sheep are indigenous to Xinjiang Altay, have adapted well to the long, cold winters and sparse forage of low protein content, and are resistant to diseases, whereas Hu sheep are typically raised in warm, humid areas and are bred for high reproductive rates.

4.2. Cold-Exposed Altay Lambs Enhance Muscle ST and NST through Liver Regulation. The GO analysis indicated that cold-exposed Altay lambs mobilized several terms in the liver related to muscle regulation, such as muscle contraction, transition between fast and slow fibers, and cardiac muscle contraction. These terms were all upregulated. In addition, the cardiac muscle contraction pathway was enriched, and the methane metabolism pathway was upregulated. A large number of DEGs that were focused on muscle contraction, including actin alpha 1 (ACTA1), myosin light chain, phosphorylatable, fast skeletal muscle (MYLFP), myosin heavy chain 1 (MYH1), myosin heavy chain 2 (MYH2), myosin light chain 1 (MYL1), myosin light chain 2 (MYL2), troponin C1, slow skeletal and cardiac type
| GO Terms | Molecular Function | Biological Process |
|----------|--------------------|--------------------|
| c-A-liver | liver of          | GO terms | cellular component (up-regulated) |
| H-liverw | H-liverw:A-liverw | GO terms | molecular function (up-regulated) |
| A-liverc | A-liverc:H-liverc | GO terms | biological process (up-regulated) |

**Figure 2:** The significant upregulated GO terms and KEGG pathways of DEGs in the liver. A-liverc-A-liverw: liver of –5°C Altay lambs (n=5) compared to 20°C Altay lambs (n=4); H-liverw-H-liverw: liver of –5°C Hu lambs (n=6) compared to 20°C Hu lambs (n=4); A-liverw-H-liverw: liver of –5°C Altay lambs compared to –5°C Hu lambs; A-liverw-H-liverw: liver of 20°C Altay lambs compared to 20°C Hu lambs.

The screen of significant enrichment is based on q value < 0.05. The green columns represent the group of A-liverw-A-liverw, the red columns represent the group of H-liverw-H-liverw, the khaki columns represent the group of A-liverc-A-liverw, and the gray columns represent the group of H-liverw-A-liverw.
Figure 3: The heatmap of DEGs in the liver. A-liverw-A-liverc: liver of −5°C Altay lambs (n = 5) compared to 20°C Altay lambs (n = 4); H-liverc-H-liverw: liver of −5°C Hu lambs (n = 6) compared to 20°C Hu lambs (n = 4); A-liverc-H-liverc: liver of −5°C Altay lambs compared to −5°C Hu lambs; A-liverw-H-liverw: liver of 20°C Altay lambs compared to 20°C Hu lambs. (a) A heatmap of top 50 DEGs. (b) A heatmap of candidate DEGs. The cluster analysis of gene expression is based on log2 FPKM data. The red color represents higher expression, and the green color represents lower expression.

Figure 4: Expression levels of four candidate genes from RT-PCR and RNA-seq. A-liverc and A-liverw: liver of Altay lambs under thermoneutral (20°C; n = 4) and cold exposure (−5°C; n = 5); H-liverc and H-liverw: liver of Hu lambs under thermoneutral (20°C; n = 4) and cold exposure (−5°C) (n = 6). The X-axis represents the tissue of the liver; the Y-axis on the left represents the relative gene FPKM levels of RNA-seq by columns and bars; the Y-axis on the right represents the relative gene expression levels of RT-PCR by columns and bars. *P < 0.05.
skeletalmuscleactivates transport and sarcoplasmic reticulum were activated. fK_hedemonstratedthattheskeletalmuscleisnotonlyinvolvedin skeletal muscle contraction, plays a major role in thermo-regulation, but this is temporary [29]. Recent studies have demonstrated that the skeletal muscle is not only involved in ST but also in NST [30, 31]. In the present study, sarco-demonstratedthattheskeletalmuscleisnotonlyinvolvedin exposure enhanced liver-mediated muscle metabolism, including muscle contraction and transition between fast and slow fibers. Studies have reported that the liver has a close metabolic relationship with the muscle and that it could regulate heat production by stimulating muscular activity [27, 28]. In the early stage of cold exposure, ST, through skeletal muscle contraction, plays a major role in thermo-regulation.

4.3. Cold-Exposed Altay Lambs Enhance Liver Metabolism. Interestingly, the methane metabolism pathway was upregulated in cold-exposed Altay lambs. Related DEGs, including phosphofructokinase, muscle (PFKM), aldolase, fructose-bisphosphate C (ALDOC), phosphoglycerate mutase 2 (PGAM2), enolase 2 (ENO2), enolase 3 (ENO3), and enolase 4 (EN04), were also enriched and involved in the GO term of glycolytic processes. PFKM is the limiting en-zyme of the glycolytic process and represents a major control point in the metabolism of glucose [41]. The function of PFKM was reported to be associated with the growth of methane-producing bacteria. EN0s catalyze the interconversion of 2-phosphoglycerate to phosphoenolpyruvate, which is the second of the two high-energy intermediates that generate ATP in glycolysis [42, 43]. Consequently, cold exposure could result in an increase in glucose metabolism and methane production in Altay lambs. However, cold exposure did not result in changes in rumen methane transcriptome profiles in Altay lambs (unpublished data). Studies have shown that high temperature could reduce methane production in sheep [44], but whether low temperature increases methane emission needs further research.

We found that the GO terms of oxygen transport, oxygen carrier activity, oxygen binding, and KEGG pathway of cardiac muscle contraction were enriched in Altay lambs. Enzyme activity in the citric acid cycle (TCA cycle) was reported to be directly proportional to the rate of oxygen utilization by muscle cells as a function of the increase in ATP requirement for myocardial contraction [45]. Appar-ently, these 6 candidate genes are related to the glycolytic pathway and TCA cycle to produce energy for thermogenesis. Several DEGs involved in lipid metabolism, such as APOD and endothelial lipase (LIPG), were upregulated in the cold-exposed Altay lambs compared to 20°C Altay lambs. LIPG clears the circulation of high-density lipoproteins and provides lipid precursors for lipid synthesis [46, 47], while the APOA4 gene is a major constituent of high-density lipoprotein particles [48] and is downregulated in the cold-exposed Altay lambs compared to 20°C Altay lambs. This indicated that cold exposure reduced the circulation of high-density lipoproteins, which resulted in a reduction in transport of cholesterol to the liver. Furthermore, APOD not only influences lipid metabolism but also plays an important role in glucose homeostasis [49, 50]. In addition, TFF2, as a regulator of energy metabolism [51], was upregulated in the cold-exposed Altay lambs compared to –5°C Hu lambs. These results indicated that cold exposure influenced the cholesterol transport in the liver of Altay lambs and enhanced glycolysis in the liver to generate heat.

4.4. The Liver May Not Be the Main Thermogenic Tissue for Hu Lambs. Through the cluster of candidate DEGs, almost all candidate genes were downregulated in the cold-exposed Hu lambs, which means that the liver may not be the main thermogenic tissue. Compared to cold-exposed Altay lambs, the response of the liver in Hu lambs to cold exposure was focused mainly on immune diseases and immune system pathways. The gene transcriptional expression level had smaller differences between breeds of 20°C lamb group. In a previous study (unpublished data), cold-exposed Hu lambs relied mainly on tail fat for heat production, rather than typical thermogenic tissues such as the liver and muscles. In the present study, cellular retinoic acid-binding protein type 1 (CRABP1) was upregulated in the –5°C Hu lambs compared to 20°C Hu lambs, but downregulated in the –5°C Altay lambs compared to –5°C Hu lambs, whereas acyl-coA thiosterase 11 (ACOT11) regulation displayed an opposite trend. CRABP1 is one of the carrier proteins of retinoic acid and was reported to be associated with fat accumulation in adipose tissue in mice [52]. ACOT11 was reported to limit the oxidation of lipid droplet-derived fatty acids, possibly by regulating the availability of substrates to be used for β-oxidation and uncoupling [53–55]. These results indicate that cold exposure mobilizes adipose tissue as the main heat-producing tissue in Hu lambs and could explain why cold
exposure did not cause enrichment and upregulation of energy metabolism-related pathways and genes in their liver.

5. Conclusions

Transcriptome sequencing of the liver of cold-exposed Altay and Hu lambs revealed different thermogenic pathways between breeds. Cold exposure induced thermogenesis in Altay lambs and activated the liver-regulated muscle contraction pathway related to ST of muscles. Eight candidate genes, namely, ACTA1, MYH1, MYH2, MYL1, MYL2, TNNC1, TNNC2, and TNNT3, were upregulated in response to cold. The Ca²⁺ signal pathway and creatine cycle were also activated, and 3 candidate genes, including ATP2A1, SLN, and CKM, were involved and upregulated in muscular NST. In addition, 6 candidate genes related to methane metabolism, PFKM, ALDOC, PGAM2, ENO2, ENO3, and ENO4, were upregulated in the liver of cold-exposed Altay lambs. However, in the cold-exposed Hu lambs, it appears that the liver is not the main tissue for thermogenesis, but has a much lesser role when compared to Altay lambs. In summary, Altay and Hu lambs have different regulation mechanisms in response to cold exposure, which are likely manifested in breed differences in cold resistance.

Data Availability

The data are available confidentially to editors and reviewers, and all transcriptome data were submitted to the NCBI Sequence Read Archive (SRA series accession: PRJNA639638).

Consent

Not applicable.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

DJ summarized the transcriptome sequencing data, performed the data analysis, and prepared the original manuscript. KXJ, WQW, and HL attended the animals and collected samples. JWZ, YSZ, PZ, and GY designed the experiments and provided the research platform. AAD and GY revised the manuscript. All authors approved the final manuscript.

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Supplementary Materials

Supplementary Material 1: Figure S1: CPCoA analyses of all samples and sequencing quality in the liver of Altay and Hu lambs. Supplementary Material 2: Table S1: summary of RNA-seq results. Supplementary Material 3: Table S2: GO terms significantly enriched in the liver at different temperatures in Altay and Hu lambs. Supplementary Material 4: Table S3: KEGG pathways significantly enriched in the liver at different temperatures in Altay and Hu lambs. Supplementary Material 5: Table S4: top 50 DEGs in the liver at different temperatures in Altay and Hu lambs. s (Supplementary Materials)

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