



Polymorphism and Comparative Expression Analysis of *THRSP* Gene in Fat-Tailed and Thin-Tailed Sheep Breeds

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ABSTRACT

Sheep is a significant agricultural animal and food source for people worldwide. The fat tail/rump is considered as an adaptive selection under harsh challenges which serves as a fat store for the animal. Thyroid hormone responsive (*THRSP*) is a crucial protein for cellular de novo lipogenesis. *THRSP* gene encodes a nuclear protein which regulates fatty acid synthesis in lipogenic tissues. Identification of single nucleotide polymorphisms (SNPs) of sheep *THRSP* gene and their association with fat deposition were investigated using Altay and White Suffolk sheep. In addition, the messenger RNA expression profiling of *THRSP* in fat-tailed and thin-tailed breeds was compared among 8 different tissues. Four SNPs have been detected in position g. 205A>C, c. 52C>T, c. 364A>T and c. 1031C>T of *THRSP* gene. Genotyping method was used to analyze genotypes among Altay sheep (fat-rumped breed) and White Suffolk (thin-tailed breed) by Sequenom MassArray. In the c.1031C>T locus, the frequency of T allele of fat-tail breed was significantly ($p<0.001$) higher than that of the thin-tailed breed using F test, which suggest that this locus may be associated with fat deposition in fat-tail breed. Gene expression levels in both subcutaneous adipose and tail fat tissues were significantly ($p<0.01$) higher than the other tissues analyzed. Levels of *THRSP* expression in liver, subcutaneous adipose and tail fat tissues in Tan sheep (thin-tailed breed) were significantly ($p<0.01$) higher than that in Altay sheep (fat-tailed breed). The results obtained might expand our understanding of the important regulating role of *THRSP* gene in lipogenesis in fat-tailed sheep breed.

INTRODUCTION

Sheep (*Ovis aries*) was probably the first domesticated animal in the Fertile Crescent region on the discovery of the archaeological evidence (Mason, 1984). There is a spectrum of phenotypically diverse populations of sheep worldwide due to their manageable size, early maturity, diverse products, to poor nutritional diets, tolerance to extreme climatic conditions and economic performance under harsh environments (Kijas *et al.*, 2009). According to the tail types, sheep can be categorized into five groups,

including fat rump, short- and long-thin tail, as well as short- and long-fat tail breeds (Zhang *et al.*, 2019).

China has the most diverse sheep breeds compared to other countries. Altay sheep is a local breed native to Xinjiang (China), famous for tasty, high meat production and strong adaptability in rigid environments. This breed is one of the most important sheep in Xinjiang which is a kind of typical fat-rumped breed (China National Commission of Animal Genetic Resources, 2011). The Altay sheep has a large rump composed entirely of white adipose tissue which accounts for about 18% of the carcass content. The fat tail/rump is considered as a selection of evolution which serves as a fat store for the animal under harsh environment. However, more energy is needed for fat than for protein (lean meat) deposition. Nowadays, reduction in fat tail size is often desirable for both sheep

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Authors' Contribution

QL designed the study. QS and QL performed the experiments, analyzed data, and wrote the first draft. RD, YW, SG and SL supported the sampling and genotyping of animals. XW, WH, XC and ZP provided analysis tools. XG and YY provided support in statistical analysis of data. HER and MC critically reviewed and revised the manuscript.

Key words

Altay sheep, Fat tail, *THRSP* gene, SNP

stakeholders and mutton consumers. Consequently, the advantages of large fat tail/rump have begun to fade due to changing consumer preferences (Ahbara *et al.*, 2018).

Several studies have been carried out to evaluate growth performance and carcass characteristics using fat-tailed and lean-tailed sheep breeds (Khaldari *et al.*, 2008; Pourlis, 2011). Advances achieved in molecular biology and genomics have made possible the study of genetic background of economically important traits. In this respect, the next-generation sequencing platforms have been employed to explore candidate genes / genomic regions linked to fat deposition in thin- and fat-tailed sheep breeds. A genome-wide scan was performed based on OvineSNP50K Beadchip in Iranian thin- and fat-tailed breeds, and two of identified regions were associated with an increase of homozygosity in the fat-tailed breeds (Moradi *et al.*, 2012). *De novo* transcriptome sequencing was used to compare sheep adipose tissue transcriptome profiles between fat-tailed and short-tailed breeds. Approximately 646 differentially expressed genes and functional pathways were identified (Wang *et al.*, 2014). Up to date; however, the genes affecting fat deposition in fat tails of domesticated sheep are still scarce.

Thyroid hormone responsive (*THRSP* or Spot14) gene encodes a nuclear protein which regulates fatty acid synthesis in lipogenic tissue (Donnelly *et al.*, 2008). It is induced by thyroid hormone, carbohydrate intake, adipose tissue differentiation, and lactation. Expression profiling of *THRSP* gene goes in line with fatty acid synthase in adipose, liver, and mammary tissue in bovine and murine species (Zeng *et al.*, 2018). Spot14 protein is expressed primarily in tissues which synthesize fatty acids. The expression levels of *THRSP* mRNA in white adipose tissue, brown adipose tissue and liver are elevated when *de novo* fatty acid synthesis is induced by dietary and hormonal stimuli (Chou *et al.*, 2007).

To the best of our knowledge, no associations between SNPs within *THRSP* gene and fat metabolism in fat-tailed and thin-tailed sheep breeds have been reported. Therefore, the present study was undertaken to identify SNP variants in *THRSP* gene and to evaluate their potential association with fat deposition in the tail of Altay and White Suffolk sheep breeds. Also, *THRSP* gene expression profiles was targeted in adipose tissues of fat-tailed and thin-tailed sheep breeds.

MATERIALS AND METHODS

Sampling and DNA extraction

All procedures involving animals were approved by the Animal Care and Use Committee at the respective institutions where the present study was conducted.

Also, such procedures involving animals were officially authorized later by the Chinese Ministry of Agriculture.

For SNP analysis study, 200 ewes of two different breeds reared in China were selected. Sampling process included 100 Altay sheep belonging to Fuyun Breeding Farm (Fuyun County, Xinjiang Uygur Autonomous Region, P.R. China) and 100 White Suffolk from Beijing Aoxin Stud Farm Co. Ltd. (Beijing, P.R. China). All the sampled individuals were in a good state of health and nutrition. Ear tissue taken from each Altay sheep was immersed in 70% ethanol under 4°C and stored at -20°C pending for DNA isolation. On the other hand, blood samples from the jugular vein of White Suffolk were collected in tubes containing acid citrate dextrose as an anticoagulant. Genomic DNA was extracted from ear tissue and whole blood by the phenol-chloroform method depending on the breed, and then dissolved in TE buffer composed of 10 mmol/l Tris-HCl (pH 8.0), 1 mmol/l EDTA (pH 8.0), and then kept at -20 °C.

For gene expression analysis study, three animals of Altay sheep reared at the animal housing facilities of Xinjiang Academy of Agriculture and Reclamation Science, Shihezi, China as well as other three individuals of Tan sheep owned by Breed Conservation Farm in the Ningxia Hui Autonomous Region, China were subjected to this experiment. The dorsal subcutaneous adipose tissue samples taken from each animal were frozen in liquid nitrogen and stored at -80°C till RNA isolation.

Primers and PCR amplification

Five pairs of primers (P1, P2, P3, P4, P5) were designed according to the mRNA sequences of sheep *THRSP* gene (GenBank Accession Number: XM_004019431) and DNA sequence of 5'UTR of goat *THRSP* gene (GenBank Accession Number: JN684754.1 or JN6847541). Molecular data corresponding to the primers amplified in the current study are given in Table 1. The DNA fragments of the studied gene were amplified using Polymerase Chain Reaction (PCR) technique. PCRs were carried out in 25 µl volume containing approximately 1.0 µl of 10 µmol/l each primer, 2.5 µl of 10×PCR buffer (50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.0), 0.1% Triton X-100), 1.0-1.8 µl of 25 mmol/l MgCl₂, 2.5 µl of 2.5 mmol/l each dNTP, 3.0 µl of 50 ng/µl ovine genomic DNA, 1.0 µl of 2.5 U/µl *Taq* DNA polymerase (Promega, Madison, WI, USA), and the rest was ddH₂O. PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min on Mastercycler[®] 5333 (Eppendorf AG, Hamburg, Germany).

Table I. Primers used for *THRSP* gene analysis.

Primer name	Primer sequence(5'→3')	Amplified regions	Product size(bp)	Annealing temperature (°C)	Reference sequences (NCBI)
P1 <i>THRSP</i> 1	F: GGGTCAATGGTGAAGACGAAGCT R: GCCAAGTACCGGTCCATGACG	5'UTR	1k	58	KT266274
P2 <i>THRSP</i> 2	F: CTCTAAATGGCTCTGCAAGCTGATG R: TAGCCCTCTTTTAGGCCAATCCTAG	5'UTR	200	55	KT266274
P3 <i>THRSP</i> 3	F: TGACCAAGCGCTACCCTAAG R: CTCCTCGGCTTTCAGGGTAA	Exon 1	400	57	XM_004019431
P4 <i>THRSP</i> 4	F: GTGATGATCCCCAGCCTTCT R: TACCCTCTTCTCAGCCT	Exon 2	230	53	XM_004019431
P5 <i>THRSP</i> 5	F: ACTCCACATGATAGAAGGCAGT R: AGTCCGAAAACCTTGCCAAAATT	3'UTR	802	57	XM_004019431
<i>THRSP</i>	F: GGAGAGATGGAAGAGGCTGAG R: CTCCTCGGCTTTCAGGGTAA	Real-time PCR	123	58	XM_004019431
GAPDH	F:ATGCCTCCTGCACCACCA R: AGTCCCTCCACGATGCCAA	Real-time PCR	76	58	NM_001190390

Table II. SNPs detected in ovine *THRSP*.

Position	THRSP				
	g. 205A>C	c. 52C>T	c. 364A>T	c. 1031C>T	
Nucleotide position					
Amino acid position	/	10	114	/	
Codon in Altay	/	C/TTG	T/ATG	/	
Codon in Suffolk	/	C/TTG	T/ATG	/	
Amino acid in Altay	/	L	L/M	/	
Amino acid in Suffolk	/	L	L/M	/	
SNP type	S	NS	NS	S	

NS = Non-synonymous; S = Synonymous

The PCR products were separated by electrophoresis on 2% agarose gels (Promega, Madison, WI, USA) in parallel with DNA Marker I (Tiangen, Beijing, P.R. China). Gels were visualized using a 1.5% agarose gel containing ethidium bromide, photographed, and analyzed using an AlphaImager™ 2200 and 1220 Documentation and Analysis Systems (Alpha Innotech Corporation, San Leandro, CA, USA).

SNP analysis

Ten individuals from each sheep breed were selected randomly. Genomic DNA from Altay sheep and White Suffolk sheep was used as a template to be amplified with the five pairs of primers (P1-P5). Sequences were aligned to search for the base pair variations. PCR products were separated on 2% agarose gels and recovered using GeneClean II kit (Promega, Madison, WI, USA). Each DNA fragment was sequenced in both directions using

an automatic ABI 3730 sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) by Sino Geno Max Co. Ltd. (Beijing, China). Sequence analysis and amino acid determination were performed by DNAMAN version 9.0 and DNASTAR lasergene 7.1.

Genotyping

Ten out of 200 animals from Altay and White Suffolk sheep were selected for genotyping. Genotyping was performed using primer extension chemistry and mass spectrometric analysis (iPlex assay, Sequenom, San Diego, CA) on the Sequenom MassArray according to the manufacturer's instructions (<http://www.sequenom.com>). Only those samples with a >95% success rate and only those SNPs with a genotype success rate of >95% were included in the subsequent analysis.

Allele and genotype frequencies were estimated by direct counting. Statistical analyses were performed by

SAS program (Ver 8.1) (SAS Institute Inc., Cary, NC, USA). Differences between the two groups of samples were assessed using *t*-test assuming unequal variances. *P* values less than 0.05 were considered to be significant. *Chi-square* test was applied to analyze the statistical significance of genotypic distributions of the two sheep breeds under study.

Total RNA extraction and cDNA preparation

Total RNA was prepared from each frozen sample and purified by using the QIAzol Lysis Reagent (RNeasy Lipid Tissue) according to the manufacturer's instructions (Qiagen, Valenci, CA, USA). About 1 µg RNA was reverse transcribed into single-strand cDNA using oligo(dT)₁₈-mer primers and M-MLV Reverse Transcriptase (Invitrogen, Karlsruhe, Germany).

Quantitative real-time PCR

Reverse transcription polymerase chain reaction (RT-PCR) was performed on a fluorescence thermal cycler (ABI PRISM® 7500HT Sequence Detection System, Applied Biosystems). A standard two-step procedure was applied. RNAs were reverse transcribed into single strand cDNAs as described above. PCR was performed in 15-µL reaction mixtures consisting of cDNA, 0.5 µM specific primer sets for each target gene, and SYBR Green PCR Master Mix (Applied Biosystems, UK). RT-PCR were cycled with the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 repetitive cycles of 95°C for 15 sec and 60°C for 1 min. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as internal control to normalize for initial RNA input. The primers used for PCR are listed in Table I. The specificity and quantity of each RT-PCR product were checked by melting curve analysis according to the following program: 95°C for 15 sec, 60°C for 15 sec, 95°C for 15 sec. Relative expression of each gene was calculated according to the 2^{-ΔΔCt} method. Differential expression of *THRSP* was analyzed using one way ANOVA at *p*<0.05. Each PCR reaction was run in triple for each independent sample.

RESULTS

Polymorphism identification and detection

Genomic DNA of the two sheep breeds (Altay and White Suffolk) was successfully amplified using primers P1-P5 for *THRSP* gene. A total of 10 PCR products amplified by each primer pair was selected randomly and subsequently cloned for sequencing. Molecular information about the SNPs detected in ovine *THRSP* gene is summarized in Table II. PCR products amplified by primers P1, P3, P4 and P5 displayed polymorphisms

which had four base pair changes (g. 205A>C, GenBank Accession Number: KT266274; c.52C>T, c.364A>T and c.1031C>T, GenBank Accession Number: XM_004019431). The g. 205A>C and c.1031C>T are located in 5' and 3'UTR, respectively. Non-synonymous c.52C>T SNP and non-synonymous c.364A>T SNP both are located in exon 1.

Table III. Allele and genotype frequencies of *THRSP* gene in Altay and Suffolk breeds.

Genotype	Altay	Suffolk
<i>THRSP</i> g. 205A>C	n = 97	n = 96
Genotype frequency	AA 0.36 (35) AC 0.51(49) CC 0.13(13)	AA 0.35(34) AC 0.47(45) CC 0.18(17)
Allele frequency	A 0.613 C 0.387	A 0.589 C 0.411
H-W test χ^2	0.411	0.0993
P	0.521	0.753
<i>THRSP</i> c. 52C>T	n = 100	n = 98
Genotype frequency	CC 0.95(95) CT 0.05(5)	CC 0.969(95) CT 0.031 (3)
Allele frequency	C 0.975 T 0.025	C 0.985 T 0.015
H-W test χ^2	0.0658	0.0237
P	0.798	0.878
<i>THRSP</i> c. 364A>T	n = 100	n = 97
Genotype frequency	AT 0.06(6) TT 0.94(94)	AT 0.041(4) TT 0.959(93)
Allele frequency	A 0.03 T 0.97	A 0.021 T 0.979
H-W test χ^2	0.0957	0.0429
P	0.757	0.836
<i>THRSP</i> c. 1031C>T	n = 98	n = 98
Genotype frequency	CC 0.510 (50) CT 0.388 (38) TT 0.102 (10)	CC 0.867 (85) CT 0.102 (10) TT 0.031 (3)
Allele frequency	C 0.704 T 0.296	C 0.918 T 0.082
H-W test χ^2	0.473	10.0
P	0.492	0.00157

These four mutations were detected by Sequenom Mass Array in the two sheep breeds with different tail

types (Altay as a fat-tailed breed and Suffolk as a thin-tailed breed). The total detection rate was 97.7%. As demonstrated in Figure 1 and Table III, A to C transversion at site g.205A>C expressed three genotypes: AA, AC and CC. While C to T transversion at site c.52C>T revealed only two genotypes: CC and CT. Similarly, A to T transition at site c.364A>T expressed two genotypes: AT and TT. On the other hand, C to T transversion at site c.1031C>T shown three genotypes: CC, CT and TT.

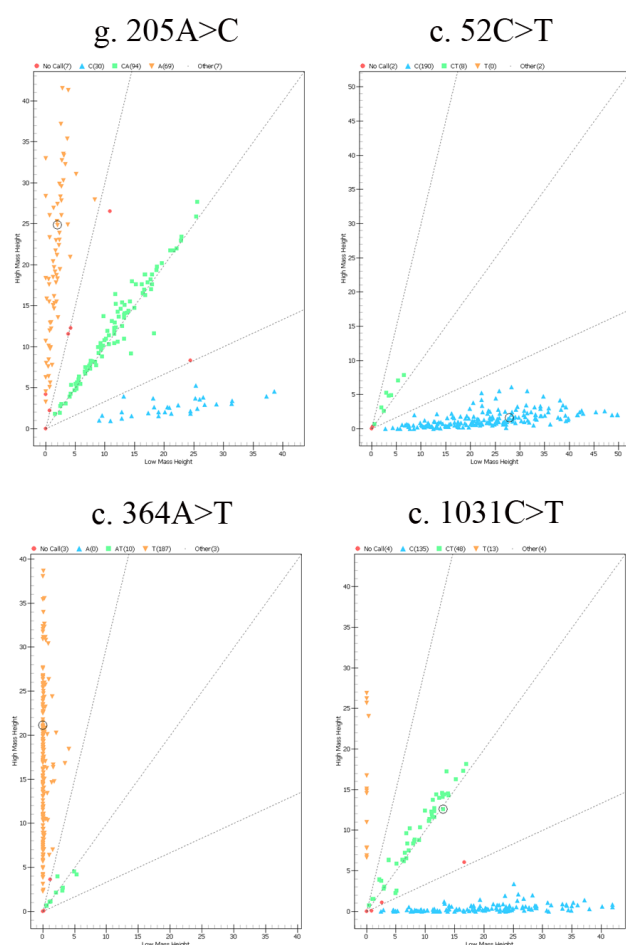


Fig. 1. The results of sequenom massarray at (g. 205A>C), (c. 52C>T), (c. 364A>T), (c. 1031C>T) in sheep *THRSP* gene.

Allele and genotype frequencies of sheep *THRSP* gene

Allelic and genotypic frequencies of *THRSP* gene in Altay and White Suffolk breeds were calculated (Table III). F test was applied to determine the statistical significance of allele frequencies between the two sheep breeds. As shown in Table III, allele A is the dominant allele in both breeds at the g. 205A>C locus. At the locus c.52C>T, allele C is largely dominant over allele T in the two studied

breeds. However, no statistically significant difference is found between both alleles in each individual breed. In respect to locus c.364A>T, a pair of alleles is identified (A and T), without significant difference between fat-tailed breed and thin-tailed breed at the respective locus located in exon 1 of *THRSP* gene. For the c.1031C>T locus, allele C is the most common in the two sheep breeds compared to allele T. The difference between both alleles is statistically significant only in the thin-tailed breed. White Suffolk is significantly deviated from Hardy-Weinberg equilibrium ($p < 0.01$). The frequency of T allele of fat-tailed breed is significantly ($p < 0.001$) higher than that of thin-tailed breed, indicating that the c.1031C>T locus may be associated with fat deposition in fat-tailed sheep breeds.

Tissue distribution of *THRSP* mRNA

The distribution of *THRSP* mRNA expression in eight tissues (heart, liver, spleen, lung, kidney, skeletal muscle, subcutaneous adipose and tail fat) was examined by RT-PCR, using a pair of specific primers amplifying a 123-bp amplicon of this gene. The house-keeping gene GAPDH was used as an internal control for normalization (Fig. 2A). Interestingly, a relatively high level of expression of *THRSP* can be only detected in the subcutaneous adipose and tail fat tissue which were extremely higher than the corresponding expression in the other six tissues (>1000 fold, $p < 0.01$). Higher mRNA levels were detected in the liver than in the spleen, lung, kidney and skeletal muscle, while there was almost no expression level shown in the heart of Altay sheep.

Table IV. Test of difference of the loci genotype distributions in Altay and Suffolk breeds.

Breeds	Suffolk	χ^2	P
Altay	SNP locus		
	<i>THRSP</i> g. 205A>C	0.713	0.7
	<i>THRSP</i> c. 52C>T	0.48	0.488
	<i>THRSP</i> c. 364A>T	0.36	0.549
	<i>THRSP</i> c. 1031C>T	29.177	4.62E-07

Expression levels of *THRSP* in adipose-related tissues

To further determine the differential expression between two breeds with different tail types, mRNA levels of *THRSP* in liver, subcutaneous adipose and tail fat tissues were determined based on RT-PCR in Altay and Tan sheep breeds. As shown in Figure 2B, the highest *THRSP* mRNA expression level is shown in the subcutaneous fat tissue, followed by tail fat and liver for the two studied breeds. Significantly ($p < 0.01$) higher

THRSP expression levels are observed in Tan compared to Altay sheep for all examined tissues.

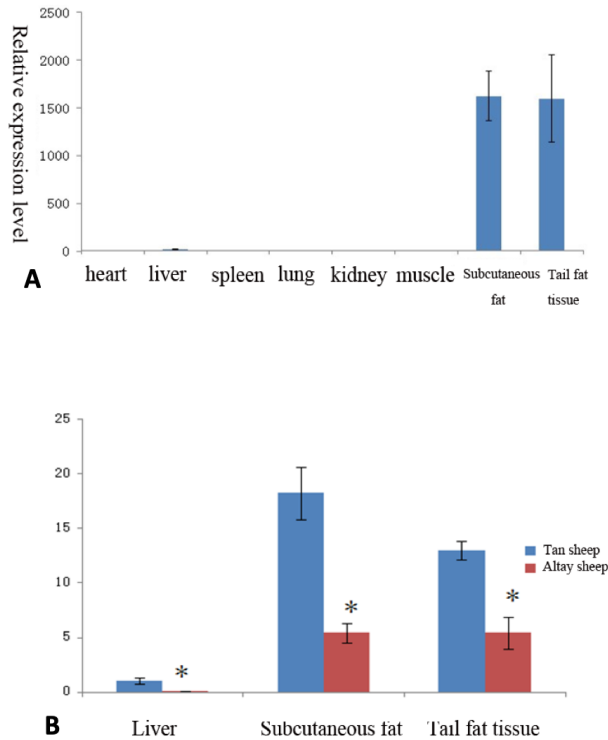


Fig. 2. Gene expression profiles of *THRSP* gene. (A) Expression levels of *THRSP* gene in heart, liver, spleen, lung, kidney, muscle, subcutaneous fat and tail fat tissue in Altay sheep (B) Expression levels of *THRSP* gene in liver, subcutaneous fat, tail fat tissue of Tan sheep and Altay sheep.

DISCUSSION

The fat-tailed sheep breeds are characterized by the distinctive large tails and hindquarters. Approximately 25% of the world sheep populations comprise fat-tailed breeds which are commonly found in the northern parts of Africa, the Middle East, Central Asia, and Western China (Pourlis, 2011). Altay sheep chosen for this study has a large rump composed entirely of white adipose tissue which is known for their ability to cope with harsh environmental conditions such as drought and famine in northern part of Xinjiang Uygur Autonomous Region. Due to improved forage availability, healthy issue and change in consumption patterns and trends, fat-tailed trait, fat tail trait is commercially undesirable now. Accordingly, today's sheep breeders are highly interested in finding useful molecular markers that can be incorporated into contemporary sheep breeding programs through marker-

assisted selection (MAS) or genomic selection (GS). So, searching for gene variants affecting the phenotypic expression of fat-tailed trait in ovine species is becoming a hot topic in molecular genetics.

Biological processes such as lipogenesis and adipogenesis are governed by a vast number of enzymes which act together along with hormones and metabolites to regulate fat cell metabolism (Cornelius *et al.*, 1994; Farmer, 2006). The main role of white adipose tissue is triacylglycerol (TAG) storage and fatty acid (FA) release. The direct action on adipose tissue should be to activate lipolytic enzymes and coordinate endocrine signals to initiate lipolysis. Adipocyte precursor differentiation is driven by a cascade of events regulated by transcription regulators and coactivators. This entire process is closely regulated at the transcriptional level (Rosen and MacDougald, 2006; Rosen and Spiegelman, 2000). Up to now, there is little published information related to fat tail syndrome especially for the Chinese local sheep breeds. Several candidate genes have been involved in association studies with fat deposition and lipid metabolism in domestic animals. One of these major genes is Peroxisome Proliferator Activated Receptor Gamma (PPARG), which is a protein coding gene. PPARG and its target genes are factors leading to greater intramuscular fat deposition in cattle (Moisa *et al.*, 2014). Also, heart-type fatty acid-binding protein (H-FABP) plays role in fat deposition and the regulation of fatty acid metabolism in Lanzhou fat-tailed sheep (Bai *et al.*, 2013). The mRNA abundance of G-protein coupled receptor 41 (GPR41), adiponectin receptors 1 and 2 (AdipoR1/2) and leptin is divergent in different fat depots from sheep (Lemor *et al.*, 2010). There were novel associations of DGAT1 gene in which the C allele had a positive effect on fat-tail weight and backfat thickness in fat-tailed sheep (Mohammadi *et al.*, 2013). *THRSP* was suggested as a transcription factor to regulate gene expression of rate-limiting enzymes in lipogenesis. Transcription is regulated via thyroid hormone and the SREBP-1c binding sites (Wu *et al.*, 2013). *THRSP* null mouse weight gain is resultant from decreased fat accumulation and is associated with improvement in glucose tolerance (Anderson *et al.*, 2009). In addition, the *THRSP* gene may contribute to fat accumulation in humans (Ortega *et al.*, 2010). In parallel, *THRSP* has been studied in different farm animals species, *THRSP* gene expression is highly correlated with the intramuscular fat content of cattle (Wang *et al.*, 2009). The mutations of *THRSP* gene had an important effect on body weight in the chicken (Cao *et al.*, 2007). Three mutational sites, C-233T, G113A and A138G of *THRSP* were detected in Chinese indigenous goat breeds, and those polymorphisms may be associated with the ecological factors and affect the goat lipogenesis

ability (Chen *et al.*, 2012).

In the present study, four polymorphic sites (g.205A>C, GenBank Accession Number: KT266274; c.52C>T, c.364A>T and c.1031C>T, GenBank Accession Number: XM_004019431) of sheep *THRSP* gene were detected. The g.205A>C and c.1031C>T located in 5' and 3'UTR, respectively. The variations identified in the coding region did not largely change the genotype frequencies between fat-tailed and thin-tailed breeds. T allele frequency of c.1031C>T in fat tail breed was higher than that of the thin tail breed, which may indicate that this locus may be associated with fat deposition in fat-tail breed. It has been commonly recognized that SNPs which occur in the 3'UTR can affect gene regulation by interfering with posttranscriptional activity, such as protein binding, polyadenylation and miRNA binding (Sethupathy *et al.*, 2007). A 3'UTR SNP in the sheep *Gdf8* gene creates a new illegitimate miRNA target site, which leads to significant down-regulation of *Gdf8* and contributes to muscular hypertrophy (Clop *et al.*, 2006). The expression of human *AGTR1* gene that contains one SNP in 3'UTR can be regulated by has-miR-155 to associate with hypertension in trisomy 21 (Sethupathy *et al.*, 2007). It has been reported that *THRSP* can be targeted by miR-3582 to affect the lactation in rat (Zhang *et al.*, 2014). As the c.1031C>T locus is located near to the complementary sites of miR-3582, so the expression profiling of *THRSP* gene may be affected by such neighborhood. Then the adipose metabolism in tail could be regulated via alteration of transcription activities. This conclusion needs to be confirmed in the future studies.

The high levels of the *THRSP* expression patterns detected in the subcutaneous adipose and tail fat tissues in this study is consistent with expression data of *THRSP* gene in other mammals (Kuemmerle and Kinlaw, 2011). Compared with Altay sheep, Tan breed has much smaller fat tail size, produces more delicious meat and better lamb skin characteristics. These advantages make Tan sheep breed the most popular breed in Northern China. We were trying to compare mRNA abundance of *THRSP* in two sheep breeds for understanding the phenotypic and functional differences of fat deposition in sheep. In this research, *THRSP* mRNA levels in adipose were significantly higher than its level in liver in both breeds, while higher *THRSP* expression levels were observed among all three tissues in Tan sheep.

CONCLUSION

Gene variants affecting the phenotypic expression of fat-tailed trait in sheep are becoming a hot topic in molecular genetics. From our study, association between

polymorphisms of *THRSP* gene and fat deposition was analyzed in fat-tailed and thin-tailed sheep breeds. *THRSP* gene expression profiles in adipose tissues between fat-tailed and thin-tailed breeds have also been evaluated. Our results might expand our understanding of *THRSP* gene regulating lipogenesis in fat-tailed sheep breed.

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Statement of conflict of interest

The authors have indicated no conflicts of interest.

REFERENCES

- Ahbara, A., Bahbahani, H., Almuthen, F., Al Abri, M., Agoub, M.O., Abeba, A., Kebede, A., Musa, H.H., Mastrangelo, S., Pilla, F., Ciani, E., Hanotte, O. and Mwacharo, J.M., 2018. Genome-wide variation, candidate regions and genes associated with fat deposition and tail morphology in Ethiopian indigenous sheep. *Front. Genet.*, **9**: 699. <https://doi.org/10.3389/fgene.2018.00699>
- Anderson, G.W., Zhu, Q., Metkowsky, J., Stack, M.J., Gopinath, S. and Mariash, C.N., 2009. The *THRSP* null mouse (*THRSP*(tm1cnm)) and diet-induced obesity. *Mol. Cell. Endocrinol.*, **302**: 99-107. <https://doi.org/10.1016/j.mce.2009.01.005>
- Bai, J.L., Xu, H.W., Zang, R.X., He, H.J., Cai, Y., Cao, X., Peng, F.J., Han, J., Wu, J.P. and Yang, J.T., 2013. Cloning of the heart fatty acid-binding protein (H-FABP) gene and its tissue-specific expression profile in the Lanzhou fat-tailed sheep, *Ovis aries*. *Small Rumin. Res.*, **112**: 114-122. <https://doi.org/10.1016/j.smallrumres.2012.12.016>
- Cao, Z.P., Wang, S.Z., Wang, Q.G., Wang, Y.X. and Li, H., 2007. Association of Spot14alpha gene polymorphisms with body weight in the chicken. *Poult. Sci.*, **86**: 1873-1880. <https://doi.org/10.1093/ps/86.9.1873>
- Chen, H.Q., Qin, J., Zhu, Y.J., Pan, Z.J., Xie, Y.N., Jiao,

- M.H., Chen, G.W., Chen, H. and Chu, M.X., 2012. The polymorphisms of goat *THRSP* gene associated with ecological factors in chinese indigenous goat breeds with different lipogenesis ability. *Asian J. Anim. Vet. Adv.*, **7**: 802-811. <https://doi.org/10.3923/ajava.2012.802.811>
- China National Commission of Animal Genetic Resources, 2011. *Animal genetic resources in China: Sheep and goats*. China Agriculture Press, Beijing.
- Chou, W.Y., Cheng, Y.S., Ho, C.I., Liu, S.t., Liu, P.y., Kuo, C.C., Chang, H.P., Chen, Y.H., Chang, G.G. and Huang, S.M., 2007. Human spot 14 protein interacts physically and functionally with the thyroid receptor. *Biochem. biophys. Res. Commun.*, **357**: 133-138. <https://doi.org/10.1016/j.bbrc.2007.03.103>
- Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibé, B., Bouix, J., Caiment, F., Elsen, J.M. and Eychenne, F., 2006. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nature Genet.*, **38**: 813-818. <https://doi.org/10.1038/ng1810>
- Cornelius, P., MacDougald, O.A. and Lane, M.D., 1994. Regulation of adipocyte development. *Ann. Rev. Nutri.*, **14**: 99-129. <https://doi.org/10.1146/annurev.nu.14.070194.000531>
- Donnelly, C., Olsen, A.M., Lewis, L.D., Eisenberg, B.L., Eastman, A. and Kinlaw, W.B., 2008. Conjugated linoleic acid (CLA) inhibits expression of the Spot 14 (*THRSP*) and fatty acid synthase genes and impairs the growth of human breast cancer and liposarcoma cells. *Nutri. Cancer*, **61**: 114-122. <https://doi.org/10.1080/01635580802348666>
- Farmer, S.R., 2006. Transcriptional control of adipocyte formation. *Cell Metab.*, **4**: 263-273. <https://doi.org/10.1016/j.cmet.2006.07.001>
- Khaldari, M., Kashan, N., Afzalzadeh, A. and Salehi, A., 2008. Growth and carcass characteristics of crossbred progeny from lean-tailed and fat-tailed sheep breeds. *S. Afri. J. Anim. Sci.*, **37**: 51-56. <https://doi.org/10.4314/sajas.v37i1.4026>
- Kijas, J.W., Townley, D., Dalrymple, B.P., Heaton, M.P., Maddox, J.F., McGrath, A., Wilson, P., Ingersoll, R.G., McCulloch, R. and McWilliam, S., 2009. A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. *PLoS One*, **4**: e4668. <https://doi.org/10.1371/journal.pone.0004668>
- Kuemmerle, N.B. and Kinlaw, W.B., 2011. *THRSP* (thyroid hormone responsive). *Atlas Genet. Cytogenet. Oncol. Haematol.*, **15**: 480-482. <https://doi.org/10.4267/2042/45032>
- Lemor, A., Mielenz, M., Altmann, M., von Borell, E. and Sauerwein, H., 2010. mRNA abundance of adiponectin and its receptors, leptin and visfatin and of G-protein coupled receptor 41 in five different fat depots from sheep. *J. Anim. Physiol. Anim. Nutri. (Berl)* **94**: e96-101. <https://doi.org/10.1111/j.1439-0396.2010.00987.x>
- Mason, I.L., 1984. *Evolution of domesticated animals*. Longman, London, New York.
- Mohammadi, H., Shahrehabak, M.M. and Sadeghi, M., 2013. Association between single nucleotide polymorphism in the ovine DGAT1 gene and carcass traits in two Iranian sheep breeds. *Anim. Biotechnol.*, **24**: 159-167. <https://doi.org/10.1080/10495398.2013.763816>
- Moisa, S.J., Shike, D.W., Faulkner, D.B., Meteer, W.T., Keisler, D. and Loor, J.J., 2014. Central role of the PPARgamma gene network in coordinating beef cattle intramuscular adipogenesis in response to weaning age and nutrition. *Gene Regul. Syst. Biol.*, **8**: 17-32. <https://doi.org/10.4137/GRSB.S11782>
- Moradi, M.H., Nejati-Javaremi, A., Moradi-Shahrbabak, M., Dodds, K.G. and McEwan, J.C., 2012. Genomic scan of selective sweeps in thin and fat tail sheep breeds for identifying of candidate regions associated with fat deposition. *BMC Genet.*, **13**: 10. <https://doi.org/10.1186/1471-2156-13-10>
- Ortega, F.J., Vazquez-Martin, A., Moreno-Navarrete, J.M., Bassols, J., Rodriguez-Hermosa, J., Girones, J., Ricart, W., Peral, B., Tinahones, F.J., Fruhbeck, G., Menendez, J.A. and Fernandez-Real, J.M., 2010. Thyroid hormone responsive Spot 14 increases during differentiation of human adipocytes and its expression is down-regulated in obese subjects. *Int. J. Obes. (Lond)* **34**: 487-499. <https://doi.org/10.1038/ijo.2009.263>
- Pourlis, A.F., 2011. A review of morphological characteristics relating to the production and reproduction of fat-tailed sheep breeds. *Trop. Anim. Hlth. Prod.*, **43**: 1267-1287. <https://doi.org/10.1007/s11250-011-9853-x>
- Rosen, E.D. and MacDougald, O.A., 2006. Adipocyte differentiation from the inside out. *Nature Rev. Mol. Cell Biol.*, **7**: 885-896. <https://doi.org/10.1038/nrm2066>
- Rosen, E.D., Spiegelman, B.M., 2000. Molecular regulation of adipogenesis. *Annul Rev. Cell Develop. Biol.*, **16**: 145-171. <https://doi.org/10.1146/annurev.cellbio.16.1.145>
- Sethupathy, P., Borel, C., Gagnebin, M., Grant, G.R., Deutsch, S., Elton, T.S., Hatzigeorgiou, A.G. and Antonarakis, S.E., 2007. Human microRNA-155 on chromosome 21 differentially interacts with its

- polymorphic target in the AGTR1 3' untranslated region: A mechanism for functional single-nucleotide polymorphisms related to phenotypes. *Am. J. Human Genet.*, **81**: 405-413. <https://doi.org/10.1086/519979>
- Wang, X.L., Zhou, G.X., Xu, X.C., Geng, R.Q., Zhou, J.P., Yang, Y.X., Yang, Z.X. and Chen, Y.L., 2014. Transcriptome profile analysis of adipose tissues from fat and short-tailed sheep. *Gene*, **549**: 252-257. <https://doi.org/10.1016/j.gene.2014.07.072>
- Wang, Y.H., Bower, N.I., Reverter, A., Tan, S.H., De Jager, N., Wang, R., McWilliam, S.M., Cafe, L.M., Greenwood, P.L., Lehnert, S.A., 2009. Gene expression patterns during intramuscular fat development in cattle. *J. Anim. Sci.*, **87**: 119-130. <https://doi.org/10.2527/jas.2008-1082>
- Wu, J., Wang, C., Li, S., Li, S., Wang, W., Li, J., Chi, Y., Yang, H., Kong, X. and Zhou, Y., 2013. Thyroid hormone-responsive SPOT 14 homolog promotes hepatic lipogenesis, and its expression is regulated by Liver X receptor α through a sterol regulatory element-binding protein 1c-dependent mechanism in mice. *Hepatology*, **58**: 617-628. <https://doi.org/10.1002/hep.26272>
- Zeng, N., Huang, R., Li, N., Jiang, H., Li, R., Wang, F., Chen, W., Xia, M. and Wang, Q., 2018. MiR-451a attenuates free fatty acids-mediated hepatocyte steatosis by targeting the thyroid hormone responsive spot 14 gene. *Mol. Cell. Endocrinol.*, **474**: 260-271. <https://doi.org/10.1016/j.mce.2018.03.016>
- Zhang, C.L., Zhao, Y.L., Wang, Y.H., Wu, H., Fang, X.T. and Chen, H., 2014. Deep RNA sequencing reveals that micrnas play a key role in lactation in rats. *J. Nutri.*, **144**: 1142-1149. <https://doi.org/10.3945/jn.114.192575>
- Zhang, T., Gao, H., Sahana, G., Zan, Y., Fan, H., Liu, J., Shi, L., Wang, H., Du, L., Wang, L. and Zhao, F., 2019. Genome-wide association studies revealed candidate genes for tail fat deposition and body size in the Hulun Buir sheep. *J. Anim. Breed. Genet.*, **136**: 362-370. <https://doi.org/10.1111/jbg.12402>