

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 IKK γ , 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- κ 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.

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 \times 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 $94^{\circ}\text{C}/50$ sec, $T_a^{\circ}\text{C}/50$ sec, $72^{\circ}\text{C}/50$ 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/ $^{\circ}\text{C}$
<i>ZFAND5</i>	Forward:5'-ATGGCTCAGGAGACTAAC-3'	55
	Reverse:5'-TTATATTCTCTGAATTTTTTCA-3'	
<i>ZGPAT</i>	Forward:5'-ATGGACGAGGAGAGCCTG-3'	61
	Reverse:5'-CTAGAACTCAGTCATCTTCTT-3'	
<i>ZDHHC7</i>	Forward:5'-ATGCCGTCCCTCAGGACAC-3'	61
	Reverse:5'-TCACACTGAGAAGCTCCGGG-3'	
<i>Beta-actin</i>	Forward:5'-CTTGATGTCACGGACGATT-3'	56
	Reverse:5'-CACGGCATTGTCACTCAACT-3'	

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 \times 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 $94^{\circ}\text{C}/50$ sec, $T_a^{\circ}\text{C}/50$ sec, $72^{\circ}\text{C}/50$ 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,

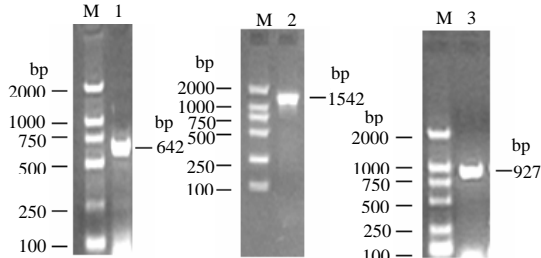


Fig.1: RT-PCR results for sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes. M, DL2000 DNA markers; 1, PCR product for sheep *ZFAND5* gene; 2, PCR product for sheep *ZGPAT* gene; 3, PCR product for sheep *ZDHHC7* gene

respectively. Finally, these three novel sheep genes were assigned to GeneIDs: 100302558, 100302028 and 100302557.

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;

Human	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Northern	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Giant	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Sheep	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Cattle	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Pig	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Rabbit	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Mouse	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGTASGSNSPT
Gray	MAQETNQTGGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQQNSGRMSPMGSASGSNSPT
	*****:*****
Human	SDSASVQRADTSLNCEGAAGSTSEKSRNPVVAALPVTQQMTEMSISREDKITTPKTEVS
Northern	SDSASVQRADTSLNCEGAAGSASEKSRNPVVAALPVTQQMTEMSISREDKITTPKTEVS
Giant	SDSASVQRADTSLNCEGAAGSTSEKSRNPVVAALPVTQQMTEMSISREDKITTPKTEVS
Sheep	SDSASVQRADASLNNCEGAAGSTSEKSRNMPVVAALPVTQQMTEMSISREDKVTTPKTEVS
Cattle	SDSASVQRADASLNNCEGAAGSTSEKSRNMPVVAALPVTQQMTEMSISREDKVTTPKTEVS
Pig	SDSASVQRADASLNNCEGAAGSTSEKSRNPVVAALPVTQQMTEMSISREDKITTPKTEVS
Rabbit	SDSASVQRADASLNNCEGAAGSTSEKSRNPVVAALPVTQQMTEMSISREDKITTPKTEVS
Mouse	SDSASVQRADAGLNNCEGAAGSTSEKSRNPVVAALPVTQQMTEMSISREDKITTPKTEVS
Gray	SDSASVQRAEASLNNCEGAAGSTSEKSRNPVVAALPVTQQMTEMSISREDKITTPKTEAS
	*****:..*****.****:*****:*****:*****:*****.*
Human	EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
Northern	EPVVTQPSPSVFPSTFQSEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
Giant	EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
Sheep	EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
Cattle	EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
Pig	EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
Rabbit	EPVVTQPSPSVSQPSTSQSEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
Mouse	EPVVTQPSPSVSQPSSSQSEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
Gray	EPVVTQPSPSVSQPSTSRNEEKAPELPKPKKNRCFMCRRKVGLTGFDCRCGNLFCGLHRY
	***** ***:..*****:*****:*****:*****:*****:*****
Human	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
Northern	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
Giant	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
Sheep	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
Cattle	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
Pig	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
Rabbit	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
Mouse	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
Gray	SDKHNCPYDYKAEAAAIRKENPVVVAEKIQRI
	*****:*****:*****:*****:*****:*****:*****

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

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Sheep      MDEESLQALRTYDAQLQQVELALGAGLDPSSELADLRQLQGDLKELIELTEASLVSVRKS
Cattle     MDEESLQALRTYDAQLQQVELALGAGLDPSSELADLRQLQGDLKELIELTEASLVSVRKS
Giant      MDEESLQALRTYDAQLQQVELALGAGLDPSSELADLRQLQGDLKELIELTEASLVSVRKS
Human      MDEESLESALQTYRAQLQQVELALGAGLDSSSEQADLRQLQGDLKELIELTEASLVSVRKS
Chimpanzee MDEESLESALQTYRAQLQQVELALGAGLDSSSEQADLRQLQGDLKELIELTEASLVSVRKS
Mouse      MDEENLETALQTYRAQLQQVELALGAGLDASEQADLRQLQGDLKELIELTEASLVSVRKS
Rat        MDEENLETALQTYRAQLQQVELALGAGLDASEQADLRQLQGDLKELIELTEASLVSVRKS
*****

Sheep      KLLAALDGERP-VQEDAEPLAFQNAIVETAIEVFPVAP-GAELETVPSPRETGGFTEPGQEE
Cattle     KLLAALDGERP-AQEDAEPLALQNAIAETAIEVFPVAP-GAELETVPSPRETGGFTEPGQEE
Giant      KLLAALDGEHP-APDDAEYLAFOKAVAEVFPVAP-GAELETVPSPRETGGFTEPGQEE
Human      RLLAALDEERFGRQEDAEYQAFREAITAEVAPAAARGSGSETVPKAEAGPESAAGGQEE
Chimpanzee RLLAALDEERFGRQEDAEYEDFREAITAEVAPAAARGSGSETVPKAEAGPESAAGGQEE
Mouse      KLLSTVDQESF-AQEDAEYLAFOKAVAEVFPVAP-CNDSETAPGSEVQPSSTSSALLE
Rat        KLLSTVDQEH---QEDAEYLAFOKAVAEVFPVAP--P-GNDSKTVGSEVQPSSTSSALLE
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Sheep      DDGEDE-EGGAALSGRKVNAPYYSAWGTLEYHNAMVVGTEEDDGSFGRVLYLYPTHKS
Cattle     DDGEDE-EGGAALSGRKVNAPYYSAWGTLEYHNAMVVGTEEDDGSFGRVLYLYPTHKS
Giant      EEDEDEDEDEEWSGRKVNAPYYSSWGTLEYHNAMVVGTEEDDGSFGRVLYLYPTHKS
Human      EGEDE----EELSGTKVSAFYSSWGTLEYHNAMVVGTEEDDGSAGRVLYLYPTHKS
Chimpanzee EGEDE----EELSGTKVSAFYSSWGTLEYHNAMVVGTEEDDGSAGRVLYLYPTHKS
Mouse      EEEDFD---LEELSGAKVNAPYYSAWGTLEYHNAMVVGTEEDDGSACRVLYLYPTHKS
Rat        EEEDFD---LEDLSGAKVNAPYYSAWGTLEYHNAMVVGTEEDDGSACRVLYLYPTHKS
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Sheep      LKPCPFFLEGKCRFQENCRFSGHQVVSDELRFQDQDPLSSLQAGSACLAKRQDGLWYFA
Cattle     LKPCPFFLEGKCRFQENCRFSGHQVVSDELRFQDQDPLSSLQAGSACLAKRQDGLWYFA
Giant      LKPCPFFLEGKCRFQENCRFSGHQVVSDELRFQDQDPLSSLRAGSACLAKRQDGLWYFA
Human      LKPCPFFLEGKCRFQENCRFSGHQVVSDELRFQDQDPLSSLQAGSACLAKRQDGLWYFA
Chimpanzee LKPCPFFLEGKCRFQENCRFSGHQVVSDELRFQDQDPLSSLQAGSACLAKRQDGLWYFA
Mouse      LKPCPFFLEGKCRFQENCRFSGHQVVSDELRFQDQDPLSSLQAGSACLAKRQDGLWYFA
Rat        LKPCPFFLEGKCRFQENCRFSGHQVVSDELRFQDQDPLSSLQAGSACLAKRQDGLWYFA
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Sheep      RITDVGSGYITVKFDSLLEAVVEGDSILPPLRTDPAGSSDSDGSDADDPYSYARVVEFG
Cattle     RITDVGSGYITVKFDSLLEAVVEGDSILPPLRTEPAGSSDSDGSDADDPYSYARVVEFG
Giant      RITDVGSGYITVKFDSLLEAVVEGDSILPPLRTEPAGSSDSDGSDADDPYSYARVVEFG
Human      RITDVGSGYITVKFDSLLEAVVEGDSILPPLRTEPAGSSDSDGSDADDPYSYARVVEFG
Chimpanzee RITDVGSGYITVKFDSLLEAVVEGDSILPPLRTEPAGSSDSDGSDADDPYSYARVVEFG
Mouse      RITDVGSGYITVKFDSLLEAVVEGDSILPPLRTEPAGSSDSDGSDADDPYSYARVVEFG
Rat        RITDVGSGYITVKFDSLLEAVVEGDSILPPLRTEPAGSSDSDGSDADDPYSYARVVEFG
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Sheep      AANPGTCSAFAGNEVHTRGIGSRLLAKMGYEFKGGLGRRAEGRVEPIHAVVLPGRKSLD
Cattle     AANPGTCSAFAGNEVHTRGIGSRLLAKMGYEFKGGLGRRAEGRVEPIHAVVLPGRKSLD
Giant      AADHGTCSAFAGNEVHTRGIGSRLLAKMGYEFKGGLGRRAEGRVEPIHAVVLPGRKSLD
Human      AVDSGTCSAFAGNEVHTRGIGSRLLAKMGYEFKGGLGRRAEGRVEPIHAVVLPGRKSLD
Chimpanzee AVDSGTCSAFAGNEVHTRGIGSRLLAKMGYEFKGGLGRRAEGRVEPIHAVVLPGRKSLD
Mouse      TVDTGTCSAFAGNEVHTRGIGSKLLVMGYEFKGGLGRRAEGRVEPIHAVVLPGRKSLD
Rat        TVDTGTCSAFAGNEVHTRGIGSKLLVMGYEFKGGLGRRAEGRVEPIHAVVLPGRKSLD
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Sheep      QCAEILQKRTIRAGQAGVSKPPKCRSRGSGPGRPPPRVDFLNEKLGGAAPGAPFVGGAA
Cattle     QCAEILQKRTIRAGQAGVSKPPKCRSRGSGPGRPPPRVDFLNEKLGGAAPGAPFVGGAA
Giant      QCAEILQKRTIRAGQAGVSKPPKCRSRGSGPGRPPPRVDFLNEKLGGAAPGAPFVGGAA
Human      QCVETLQKQTRVKGAGTNKPPRCRGRGARPPGRPAFPRNVDFLNEKLGGAAPGAPFVGGAA
Chimpanzee QCVETLQKQTRVKGAGTNKPPRCRGRGARPPGRPAFPRNVDFLNEKLGGAAPGAPFVGGAA
Mouse      QCAEILQKRTIRAGQAGVSKPPKCRSRGSGPGRPPPRVDFLNEKLGGAAPGAPFVGGAA
Rat        QCAEILQKRTIRAGQAGVSKPPKCRSRGSGPGRPPPRVDFLNEKLGGAAPGAPFVGGAA
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Sheep      PFGR-SGKEVYHASRSTKRALSLRLLQTEEKIEQTQRAIRIQEALARNAGRHSVITTAQL
Cattle     PFGR-SGKEVYHASRSTKRALSLRLLQTEEKIEQTQRAIRIQEALARNAGRHSVITTAQL
Giant      PFGRSSKEMYHASKSAKRALSLRLLQTEEKIEQTQRAIRIQEALARNAGRHSVITTAQL
Human      PAGR-RSKDMYHASKSAKRALSLRLLQTEEKIEQTQRAIRIQEALARNAGRHSVITTAQL
Chimpanzee PAGR-RSKDMYHASKSAKRALSLRLLQTEEKIEQTQRAIRIQEALARNAGRHSVITTAQL
Mouse      TPER-RNKDMYHASKSAKRALSLRLLQTEEKIEQTQRAIRIQEALARNAGRHSVITTAQL
Rat        TPER-RNKDMYHASKSAKRALSLRLLQTEEKIEQTQRAIRIQEALARNAGRHSVITTAQL
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Sheep      QEKLGAQRQLGQLRAQEAGLQREQRKADTHKKMTEF
Cattle     QEKLGAQRQLGQLRAQEAGLQREQRKADTHKKMTEF
Giant      QERLAGAQRQLGQLRAQEAGLQREQRKADTHKKMTEF
Human      QEKLGAQRQLGQLRAQEAGLQREQRKADTHKKMTEF
Chimpanzee QEKLGAQRQLGQLRAQEAGLQREQRKADTHKKMTEF
Mouse      QEKLGAQRQLGQLRAQEADLQREQRKADTHKKMTEF
Rat        QEKLGAQRQLGQLRAQEADLQREQRKADTHKKMTEF
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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 ZDHHHC7 protein has high homology with the Zinc finger, DHHC-type containing 7 (ZDHHHC7) 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 ZDHHHC7 proteins, three phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software as shown in Fig. 5-7.

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Human      MQPSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
Northern   MQPSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
White-tufted-ear MQPSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
Rat        MQPSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
Mouse      MQPSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
Dog        MPSSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
Giant      MPSSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
Sheep      MPSSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
Cattle     MPSSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
Pig        MPSSGHRRLRDVEHHFLAENDNYDSSSSSSSEADVADRWFIRDGCGMICAVMTWLLVAY
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Human      ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
Northern   ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
White-tufted-ear ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
Rat        ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
Mouse      ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
Dog        ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
Giant      ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
Sheep      ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
Cattle     ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
Pig        ADFVVT FVMLLPSKDFWYSVNVGVI FNC LAVLALSSHLR TMLTDPG AVPKGNATKEYMES
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Human      LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
Northern   LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
White-tufted-ear LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
Rat        LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
Mouse      LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
Dog        LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
Giant      LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
Sheep      LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
Cattle     LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
Pig        LQLKPGEVIYKCPKCCCIKPERAHHCSICKRCIRKMDHHC PWVNN CVGEKNQRFVFLFTM
*****:*****:*****:*****:*****:*****

Human      YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
Northern   YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
White-tufted-ear YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGS
Rat        YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
Mouse      YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
Dog        YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
Giant      YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
Sheep      YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
Cattle     YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
Pig        YIALSSVHALILCGLQFIS CVRGQWTECSDFSPPI TVILLIFL CLEGLLFFFTAVMFGT
*****:*****:*****:*****:*****:*****

Human      QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
Northern   QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
White-tufted-ear QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
Rat        QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
Mouse      QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
Dog        QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
Giant      QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
Sheep      QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
Cattle     QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
Pig        QIHSICNDETEIERLKSEKPTWERRLRWEGMKS VFGGPPSLLW MNPVFGFRFRRLQTRPR
*****:*****:*****:*****:*****:*****

Human      KGGPEFSV
Northern   KGGPEFSV
White-tufted-ear KGSPEFSV
Rat        KGGPEFSV
Mouse      KGGPEFSV
Dog        KGGPEFSV
Giant      KGGPEFSV
Sheep      KGAPPEFSV
Cattle     KGAPPEFSV
Pig        KGGPEFSV
**..*****
    
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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

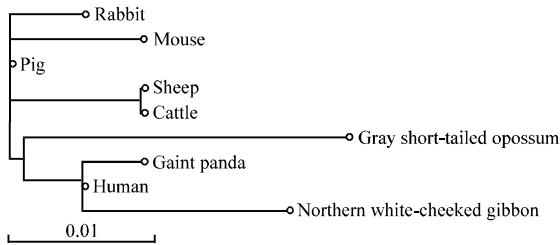


Fig. 5: The phylogenetic analysis for nine kinds of *ZFAND5* genes

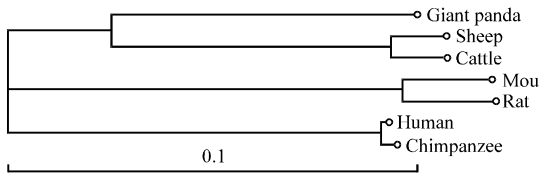


Fig. 6: The phylogenetic analysis for seven kinds of *ZGPAT* genes

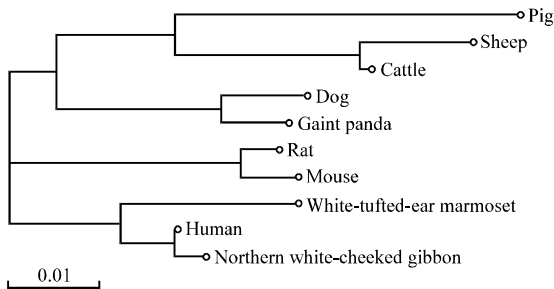


Fig. 7: The phylogenetic analysis for ten kinds of *ZDHHC7* genes

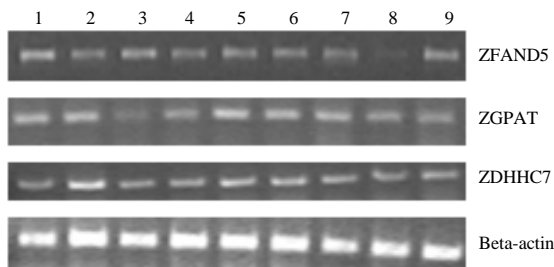


Fig. 8: Tissue expression distribution of sheep *ZFAND5*, *ZGPAT* and *ZDHHC7* genes. The beta-actin expression is the internal control. 1: muscle; 2: heart; 3: lung; 4: spleen; 5: skin; 6: fat; 7: liver; 8: kidney and 9: ovary

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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