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Full Length Article

Tanscriptomic Analyses of Subcutaneous Fat and Muscle in Lanzhou Fat-Tailed Sheep

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Abstract

The level of fat deposition in carcass is an important factor affecting meat quality. Lanzhou fat-tailed sheep, an important local breed distributed in northwestern china, are famous for the considerably meat quality and plentiful fat, particularly in the tail. In this text, transcriptome profiles of subcutaneous fat (SCF) and muscle tissues were determined by RNA-sequencing in order to obtain a better understanding of fat deposition. As a result, 25,634,948 and 42,178,378 high-quality reads were obtained for SCF and muscle tissues, respectively, of which 89.51 and 89.21% can be aligned. FABP4 was the most highly expressed gene in SCF, while ACTA1 was the most highly expressed in muscle among all the known genes related to adipose deposition. Gene Ontology (GO) analysis showed that DEGs were enriched in different terms related to metabolism. Pathway analysis of DEGs revealed their association with processes relevant to fat deposition based on the Kyoto Encyclopedia of Genes and Genomes (KEGG). By using cufflinks, 960 novel transcripts were identified in each sample (SCF, n = 321 and muscle, n = 359), with 280 additional novel transcripts common to both libraries. Compared with the muscle tissue, in this text, 1915 DEGs with 1264 up-regulated and 651 down-regulated in the SCF were detected. It was suggested that the levels of the majority of transcripts are extremely conserved in the different tissues. These results provide new insight into exploring the specific fat deposition in Lanzhou fat-tailed sheep, and some basic date for further excavating the molecular mechanisms.

Keywords: Muscle; Lanzhou fat-tailed sheep; Subcutaneous adipose; Transcriptome

Introduction

Adipose is a kind of principal tissue involved in the regulation of fat deposition in livestock, which is composed primarily of adipocytes that are surrounded by fibroblasts, fibroblastic precursor cells, endothelial cells, nerves and immune cells (Wang *et al*., 2017). The Lanzhou fat-tailed sheep is an important sheep breed, and is well-known in China for its tail fat (Ma *et al*., 2018). It is a hybrid of Mongolian and Tong sheep, and gradually became a prominent local variety by long term artificial selection. Lanzhou fat-tailed sheep is distributed in north-western China, including Gansu and Ningxia provinces. This sheep is regarded as an adaptive response to harsh environment, such as crude feed and disease. The fat stored in such a "huge" tail is a valuable reserve for sheep during migration and the season that is lack of food. Actually, the fat deposition mainly distributes in the section of tail of Lanzhou fat-tailed sheep, which nearly accounts for 13.82% of carcass weight. Although the breed is characterized by large fat tails and good meat quality (Miao *et al*., 2015a), Lanzhou fat-tailed sheep shows low fecundity and is consequently facing the danger of extinction.

It was suggested that excess intra-abdominal fat storage is well known to be associated with increased metabolic risks, such as cardiovascular disease, hypertension and type II diabetes (Pouliot *et al*., 1992). Actually, preadipocyte proliferation, differentiation and subtype abundance can be influenced by regional gene expression differences, which mainly contribute to regional variations in fat tissue function (Van-Harmelen *et al*., 2004). However, the exact physiological, metabolic or endocrine mechanisms underlying the beneficial effects of lower body fat deposition in Lanzhou fat-tailed sheep still remain unknown. Therefore, it seems that a comprehensive understanding of the molecular mechanism underlying fat deposition is very important to control the fat mass in carcass through sheep breeding. In addition, as a breed is on the verge of extinction, the detailed analysis of important genes regulating metabolism in adipose tissue is seemed extremely important and urgent nowadays.

Until now, the transcriptome profiles of tissues in sheep have been determined by deep sequencing, such as

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heart, skin, muscle, mammary gland, and adipose tissue (Cox *et al*., 2012; Yue *et al*., 2015; Suarez-Vega *et al*., 2016). Actually, it is emphasized that animal species and breeds can dominate the features of a given adipose tissue depot (Hausman *et al*., 2014). In order to better understand the genetic regulation of fat deposition in Lanzhou fat-tailed sheep, therefore, RNA-Seq technology are used for analyzing transcriptome profiles in this text. Transcriptome profiles of subcutaneous fat (SCF) and muscle tissues in Lanzhou fat-tailed sheep were also analyzed comparatively by using RNA-Seq technology. In addition, some crucial genes that associated with fat metabolism were identified. These results provide basic date for further study of the molecular mechanisms of fat deposition.

Materials and Methods

Animals and Tissue Preparation

Animal experiments were permitted by Gansu Agricultural University Animal Protection Association. Sheep were slaughtered obey the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China) (Zhang *et al.*, 2016). In this article, Fifteen Lanzhou fat-tailed sheep (four-month old) were selected from healthily purebred herds. The tissue of subcutaneous fat and muscle were collected after the sheep were slaughtered and stored at -80°C until use (Zhou *et al*., 2014).

RNA Isolation, Library Construction and Sequencing

Total RNA and mRNA were isolated using Trizol Reagent and Oligotex mRNA Mini Kit (Qiagen), respectively. cDNA libraries were constructed according to the guidance of the TruSeq™ RNA Sample Preparation Guide. Library size and purity were checked using DNA 1000 kit on a Bioanalyzer 2100 system and quantification was performed using a Qubit™ dsDNA HS kit and a Qubit® 2.0 Fluorometer (Miao *et al*., 2015a). The cDNA library were sequenced on an Illumina HiSeq 2000 platform for paired-end sequencing at Shanghai Biotechnology Corporation (SBC).

Sequencing Data Preprocessing and Read Mapping

Raw sequencing reads were filtered to remove reads of low quality, reads containing 'N' bases, adapter sequences and ribosomal RNA sequences using fastx, finally obtaining clean reads (Yu *et al*., 2012). After trimming, clean reads had a minimum length of ≥ 35 bases. The Ovine reference genome sequence were downloaded from the NCBI livestock genomics website (http://www.livestockgenomics.csiro.au/sheep/oarV2.0_chromosomes.tar). High-quality reads were aligned to the Ovine reference genome using Tophat during mapping (Wang *et al*., 2018), mismatches of no more than two bases were permitted in the alignment. Reads that could not be aligned to the reference sequence, were subdivided into shorter segments and realigned to the reference sequence (splice junction mapping). Different insert sizes between paired reads (1 bp–10 kb) were set to align to splicing junction reads to the genome (Chen *et al*., 2011).

Analysis of Differentially Expressed Genes (DEGs)

Gene expression levels were calculated based on the fragments per kilobase of exon model per million mapped reads (FPKM) method (Wang *et al*., 2016). The gene expression level was directly compared between different samples (Cheng *et al*., 2016). The threshold of DEGs between the two samples were defined by FDR value ≤ 0.001 and |log2FPKM_SCF/FPKM_Muscle | ≥ 1.

Analysis of Alternative Splicing

Alternative splicing (AS) is one of the important factors leading to large differences in the number of eukaryotic genes and proteins. With TopHat software, five types of AS events were analyzed, including 5' alternative splice site (A5SS), 3' alternative splice site (A3SS), exon skipping (ES), intron retention (IR), mutually exclusive exon (MEX) (Wang *et al*., 2015).

Identification of Novel Transcripts

Novel transcriptional fragments were identified by the method of Cufflinks Reference Annotation Based Transcript Assembly. Intergenic regions were defined between adjacent genes using Ovine genome data. A transcript unit was connected using transcriptionally active region (TAR), which was joined by at least one set of paired-end reads. Novel transcript units located in intergenic regions with a continuous mapping length ≥ 150 bp and average coverage ≥ 2 (He *et al*., 2013).

GO Category and KEGG Pathway Analyses

Gene enrichment analyzed by Gene Ontology (GO) (Miao *et al*., 2015b). Classification of GO categories was determined by using the Fisher's exact test with FDR-corrected p-value. p-value < 0.05 were considered to indicate GO enrichment (Dunnick *et al*., 2012). Pathway enrichment was determined using the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Savage *et al*., 2015). The significance enrichment was defined by the p-value (p-value < 0.05) (Savage *et al*., 2015).

Results

Summary of Raw Sequence Data

In this research, cDNA libraries of SCF and muscle tissues were constructed from Lanzhou fat-tailed sheep and were subjected to sequencing. In total, 27,189,803 (SCF) and 45,701,098 (muscle) raw sequence reads were obtained. The

total read length was 7.28 gigabases (Gb), representing more than two-fold coverage of the sheep genome. After quality control, high quality clean reads were acquired from both samples (Table 1).

All short reads onto the whole reference genome (Ovis. Genome v 2.0) were aligned. As shown in Table 2, 89.51% (22, 946, 127/25, 634, 948) and 89.21% (37, 626, 582/42, 178, 378) of SCF and muscle clean reads, respectively, were aligned to the Oar_v 3.1 sheep reference genome. The reads in each sample uniquely mapped to the sheep genome is about eighty percent, ensuring reliable downstream analysis. Mapping of clean reads to sheep chromosomes showed that chromosomes 1, 2 and 3 had the most mapped reads (Fig. 1).

Table 1: Summary of sequence data

Sample	SCF	Muscle
Raw reads	27,189,803	45,701,098
Quality trimmed	26,747,836 (98.37%)	44,685,402 (97.78%)
Adaptor trimmed	26,942,149 (99.09%)	43,811,272 (95.86%)
Clean reads	25,634,948 (94.28%)	42,178,378 (92.29%)

Table 2: Summary of the alignment of sequencing reads to the *Ovis aries* genome

Sample	SCF	Muscle
All reads	25,634,948	42,178,378
Mapped reads	22,946,127 (89.51%)	37,626,582 (89.21%)
Mapped pair reads	21,675,604 (94.46%)	36,056,986 (85.83%)
Mapped broken-pair reads	1,270,523 (5.54%)	1,569,596 (4.17%)
Unmapped reads	2,688,821 (10.49%)	4,551,796 (10.79%)
Mapped unique reads	20,701,256 (80.75%)	34,528,113 (81.86%)
Mapped multi reads	2,244,871 (8.76%)	3,098,469 (7.35%)

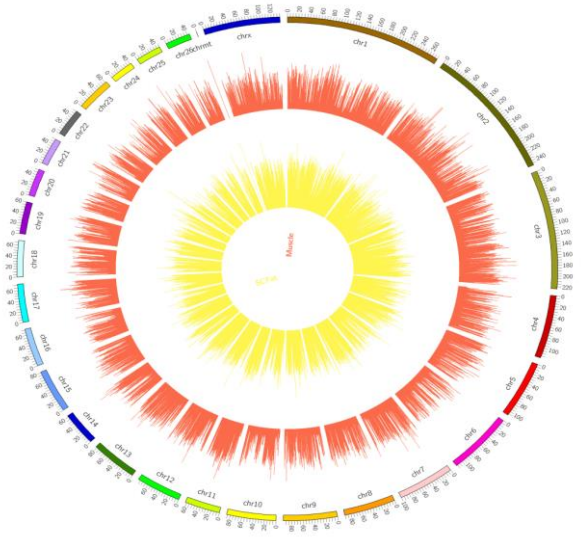

Fig. 1: Mapping of clean reads to sheep chromosomes. The inner circle represents the SCF group (yellow), the middle circle represents the muscle group (red) and the outer circle chromosomes

Analysis of Differentially Expressed Genes (DEGs)

Expressed genes (n=15763) were identified in the two types of Ovine tissues by RNA-seq analysis. In total 14,844 (90.89%) and 15,058 (92.20%) of genes expressed in the SCF and muscle samples respectively, of which 14,139 (86.57%) were common expressed genes between the two samples (Fig. 2). In this study, the scatter plot showed that the correlation of transcript expression was similar in the two tissues (Fig. 3).

The most expressed annotated transcripts in the SCF and muscle groups included the following genes: FABP4, SCD, FTH1, ADIPOQ, RPLP1, LOC101114719, ACTA1, LOC101119370, CKM, MYLPF and LOC780509. FABP4 was the most highly expressed gene in SCF (FPKM =28428.9), while ACTA1 was the most highly expressed in muscle (FPKM = 36944.1). There were genes highly expressed in SCF were also relatively abundant in muscle, such as LOC101119370, however, other genes highly expressed in muscle were detected at relatively low levels in SCF, with some of the genes completely absent, including CKM, MYL2 and MYL1.

There were 1915 DEGs with 1264 genes up-regulated and 651genes down-regulated in SCF compared with muscle samples (Fig. 4). According to their expression levels, DEGs were divided into five groups: high (FPKM > 5000), medium high (FPKM ≥ 2500–5000), medium (FPKM ≥ 500–2500), medium low (FPKM ≥ 50–500) and low (FPKM <50) expression level genes (Table 3).

Alternative Splicing (AS) and Novel Transcript Prediction

There were 9,934 and 10,626 AS events in two libraries, respectively, including A3SS, A5SS, IR, SE and MEX. SE accounted for 42.0% and 46.2% in the SCF and muscle transcripts, MXE occurred only 2.6% and 2.9% in the SCF and muscle transcripts (Fig. 5). The number of AS events mainly take place on chromosomes 1, 2 and 3, with the least number of events on chromosome 26 (Fig. 6).

960 novel transcript units using cufflinks (version: 2.0.2) were detected. A total of 321 (SCF) and 359 (muscle) novel transcript units were unique. In addition, 280 common novel transcripts were detected in both libraries.

Functional Annotation of DEGs

A total of 827 clusters were annotated with GO terms. The identified DEGs were divided into three functional groups: cellular component (15%), molecular function (20.8%) and biological process (64.2%). The top 40 functional categories included immune response, cytosol, translation, fatty acid biosynthetic process, etc. DEGs were enriched in different terms related to metabolism. Such as, DEGs were enriched in 'fatty acid biosynthetic process', which could mediate

free fatty acid synthesis, and plays important role in promoting fatty acid metabolism.

DEGs from the SCF and muscle groups analyzed by KEGG pathway, including fatty acid metabolism (22 genes, P-value = 4.26E-05, FDR = 0.00056), as well as fatty acid degradation (16 genes, P-value = 0.004621, FDR = 0.02681), which may contribute to fat deposition and fatty acid metabolism.

Discussion

In total, 7.28 Gb of sequence data were obtained, more than 6.78 Gb of clean read sequences were analyzed in this study. There were 10.49% and 10.79% unmapped reads in SCF and muscle, respectively, which might depend on the sequencing errors and the mapping criteria. The majority of the most highly expressed genes in the SCF transcriptome were involved in fatty acid metabolism, including FABP4, SCD, FTH1, RPLP1and ADIPOQ, which was consistent with the reports in humans (Mukamal *et al*., 2013). Results indicated that ADIPOQ and FABP4 expressed in Lanzhou fat-tailed sheep. Actually, these genes can also express in other sheep, such as Polled Dorset sheep (Liu *et al*., 2015).

RNA-seq is widely used as a powerful method to identify DEGs (Moreira *et al*., 2015). However, few reports have investigated DEGs using next-generation sequencing in Lanzhou fat-tailed sheep. Preliminary analysis of DEGs between SCF and muscle tissues was first presented in this text. Compared with the muscle tissue, in this text, 1915 DEGs with 1264 up-regulated and 651 down-regulated in the SCF were detected. It was suggested, from the high correlation of gene expression levels between the samples (r =0.804), that the levels of the majority of transcripts are extremely conserved in the different tissues. This was consisted with the results obtained from other mammalian species (Zhang *et al*., 2013).

Five alternative splicing types were analyzed in this study, including A3SS, A5SS, SE, RI and MEX. SE is the most common type of AS, which is similar to the results concluded from humans and yeast (Chen *et al*., 2011). Similar to the skin tissue of Chinese Tan Sheep (Kang *et al*., 2013), MXE was also a rare event in SCF and muscle transcripts of Lanzhou fat-tailed sheep. Compared with other species, it was implied that Lanzhou fat-tailed have different alternative splicing patterns in SCF and muscle tissues. In addition, there are 20,560 AS events detected in the two samples and primarily distributed on chromosomes 1, 2 and 3.

Among the differentially expressed genes, FABP4 belongs to a family of intracellular fatty acid binding proteins, which are critical to intracellular fatty acid transport. Actually, FABP4 are also considered to be candidate genes for muscle content in pigs (Wang *et al*., 2012). ADIPOQ, exclusively expressed in adipose tissue, also modulates a lot of metabolic processes, such as glucose regulation and fatty acid oxidation. As a gene highly

Table 3: Gene expression and annotation by FPKM

FPKM	Gene number in SCF (%)	Gene number in muscle (%)
≥ 5000	10 (0.52%)	15 (0.78%)
≥ 2500–5000	33 (1.72%)	17 (0.89%)
≥ 500–2500	154 (8.04%)	107 (5.59%)
≥ 50–500	929 (48.52%)	637 (33.26%)
< 50	789 (41.2%)	1139 (59.48%)

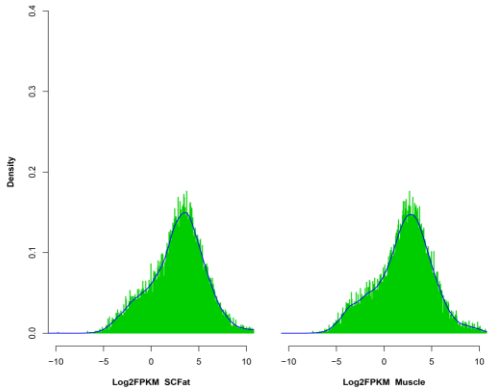

Fig. 2: Gene expression profiles. Gene expression profiles in SCF (left) and muscle (right) tissues of Lanzhou fat-tailed sheep. The x-axis represents $\log_2$ FPKM as a measure of gene expression level and the y-axis indicates transcript density

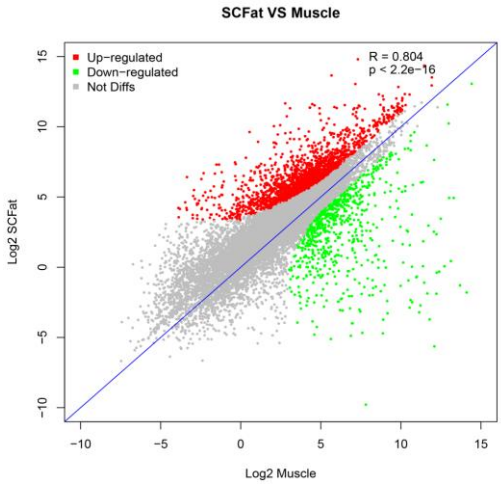

Fig. 3: Scatter plot of SCF and muscle transcript expression levels. The x-axis shows $\log_2$ FPKM muscle and y-axis $\log_2$ FPKM SCF values. Red dots indicate up-regulated genes, green dots down-regulated genes, and grey dots no difference in expression between the two libraries

expressed in the SCF library, SCD is up-regulated in the SCF group. This result is consistent with the previous study on cattle (Ohsaki *et al*., 2009). The rate-limiting enzyme SCD is not only responsible for the conversion of saturated into monounsaturated fatty acids, but also affects the fatty acid composition of membrane phospholipids, triglycerides and cholesterol esters. Pathway analysis of DEGs revealed their association with processes relevant

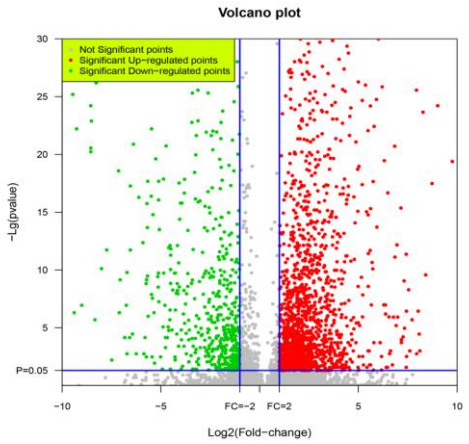

Fig. 4: Volcano plot showing genes differentially expressed in the SCF and muscle libraries. X-axis, $\log_2$ (Fold Change). Y-axis, –Lg (p-value). Green dots indicate significantly down-regulated genes and red dots significantly up-regulated genes, while grey dots indicate no significant difference in expression levels

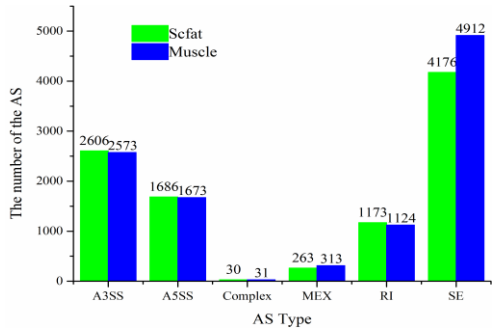

Fig. 5: Distribution of alternative splicing events. X-axis, AS type. Y-axis, number of alternative splicing events. Green, AS events in SCF. Blue, AS events in muscle. The major AS types observed were SE, A3SS and A5SS

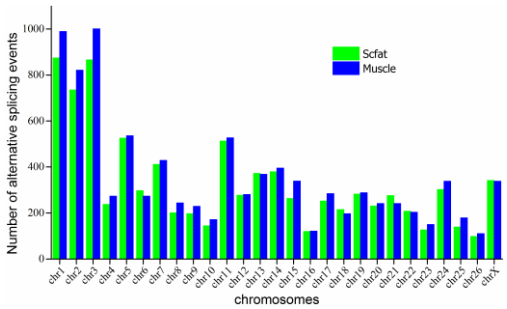

Fig. 6: Alternative splicing events on sheep chromosomes in SCF and muscle X-axis, chromosomes. Y-axis, number of alternative splicing events. Blue, AS events in SCF. Red, AS events in muscle. AS events were distributed primarily on chromosomes 1, 2 and 3

to fat deposition. Compared with muscle, the identified DEGs in SCF are thought to be extremely important to adipose deposition and differentiation. Further studies will be conducted by using quantitative real-time PCR for verification of the differentially expressed genes. Further studies are needed to verify the differentially expressed genes based on quantitative real-time PCR technology. The important genes for regulating metabolism in adipose tissue are also need to be further identified thoroughly, as well as the molecular mechanism of lipogenesis in fat tissues.

Conclusion

In this study, the transcriptome profiles of SCF and muscle tissue in Lanzhou fat-tailed sheep were analyzed comparatively based on RNA-seq technology. The results not only greatly expand our understandings of the phenotypic and functional differences in fat deposition in Lanzhou fat-tailed sheep, but also provide basic data for future investigations of the molecular mechanisms of fat deposition, as well as meat sheep breeding practice.

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References

Chen, C., H. Ai, J. Ren, W. Li, P. Li, R. Qiao, J. Yang, M. Yang, J. Ma and L. Huang, 2011. A global view of porcine transcriptome in three tissues from a full-sib pair with extreme phenotypes in growth and fat deposition by paired-end RNA sequencing. *BMC Genom.*, 12: 1–16

Cheng, X., S.G. Zhao, Y. Yue and Z. Liu, 2016. Comparative analysis of the liver tissue transcriptomes of Mongolian and Lanzhou fat-tailed sheep. *Genet. Mol. Res.*, 15: 1–13

Cox, L.A., J.P. Glenn, K.D. Spradling, M.J. Nijland, R. Garcia, P.W. Nathanielsz and S.P. Ford, 2012. A genome resource to address mechanisms of developmental programming: determination of the fetal sheep heart transcriptome. *J. Physiol.*, 590: 2873–2884

Dunnick, J.K., A. Brix, H. Cunny and M. Valla, 2012. Characterization of polybrominated diphenyl ether toxicity in Wistar Han rats and use of liver microarray data for predicting disease susceptibilities. *Toxicol. Pathol.*, 40: 93–106

Hausman, G.J., U. Basu, S. Wei, D.B. Hausman and M.V. Dodson, 2014. Preadipocyte and adipose tissue differentiation in meat animals: influence of species and anatomical location. *Annu. Rev. Anim. Biosci.*, 2: 323–351

He, H. and X.L. Liu, 2013. Characterization of transcriptional complexity during longissimus muscle development in bovines using high-throughput sequencing. *PLoS One*, 8: e64356

Kang, X.L., G. Liu, Y.F. Liu and Q.Q. Xu, 2013. Transcriptome profile at different physiological stages reveals potential mode for curly fleece in Chinese tan sheep. *PLoS One*, 8: e71763

Liu, N., J.N. He, W.M. Yu and K.D. Liu, 2015. Transcriptome analysis of skeletal muscle at prenatal stages in Polled Dorset versus small-tailed Han sheep. *Genet. Mol. Res.*, 14: 1085–1095

Ma, L., Z. Li, Y. Cai, H. Xu, R. Yang and X. Lan, 2018. Genetic variants in fat and short-tailed sheep from high-throughput RNA-sequencing data. *Anim. Genet.*, 49: 483–487

Miao, X., Q. Luo, X. Qin, Y. Guo and H. Zhao, 2015a. Genome-wide mRNA-seq profiling reveals predominant down-regulation of lipid metabolic processes in adipose tissues of Small Tail Han than Dorset sheep. *Biochem. Biophys. Res. Commun.*, 467: 413–420

Miao, X.Y., Q.M. Luo and X.Y. Qin, 2015b. Genome-wide transcriptome analysis of mRNAs and microRNAs in Dorset and Small Tail Han sheep to explore the regulation of fecundity. *Mol. Cell Endocrinol*., 402: 32–42

Moreira, R., P. Pereiro, C. Canchaya, D. Posada, A. Figueras and B. Novo, 2015. RNA-Seq in *Mytilus galloprovincialis*: comparative transcriptomics and expression profiles among different tissues. *BMC Genomics*, 16: 1–18

Mukamal, K.J., J.B. Wilk and M.L. Biggs, 2013. Common FABP4 genetic variants and plasma levels of fatty acid binding protein 4 in older adults. *Lipids*, 48: 1169–1175

Ohsaki, H., A. Tanaka, S. Sasazaki, K. Oyama, M. Taniguchi, F. Mukai and H. Mannen, 2009. Effect of SCD and SREBP genotypes on fatty acid composition in adipose tissue of Japanese Black cattle herds. *Anim. Sci.*, 80: 225–232

Pouliot, M.C., J.P. Despres, A. Nadeau, S. Moorjani, D. Prud'Homme, P.J. Lupien, A. Tremblay and C. Bouchard, 1992. Visceral obesity in men. Associations with glucose tolerance, plasma insulin and lipoprotein levels. *Diabetes*, 41: 826–834

Savage, S.R., C.A. Bretz and J.S. Penn, 2015. RNA-Seq reveals a role for NFAT-signaling in human retinal microvascular endothelial cells treated with TNFa. *PloS One*, 10: 1–10

Suarez-Vega, A., B. Gutierrez-Gil, C. Klopp, G. Tosser-Klopp and J.J. Arranz, 2016. Comprehensive RNA-Seq profiling to evaluate lactating sheep mammary gland transcriptome. *Sci. Data*, 3: 1–11

Van-Harmelen, V., K. Rohrig and H. Hauner, 2004. Comparison of proliferation and differentiation capacity of human adipocyte precursor cells from the omental and subcutaneous adipose tissue depot of obese subjects. *Metab. Clin. Exp.*, 53: 632–637

Wang, H., X.Y. Li, R.Y. Zhou, J.Z. Xi, Y.H. Wei, L.H. Li and Z.H. Zhang, 2018. Genome-wide transcriptome profiling in ovaries of small-tail Han sheep during the follicular and luteal phases of the oestrous cycle. *Anim. Reprod. Sci.*, 197: 212–221

Wang, H., Y. Lei, L. Yan, L. Wan, X. Ren, S. Chen, X. Dai, W. Guo, H. Jiang and B. Liao, 2016. Functional genomic analysis of aspergillus flavus interacting with resistant and susceptible peanut. *Toxins*, 8: 1–16

Wang, L., J. Jiang and L. Li, 2012. Different expression patterns of heart and adipocyte fatty acid-binding protein gene during porcine skeletal muscle development. *J. Anim. Vet. Adv.*, 11: 700–703

Wang, Y.H., C.L. Zhang, M. Plath, X.T. Fang, X.Y. Lan, Y. Zhou and H. Chen, 2015. Global transcriptional profiling of longissimus thoracis muscle tissue in fetal and juvenile domestic goat using RNA sequencing. *Anim. Genet.*, 46: 655–665

Wang, Z.C., X.M. Qiu, D.R. Kong and X.X. Zhou, 2017. Comparative RNA-Seq analysis of differentially expressed genes in the testis and ovary of *Takifugu rubripes*. *Comp. Biochem. Physiol. D Genom. Proteom.*, 22: 50–57

Yu, G.J., M. Wang, J. Huang, Y.L. Yin, Y.J. Chen, S. Jiang, Y.X. Jin, X.Q. Lan, B.H. Wong, Y. Liang and H. Sun, 2012. Deep insight into the ganoderma lucidum by comprehensive analysis of its transcriptome. *PLoS One*, 7: e44031

Yue, Y.J., J.B. Liu, M. Yang, J.L. Han, T.T. Guo, J. Guo, R.L. Feng and B.H. Yang, 2015. De novo assembly and characterization of skin transcriptome using RNA-seq in sheep. *Genet. Mol. Res.*, 14: 1371–1384

Zhang, C.L., G.Z. Wang, J.M. Wang, Z.B. Ji, Z.H. Liu, X.H. Pi and C.X. Chen, 2013. Characterization and comparative analyses of muscle transcriptomes in dorper and small-tailed han sheep using RNA-seq technique. *PLoS One*, 8: e72686

Zhang, W.Z., X.M. Xiong, X.J. Zhang, S.M. Wan, N.N. Guan, C.H. Nie, B.W. Zhao, C.D. Hsiao, W.M. Wang and Z.X. Gao, 2016. Mitochondrial genome variation after hybridization and differences in the first and second generation hybrids of bream fishes. *PLoS One*, 11: e0158915

Zhou. Y., J.J. Sun, C.J. Li, Y.H. Wang, L. Li, H.F. Cai, X.Y. Lan, C.Z. Lei, X. Zhao and H. Chen, 2014. Characterization of transcriptional complexity during adipose tissue development in bovines of different ages and sexes. *PLoS One*, 9: e101261

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