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Transcriptome profile analysis of adipose tissues from fat and short-tailed sheep

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ABSTRACT

Recent studies in domestic animals have used RNA-seq to explore the transcriptome of different tissues in a limited number of individuals. In the present study, de novo transcriptome sequencing was used to compare sheep adipose tissue transcriptome profiles between a fat-tailed breed (Kazak sheep; KS) and a short-tailed (Tibetan sheep; TS). The RNA-seq data from these two groups revealed that 646 genes were differentially expressed between the KS and TS groups, including 280 up-regulated and 366 down-regulated genes. We identified genes relevant to fat metabolism in adipose tissues, including two top genes with the largest fold change (*NELL1* and *FMO3*). Pathway analysis revealed that the differentially expressed genes between the KS and TS breeds belong to fatty acid metabolism relevant pathways (e.g. fat digestion and absorption, glycine, serine, and threonine metabolism) and cell junction-related pathways (e.g. cell adhesion molecules) which contribute to fat deposition. This work highlighted potential genes and gene networks that affect fat deposition and meat quality in sheep.

1. Introduction

Adipose tissue is one of the principal tissues involved in the regulation of fat deposition and lipid metabolism in domestic animals, such as pigs and sheep. The fat-tailed sheep, known for distinctive large tails and hindquarters, are commonly found in the northern parts of Africa, the Middle East, Central Asia, and Western China (Pourlis, 2011). Fat-tailed sheep comprise approximately 25% of the world's sheep population (Davidson, 1999). Although they are a source of various products (milk, meat, and fat, but also hides and hair) to the locals, their unique characters and contributions (for example, as fat and meat source) have long been neglected by the researchers (Almeida, 2011). The fat-tail characteristic of these sheep is considered an adaptive response to the harsh challenges of desert life and is a valuable reserve for sheep during migration and winter when pasture is scarce (Atti et al., 2004). Tail fat is used as a source of food (or ghee), and is a considerable portion of the dietary energy for local inhabitants. In addition, in certain areas, fat-tailed sheep breeds are appreciated for their lean meat with better quality in terms of lower intermuscular fat. By contrast, the short and small fat tails compensate by a higher fat deposition rate in their subcutaneous and intramuscular fat (Kashan et al., 2005).

The size of the tails of sheep varies between and within breeds (Pourlis, 2011). The Kazak sheep (KS) and Tibetan sheep (TS) are two native breeds raised in the extremely arid regions of western China. KS exhibits typical fat tails, while Tibetan sheep demonstrate short and thin tails. KS live at an altitude of more than 2000 m, and are mainly distributed in vast areas of Central Asia. The TS graze primarily in the areas of the Tibetan Plateau, at an altitude of higher than 3000 m. An illustration of tails in the KS and TS breeds is shown in Fig. S1.

Several recent studies have employed the next-generation sequencing (NGS) platforms to explore differentially expressed genes in the adipose tissues in pigs (Cánovas et al., 2010; Li et al., 2012; Ramayo-Caldas et al., 2012; Wang et al., 2013), cattle (Jin et al., 2012; Lee et al., 2013), chicken (Cui et al., 2012; Huang et al., 2010) and dogs (Grant et al., 2011). There have been no transcriptome studies of adipose tissues in sheep, except for a conference report studying brown and white fat at the chromatin and transcription levels (Tellam, 2013). Furthermore, our understanding of fat-tailed sheep domestication at the molecular level is still limited (Rocha et al., 2011). Although several candidate genes, including *BCO2* (Våge and Boman, 2010), *DGAT1* (Mohammadi et al., 2013), *H-FABP* (Bai et al., 2013), *leptin*, *AdipoR1* and *Adipo*R2

Abbreviations: qPCR, quantitative polymerase chain reaction; GO, gene ontology; COG, clusters of orthologous groups; BLAST, Basic Local Alignment Search Tool; KEGG, Kyoto Encyclopedia of Genes and Genomes pathway database; KS, Kazak sheep; TS, Tibetan sheep; DEGs, differentially expressed genes; CDS, coding sequence; EST, expressed sequence tag; QTL, quantitative trait loci; RNA-seq, RNA sequencing; FPKM, fragment per kilobase of exon model per million mapped reads; FDR, false discovery rate.

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(Lemor et al., 2010), associated with fat deposition in sheep tails were selected to investigate their expression profiles in adipose tissues in various fat-tailed sheep breeds, the genetic determinants underlying the formation of fat tails on a genome-wide level remain to be identified. Herein, to identify genes potentially associated with the fat deposition in sheep tails, we generated de novo adipose tissue transcriptome data from the KS and TS breeds because these two breeds both survive in the extremely arid and cold regions of Western China, but exhibit distinct differences in fat deposition in the tail region.

2. Materials and methods

2.1. Ethics statement

The experiment was conducted following the guidelines of the Animal Ethics Committee at Northwest A&F University under document no. 2011-31101684. The sampling procedures complied with the "Guidelines on Ethical Treatment of Experimental Animals" (2006) No. 398, set by the Ministry of Science and Technology, China.

2.2. Animals and phenotypes

Six unrelated adult individuals (three were males and three were females, aged 2 years) for KS and TS breeds were randomly selected from a sheep farm located in Ningxia autonomous region, China. These two breeds have been raised in this farm for more than 20 years. The length, width and circumference of the tails from each selected animal were measured and recorded.

2.3. RNA extraction and library preparation

Adipocyte tissues from fat tails were sampled by biopsy and stored in liquid nitrogen until further processing. The Trizol Reagent (Invitrogen) was used to extract the total RNA according to the manufacturer's instructions. The RNA concentration and quality were assessed by the Agilent 2100 bioanalyzer. Subsequently, equal amounts of RNA from each breed (KS = 6, TS = 6) were pooled for library preparation. Fragmentation buffer (100 mM $ZnCl_2$ in 100 mM Tris–HCl pH 7.0) was added to cut the mRNAs into short fragments. A First-Strand cDNA Synthesis Kit (Invitrogen) reverse transcribed the fragmented RNA into cDNA, in the presence of a random hexamer-primer (Invitrogen) and dNTPs for 50 min at 42 °C. DNA polymerase I was used to synthesize the second-strand cDNA, in a buffer containing dNTPs and RNaseH. The short DNA fragments were purified using the QiaQuick PCR extraction kit (Qiagen) and resolved with an elution buffer (10 mM Tris–Cl, pH 8.5) for end reparation and addition of a poly(A) fragment to both ends. Thereafter, the short fragments were connected with sequencing adapters and separated in gels by electrophoresis. The fragments with a size suitable for NGS were excised from the gels and eluted for PCR amplification by using adapter primers.

2.4. Transcriptome sequencing and de novo assembly

The two cDNA libraries were sequenced from both the 5′ and 3′ paired ends on the Illumina Genome Analyzer II platform following the manufacturer's instructions. The low-quality raw sequences were removed, and the remaining short reads, after trimming off with their adaptor sequences, were assembled in a de novo process using three assemblers: Trinity (Grabherr et al., 2011), trans-ABySS (Góngora-Castillo et al., 2012), and SOAP de-novo (Luo et al., 2012). After assembly, contigs longer than 100 bases were used for subsequent analysis.

2.5. Functional annotation

BLASTx was used to align the assembled contigs from the three assemblers to the protein and nucleotide databases including NCBI nr, clusters of orthologous groups (COG), gene ontology (GO), SwissProt and Kyoto Encyclopedia of Genes and Genomes pathway database (KEGG). The e-value cut-off was set at 1E−5 for further analysis. Each assembled contig was assigned with the gene name and related function based on the best BLASTx hit (the smallest e-value). Assembled contigs assigned to the same gene were further compared, and the contig with the best e-value was adopted. If the two assembled sequences had the same e-value, the one with the largest region of sequence identity was selected.

2.6. Screening of differentially expressed genes (DEGs)

The mapped fragments were normalized for RNA length according to fragment per kilobase of exon model per million mapped reads (FPKM) for each gene between the two pooled samples, which facilitates the comparison of transcript levels between samples (Mortazavi et al., 2008). The cutoff value for determining gene transcriptional activity was determined based on a 95% confidence interval for all FPKM values for each gene. DEGs between the two pooled samples were selected using the following filter criteria: FDR (false discovery rate) ≤ 0.001 and the absolute value of $\log_2^{(FPKM\_TS/FPKM\_KS)} \geq 2$, meaning that the expression difference for each DEG between two samples should be at least four-fold.

2.7. Validation of RNA-seq results by qPCR

Twelve DEGs were selected arbitrarily for verification of the RNA-seq data. The RNA samples used for preparing RNA pools for RNA-seq were selected (six samples for each phenotype). Two micrograms of total RNA was reverse transcribed to first-strand cDNA using reverse transcriptase (Invitrogen). The qPCR was carried out on an iQ5 system (Bio-Rad) using SYBR Premix Ex Taq (TaKaRa, Dalian, China), according to the manufacturer's instructions. All cDNA samples were examined in triplicate. qPCR using specified oligo-dT primers (Table S1), which were designed by Primer3 (Untergasser et al., 2012), determined the expression levels of specific mRNAs. The reaction mixture (25 μL) comprised 2.0 μL cDNA (1:4 dilution), 12.5 μL SYBR Premix Ex TaqTM II (TaKaRa), 1.0 μL specific forward primer (10 μM), 1.0 μL universal primer (10 μM), and 8.5 μL water. The PCR reaction comprised a denaturation step at 94 °C for 180 s, followed by 40 cycles of 10 s at 94 °C, 30 s at 60 °C, and 45 s at 70 °C. The specificity of amplification for each selected gene was evaluated by both melting curve analysis and agarose gel electrophoresis. Three replicates of the intra-assay between runs were performed to assess the coefficient of variation of the mean Ct values. The relative expression levels were normalized to the endogenous control gene *β-actin*, and expression ratios were calculated by using the $2^{-\Delta\Delta Ct}$ method.

2.8. Pathway enrichment analysis of DEGs

Biological pathways enriched for the identified DEGs through KEGG pathway analyses were carried out using Cytoscape software (version 2.6.2) (http://www.cytoscape.org/) with the ClueGO plugin (http://www.ici.upmc.fr/cluego/cluegoDownload.shtml) (Bindea et al., 2009).

3. Results

3.1. Phenotypic differences between tails of the two sheep breeds

The length, width and circumference of tails for each individual used in this study were measured. The tail measurements demonstrated significant differences between the two distinct phenotypes (Fig. 1). The circumference of the KS breed's fat-tail is approximately five times larger than that of the TS breed, while the KS breed's fat-tail is four times wider than that of the TS breed.