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Molecular Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Sheep (*Ovis aries*) Genes-*ZFAND5*, *ZGPAT* and *ZDHHC7*

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Abstract: The complete coding sequences of three sheep genes *ZFAND5*, *ZGPAT* and *ZDHHC7* were amplified using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The sheep *ZFAND5* gene encodes a protein of 213 amino acids that shares high homology with the Zinc finger, AN1-type Domain 5 (ZFAND5) proteins of eight species; cattle (100%), pig (99%), human (99%), rabbit (99%), mouse (98%), giant panda (98%), gray short-tailed opossum (97%) and Northern white-cheeked gibbon (97%). The sheep *ZGPAT* gene encodes a protein of 513 amino acids that shares high homology with the Zinc finger CCCH-type with G patch domain-containing protein (ZGPAT) proteins of seven species; cattle (97%), giant panda (84%), rabbit (99%), human (79%), rat (76%), mouse (77%) and chimpanzee (78%). The sheep *ZDHHC7* gene encodes a protein of 308 amino acids that shares high homology with the Zinc finger, DHHC-type containing 7 (ZDHHC7) proteins of nine species; cattle (99%), dog (93%), pig (93%), human (93%), giant panda (93%), Northern white-cheeked gibbon (92%), mouse (92%), white-tufted-ear marmoset (92%) and rat (92%). Finally, these three novel sheep genes were assigned to GeneIDs; 100302558, 100302028 and 100302557. The phylogenetic analysis revealed that the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes all have a closer genetic relationship with the *ZFAND5*, *ZGPAT* and *ZDHHC7* genes of cattle. Tissue expression profile analysis was also carried out and results demonstrated that sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes were all generally but differentially expressed in detected tissues.

Key words: Sheep, ZFAND5, ZGPAT, ZDHHC7, tissue expression, China

INTRODUCTION

Zinc finger, AN1-type Domain 5 (ZFAND5) contains an A20-like Zinc finger domain (ZnF-A20) at its N terminus and an AN1-like domain (ZnF-AN1) at its C terminus. Similar to A20, ZFAND5 interacted with IKKgamma, RIP and TRAF6 in co-immunoprecipitation experiments (Huang *et al*., 2004). Latest research demonstrated that ZFAND5 is a potent inhibitory factor for osteoclast differentiation and that the mechanism is unlikely due to direct attenuation of the NF-kappa B pathway (Hishiya *et al*., 2005, 2006). Zinc finger CCCH-type with G patch domain-containing protein (ZGPAT), a Zinc finger and G-patch domain-containing protein, acts as a transcription repressor through the recruitment of the nucleosome remodelling and deacetylase complex. Transcriptional target analysis revealed that ZGPAT regulates several cellular signalling pathways including EGFR pathways that are critically involved in cell proliferation, survival and migration. This gene inhibits cell proliferation and suppresses breast carcinogenesis and that ZGPAT depletion leads to a drastic tumour growth *in vivo*. ZGPAT is downregulated in breast carcinomas and that its level of expression is negatively correlated with that of EGFR. These indicate that ZGPAT is a novel transcription repressor and a potential tumour suppressor (Li *et al*., 2009).

Zinc finger, DHHC-type containing 7 (ZDHHC7) also, a Zinc finger domain-containing protein, appears to have a role in maintaining sertoli cell differentiated functions and mediating FSH actions (Chaudhary and Skinner, 2002).

As mentioned, *ZFAND5*, *ZGPAT* and *ZDHHC7* genes are three genes which have important functions. Until today, *ZFAND5*, *ZGPAT* and *ZDHHC7* genes had been reported in human and other animals but the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes have not been reported yet.

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In present experiment, researchers will isolate the coding sequences of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes based on the coding sequence information of *ZFAND5*, *ZGPAT* and *ZDHHC7* genes from human or other mammals and their highly homologous sheep ESTs sequence information, subsequently perform sequence and tissue expression profile analysis for these genes. These will establish the primary foundation of understanding these three sheep genes.

MATERIALS AND METHODS

Animals and sample preparation: Five adult Yunnan local sheep were slaughtered. Spleen, skin, lung, fat, muscle, heart, liver, kidney and ovary samples were collected, frozen in liquid nitrogen and then stored at -80°C. The total RNA was extracted using the total RNA extraction kit (Gibco, USA). First-strand cDNA synthesis was performed as that described by Liu *et al*. (2004). These 1st-strand cDNA samples were used to perform RT-PCR for the isolation of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes and for the tissue expression profile analysis.

Isolation of the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes: The primers for sheep *ZFAND5* gene isolation were designed based on the coding sequence information of human *ZFAND5* gene and its highly homologous sheep EST sequences; EE756605 and EE814109. Similarly, the primers for sheep *ZGPAT* gene isolation were designed based on the coding sequence information from human *ZGPAT* gene and its highly homologous sheep EST sequences; EE809332 and EE802245. The primers for sheep *ZDHHC7* gene isolation were designed based on the coding sequence information from human and mouse *ZDHHC7* genes and their highly homologous sheep EST sequences; EE832186 and EE809375. These primer sequences and their annealing temperature for RT-PCR reaction were shown in Table 1. The RT-PCR was performed to isolate these three sheep genes using the pooled cDNAs from different tissues above. The 25 μL reaction system was; 2.0 μL cDNA, 2.5 μL 2 mM mixed dNTPs, 2.5 μL 10×Taq DNA polymerase buffer, 2.5 μL 25 mM $MgCl_2$, 2.0 μL 10 μM forward primer, 2.0 μL 10 μM reverse primer, 2.0 units of Taq DNA polymerase (1 U 1 μL^{-1}) and 9.5 μL sterile water. The PCR program initially started with a 94°C denaturation for 4 min followed by 35 cycles of 94°C/50 sec, Ta°C/50 sec, 72°C/50 sec then 72°C extension for 10 min, finally 4°C to terminate the reaction. These PCR products for sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes were then cloned into PMD18-T vector and sequenced bidirectionally with the commercial fluorometric method. At least five independent clones were sequenced for every gene.

Table 1: Primers for sheep *ZFAND5*, *ZGPAT*, *ZDHHC7* and *beta-actin* genes and their annealing temperatures

Genes	Primer sequences	Ta/°C
ZFAND5	Forward:5'-ATGGCTCAGGAGACTAAC-3'	
	Reverse:5'-TTATATTCTCTGAATTTTTTCA-3'	55
ZGPAT	Forward:5'-ATGGACGAGGAGAGCCTG-3'	
	Reverse:5'-CTAGAACTCAGTCATCTTCTT-3'	61
ZDHHC7	Forward:5'-ATGCCGTCCTCAGGACAC -3'	
	Reverse:5'-TCACACTGAGAACTCCGGG-3'	61
Beta-actin	Forward:5'-CTTGATGTCACGGACGATTT-3'	
	Reverse:5'-CACGGCATTGTCACCAACT-3'	56

RT-PCR for tissue expression profile analysis: RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Liu and Gao, 2009; Yonggang and Shizheng, 2009; Liu, 2009). The researchers selected the housekeeping gene *beta-actin* (Accession No.: NM_001009784) as a positive control. The primers of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes which were used to perform the RT-PCR for tissue expression profile analysis were same as the primers for isolation RT-PCR. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The 25 μL reaction system was; 1 μL cDNA (100 ng μL^{-1}), 5 pmoles each oligonucleotide primer, 2.5 μL 2 mmol L^{-1} mixed dNTPs, 2.5 μL 10×Taq DNA polymerase buffer, 2.5 μL 25 mmol L^{-1} $MgCl_2$, 1.0 unit of Taq DNA polymerase and finally add sterile water to volume 25 μL. The PCR program initially started with a 94°C denaturation for 4 min followed by 25 cycles of 94°C/50 sec, Ta°C /50 sec, 72°C/50 sec then 72°C extension for 10 min, finally 4°C to terminate the reaction.

Sequence analysis: The cDNA sequence prediction was conducted using GenScan software (http://genes.mit.edu/GENSCAN.html). The protein prediction and analysis were performed using BLAST tool at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and the ClustalW software (http://www.ebi.ac.uk/ clustalw).

RESULTS AND DISCUSSION

RT-PCR results for sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes: Through RT-PCR with pooled tissue cDNAs for sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes, the resulting PCR products were 642, 1542 and 927 bp (Fig. 1).

Sequence analysis: These cDNA nucleotide sequence analysis using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) revealed that these three genes were not homologous to any of the known sheep genes and they were then deposited into the GenBank database (Accession No.: FJ937959, FJ943995

and FJ937952). The sequence prediction was carried out using the GenScan software and results showed that the 642, 1542 and 927 bp cDNA sequences represent three single genes which encoded 213, 513 and 308 amino acids, respectively. Finally, these three novel sheep genes were assigned to GeneIDs: 100302558, 100302028 and 100302557.

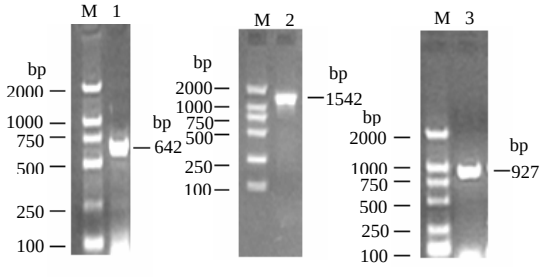

Fig.1: RT-PCR results for sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes. M, DL2000 DNA markers; 1, PCR product for sheep *ZFAND5* gene; 2, PCR product for sheep *ZGPAT* gene; 3, PCR product for sheep *ZDHHC7* gene

Further BLAST analysis of these proteins revealed that the sheep ZFAND5 protein has high homology with the Zinc finger, AN1-type domain 5 (ZFAND5) proteins of eight species; cattle (Accession No.: NP_001094515; 100%), pig (Accession No.: NP_001090975; 99%), human (Accession No.: NP_005998; 99%), rabbit (Accession No.: XP_002708253; 99%), mouse (Accession No.: NP_033577; 98%), Giant panda (Accession No.: XP_002914790; 98%), gray short-tailed opossum (Accession No.: XP_001365007; 97%) and Northern white-cheeked gibbon (Accession No.: XP_003267457; 97%) (Fig. 2).

The sheep ZGPAT protein has high homology with the Zinc finger CCCH-type with G patch domain-containing protein (ZGPAT) proteins of seven species;

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Human          MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Northern       MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Giant          MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Sheep          MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Cattle         MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Pig            MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Rabbit         MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Mouse          MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Gray           MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGSASGSNSPT
               ************************************************:********

Human          SDSASVQRADTSLNNCEGAAGSTSEKSRNVPVAALPVTQQMTEMSISREDKITTPKTEVS
Northern       SDSASVQRADTSLNNCEGAAGSASEKSRNVPVAALPVTQQMTEMSISREDKITTPKTEVS
Giant          SDSASVQRADTSLNNCEGAAGSTSEKSRNVPVAALPVTQQMTEMSISREDKITTPKTEVS
Sheep          SDSASVQRADASLNNCEGAAGSTSEKSRNMPVAALPVTQQMTEMSISREDKVTTPKTEVS
Cattle         SDSASVQRADASLNNCEGAAGSTSEKSRNMPVAALPVTQQMTEMSISREDKVTTPKTEVS
Pig            SDSASVQRADASLNNCEGAAGSTSEKSRNVPVAALPVTQQMTEMSISREDKITTPKTEVS
Rabbit         SDSASVQRADASLNNCESAAGSTSEKSRNVPVAALPVTQQMTEMSISREDKITTPKTEVS
Mouse          SDSASVQRADAGLNNCEGAAGSTSEKSRNVPVAALPVTQQMTEMSISREDKITTPKTEVS
Gray           SDSASVQRAEASLNNCEGAAGSTSEKSRNVPVAALPVTQQMTEMSISREDKITTPKTEAS
               *********::.*****.****:******:**********************:******.*

Human          EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
Northern       EPVVTQPSPSVFQPSTFQSEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
Giant          EPVVTQPSPSVSQPSTSQGEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
Sheep          EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
Cattle         EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
Pig            EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
Rabbit         EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
Mouse          EPVVTQPSPSVSQPSSSQSEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
Gray           EPVVTQPSPSVSQPSTSRNEEKAPELPKPKKNRCFMCRKKVGLTGFDCRCGNLFCGLHRY
               ********** ***: :.****************************************

Human          SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
Northern       SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
Giant          SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
Sheep          SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
Cattle         SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
Pig            SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
Rabbit         SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
Mouse          SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
Gray           SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI
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Fig. 2: The alignment of the protein encoded by sheep *ZFAND5* gene and ten other kinds ZFAND5 proteins. Gray represents gray short-tailed opossum; Giant represents Giant panda; Northern represents Northern white-cheeked gibbon

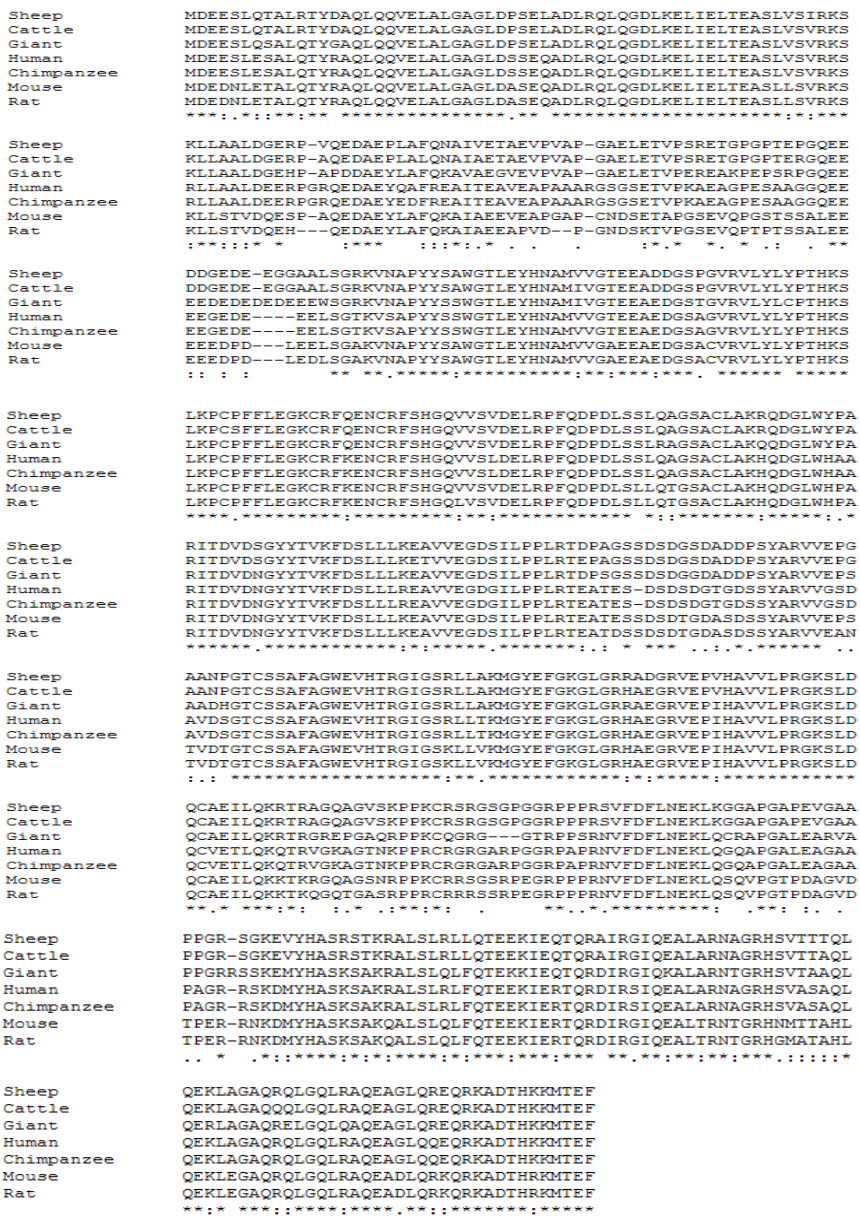

Fig. 3: The alignment of the protein encoded by sheep *ZGPAT* gene and nine other kinds of ZGPAT proteins. Giant represents Giant panda

cattle (Accession No.: NP_001019685; 97%), Giant panda (Accession No.: XP_002925738; 84%), rabbit (Accession No.: XP_002708808; 99%), human (Accession No.: AAH32612; 79%), rat (Accession No.: NP_001009656; 76%), mouse (Accession No.: NP_659143; 77%) and chimpanzee (Accession No.: XP_003317102; 78%) (Fig. 3).

The sheep ZDHHC7 protein has high homology with the Zinc finger, DHHC-type containing 7 (ZDHHC7) proteins of nine species-cattle (Accession No.: XP_874326; 99%), dog (Accession No.: XP_546796; 93%), pig (Accession No.: XP_003126871; 93%), human (Accession No.: NP_060210; 93%), Giant panda (Accession No.: XP_002913433; 93%), Northern white-cheeked gibbon (Accession No.: XP_003272546; 92%), mouse (Accession No.: NP_598728; 92%), white-tufted-ear marmoset (Accession No.: XP_002761264; 92%) and rat (Accession No.: NP_596885; 92%) (Fig. 4).

Based on the results of the alignment of ZFAND5, ZGPAT and ZDHHC7 proteins, three phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software as shown in Fig. 5-7.

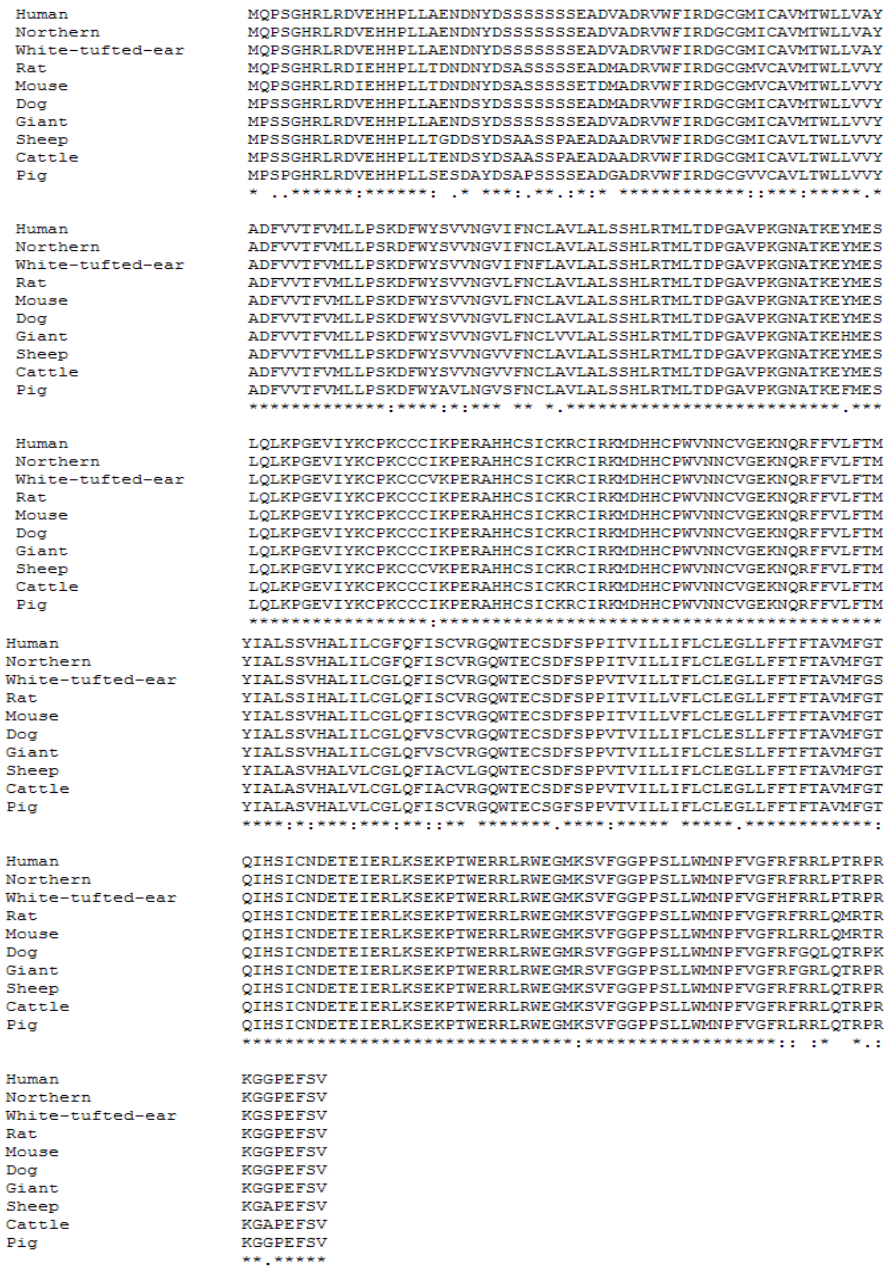

Fig. 4: The alignment of the protein encoded by sheep *ZDHHC7* gene and ten other kinds of ZDHHC7 proteins. White-tufted-ear represents white-tufted-ear marmoset; Giant represents giant panda; Northern represents Northern white-cheeked gibbon

The phylogenetic analysis revealed that the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes all have a closer genetic relationship with the *ZFAND5*, *ZGPAT* and *ZDHHC7* genes of cattle.

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes are all generally but differentially expressed in tissues including spleen, lung, muscle, kidney, ovary, skin, liver, heart and fat (Fig. 8). In the current study, we firstly get the coding sequences of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes by RT-PCR. With the development of modern bioinformatics, establishment of specific sheep NCBI EST database and different convenient analysis tools, researchers can easily find the useful ESTs which were highly homologous to the coding sequences of human genes. Based on these sheep EST sequences, researchers

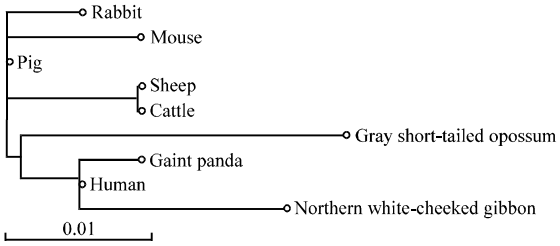

Fig. 5: The phylogenetic analysis for nine kinds of *ZFAND5* genes

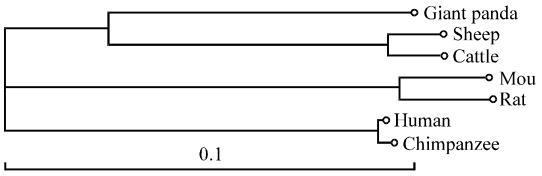

Fig. 6: The phylogenetic analysis for seven kinds of *ZGPAT* genes

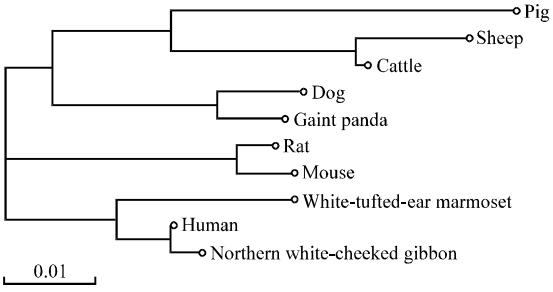

Fig. 7: The phylogenetic analysis for ten kinds of *ZDHHC7* genes

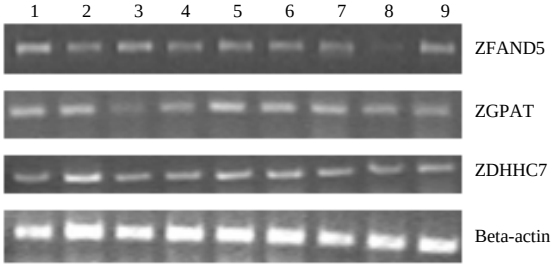

Fig. 8: Tissue expression distribution of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes. The beta-actin expression is the internal control.1: muscle; 2: heart; 3: lung; 4: spleen; 5: skin; 6: fat; 7: liver; 8: kidney and 9: ovary

can obtain the complete coding sequences of some novel sheep genes through the some experimental methods such as RT-PCR. From the clone and sequence analysis of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes, it could be seen that this is an effective method to isolate some novel sheep genes. Through sequence analysis, researchers found that the encoding protein of the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes are highly homologous with ZFAND5, ZGPAT and ZDHHC7 proteins of human and some other animals. This implied that the *ZFAND5*, *ZGPAT* and *ZDHHC7* genes were highly conserved in some species and the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes will have similar functions as the *ZFAND5*, *ZGPAT* and *ZDHHC7* genes of human and other animals.

The researchers also found that the sheep ZFAND5, ZGPAT and ZDHHC7 proteins do not show complete identity to some animals. This implied that the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes will have some differences in functions to those of other animals.

The phylogenetic analysis revealed that the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes all have a closer genetic relationship with the *ZFAND5*, *ZGPAT* and *ZDHHC7* genes of cattle. This implied that we can use cattle as a model organism to study the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes or use sheep as a model organism to study the cattle *ZFAND5*, *ZGPAT* and *ZDHHC7* genes.

From the tissue distribution analysis in this experiment it can be seen that the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes were obviously differentially expressed in some tissues.

As researchers did not study functions at protein levels yet, there might be many possible reasons for differential expression of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes. The suitable explanation for this under current conditions is that at the same time those biological activities related to the mRNA expression of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes were presented diversely in different tissues.

CONCLUSION

In this study, the researchers first isolated the sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes and performed necessary sequence and tissue expression profile analysis. This established the primary foundation for further insight into these novel sheep genes.

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