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Molecular Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Sheep (*Ovis aries*) Genes *SLC39A1*, *SLC39A2* and *SLC39A7*

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Abstract: The complete coding sequences of three sheep genes *SLC39A1*, *SLC39A2* and *SLC39A7* were amplified using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Sequence analysis revealed that sheep *SLC39A1* gene encodes a protein of 324 amino acids that shares high homology with the solute carrier family 39 (zinc transporter), member1 (SLC39A1) proteins of eleven species-goat (99%), cattle (99%), pig (95%), horse (94%), rhesus monkey (94%), chimpanzee (94%), human (94%), rabbit (94%), crab-eating macaque (93%), mouse (93%) and rat (92%). The sheep *SLC39A2* gene encodes a protein of 309 amino acids that shares high homology with the solute carrier family 39 (zinc transporter), member 2 (SLC39A2) proteins of eleven species-goat (98%), cattle (95%), horse (81%), giant panda (80%), human (79%), rhesus monkey (78%), chimpanzee (78%), rabbit (77%), Northern white-cheeked gibbon (77%), mouse (75%) and rat (74%). The sheep *SLC39A7* gene encodes a protein of 469 amino acids that shares high homology with the solute carrier family 39 (zinc transporter), member 7(SLC39A7) proteins of thirteen species-cattle (98%), dog (93%), pig (94%), chimpanzee (93%), human (93%), horse (93%), rat (93%), rhesus monkey (93%), white-tufted-ear marmoset (92%), Northern white-cheeked gibbon (92%), sumatran orangutan (92%), rabbit (89%) and mouse (86%). Finally, these three novel sheep genes were assigned to GeneIDs: 100302552, 100302553 and 100302555. The phylogenetic analysis revealed that the sheep *SLC39A1* and *SLC39A2* genes both have closer genetic relationships with the *SLC39A1* and *SLC39A2* genes of goat. The sheep *SLC39A7* gene has a closer genetic relationship with the *SLC39A7* gene of cattle. Tissue expression profile analysis was also carried out and results demonstrated that sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes were all generally but differentially expressed in detected tissues.

Key words: Sheep, SLC39A1, SLC39A2, SLC39A7, tissue expression, genetic relatiuonship, China

INTRODUCTION

There had been two superfamilies of mammalian zinc transporters identified to be the Solute carrier (Slc) 30a and Slc39a families (Kambe *et al*., 2008; Guerinot, 2000). Slc30a members, named ZnTs, function in zinc efflux and compartmentalization and are cation diffusion facilitator proteins (Palmiter and Huang, 2004). Members of the Slc39a family, named ZIPs, function in the uptake of zinc and other metals (Taylor and Nicholson, 2003). Solute carrier family 39 (zinc transporter), member 1 (SLC39A1), solute carrier family 39 (zinc transporter), member 2 (SLC39A2) and solute carrier family 39 (zinc transporter), member 7 (SLC39A7) are three members of the Slc39a family. However, recent studies have demonstrated that these three genes had many more important functions. Experimental data revealed that knockout of Zn transporters SLC39A1 and SLC39A3 attenuates seizure-induced CA1 neurodegeneration. SLC39A1 overexpression has a functional effect on the malignant potential of prostate cancer cells via inhibition of NF-kappaB-dependent pathways and this supports the concept that SLC39A1 may function as a tumor suppressor gene (Qian *et al*., 2011; Golovine *et al*., 2008). Experimental data also revealed that a novel SLC39A2 Gln/Arg/Leu codon 2 polymorphism is associated with carotid artery disease in aging (Giacconi *et al*., 2008) and SLC39A7 mediated intracellular zinc transport contributes to aberrant growth factor signaling in antihormone-resistant breast cancer cells (Taylor *et al*., 2008). As mentioned above, *SLC39A1*, *SLC39A2* and *SLC39A7* genes are three genes which have important functions. Until today, *SLC39A1*, *SLC39A2* and *SLC39A7* genes had been reported in human and other animals but the sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes have not been reported yet. In present experiment, there will isolate

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the coding sequences of sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes based on the coding sequence information of *SLC39A1*, *SLC39A2* and *SLC39A7* genes from human or other mammals and their highly homologous sheep ESTs sequence information, subsequently perform some necessary sequence analysis 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 first-strand cDNA samples were used to perform RT-PCR for the isolation of sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes and for the tissue expression profile analysis.

Isolation of the sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes: The primers for sheep *SLC39A1* gene isolation were designed based on the coding sequence information of human *SLC39A1* gene and its highly homologous sheep EST sequences: DY495423 and EE830071. Similarly, the primers for sheep *SLC39A2* gene isolation were designed based on the coding sequence information from human *SLC39A2* gene and its highly homologous sheep EST sequence: EE761386. The primers for sheep *SLC39A7* gene isolation were designed based on the coding sequence information from human and mouse *SLC39A7* genes and their highly homologous sheep EST sequences: DY498275 and DY521419. 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) 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, Ta°C/50 and 72°C/50 sec then 72°C extension for 10 min, finally 4°C to terminate the reaction. These PCR products for sheep *SLC39A1*, *SLC39A2* and *SLC39A7* 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 *SLC39A1*, *SLC39A2*, *SLC39A7* and *β-actin* genes and their annealing temperature

Genes	Primer sequence	Ta/°C
SLC39A1	Forward: 5'-ATGGGGCCCTGGGGAGAG-3'	63
	Reverse: 5'-CTAGATTTGGATAAAGAGCAGG-3'	
SLC39A2	Forward: 5'-ATGGAACCACTACTAGGAG-3	58
	Reverse: 5'-TCAGGCCCACAAGGCAAT-3	
SLC39A7	Forward: 5'-ATGGCCAGAGGCCTGGGG-3	63
	Reverse: 5'-TCACTGGAGGTGGGCAATCA-3	
β-actin	Forward: 5'-CTTGATGTCACGGACGATTT-3'	56
	Reverse: 5'-CACGGCATTGTCACCAACT-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). Researchers selected the housekeeping gene *β-actin* (Accession No.: NM_001009784) as a positive control. The primers of sheep *SLC39A1*, *SLC39A2* and *SLC39A7* 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}), 5pmoles 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, Ta°C /50 and 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

RT-PCR results for sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes: Through RT-PCR with pooled tissue cDNAs for sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes, the resulting PCR products were 975, 930 and 1410 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.: FJ937953, FJ937951 and FJ937956). The sequence prediction was carried out using the GenScan software and results showed that the 975, 930 and 1410 bp cDNA sequences represent three single genes which encoded 324, 309 and 469 amino acids, respectively.

Finally, these three novel sheep genes were assigned to GeneIDs: 100302552, 100302553 and 100302555. Further BLAST analysis of these proteins revealed that the sheep SLC39A1 protein has high homology with the solute carrier family 39 (zinc transporter), member (SLC39A1) proteins of eleven species goat (Accession No.: AEB39598; 99%), cattle (Accession No.: NP_001030458; 99%), pig (Accession No.: XP_001929540; 95%), horse (Accession No.: XP_001493953; 94%), rhesus monkey (Accession No.: XP_001112361; 94%), chimpanzee (Accession No.: XP_001148498; 94%), human (Accession

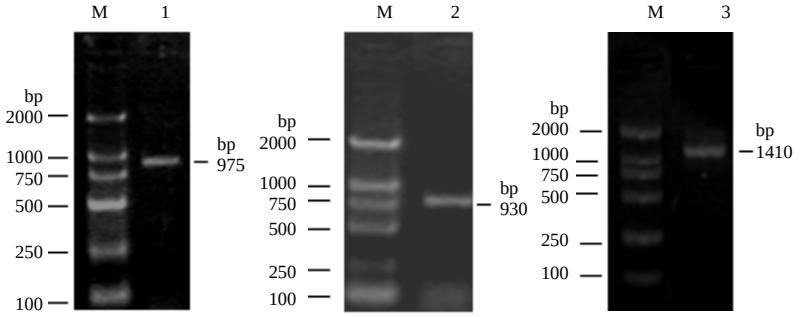

Fig. 1: RT-PCR results for sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes. M, DL2000 DNA markers; 1, PCR product for sheep *SLC39A1* gene; 2, PCR product for sheep *SLC39A2* gene; 3, PCR product for sheep *SLC39A7* gene

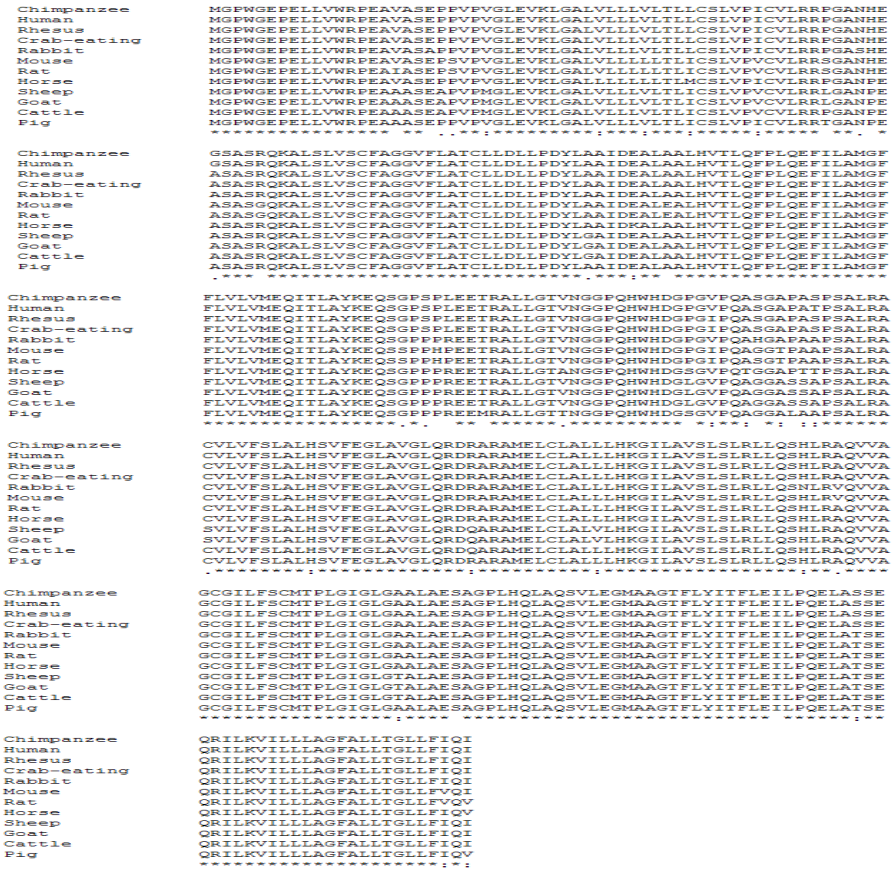

Fig. 2: The alignment of the protein encoded by sheep *SLC39A1* gene and eleven other kinds SLC39A1 proteins. Crab-eating, crab-eating macaque; Rhesus and rhesus monkey

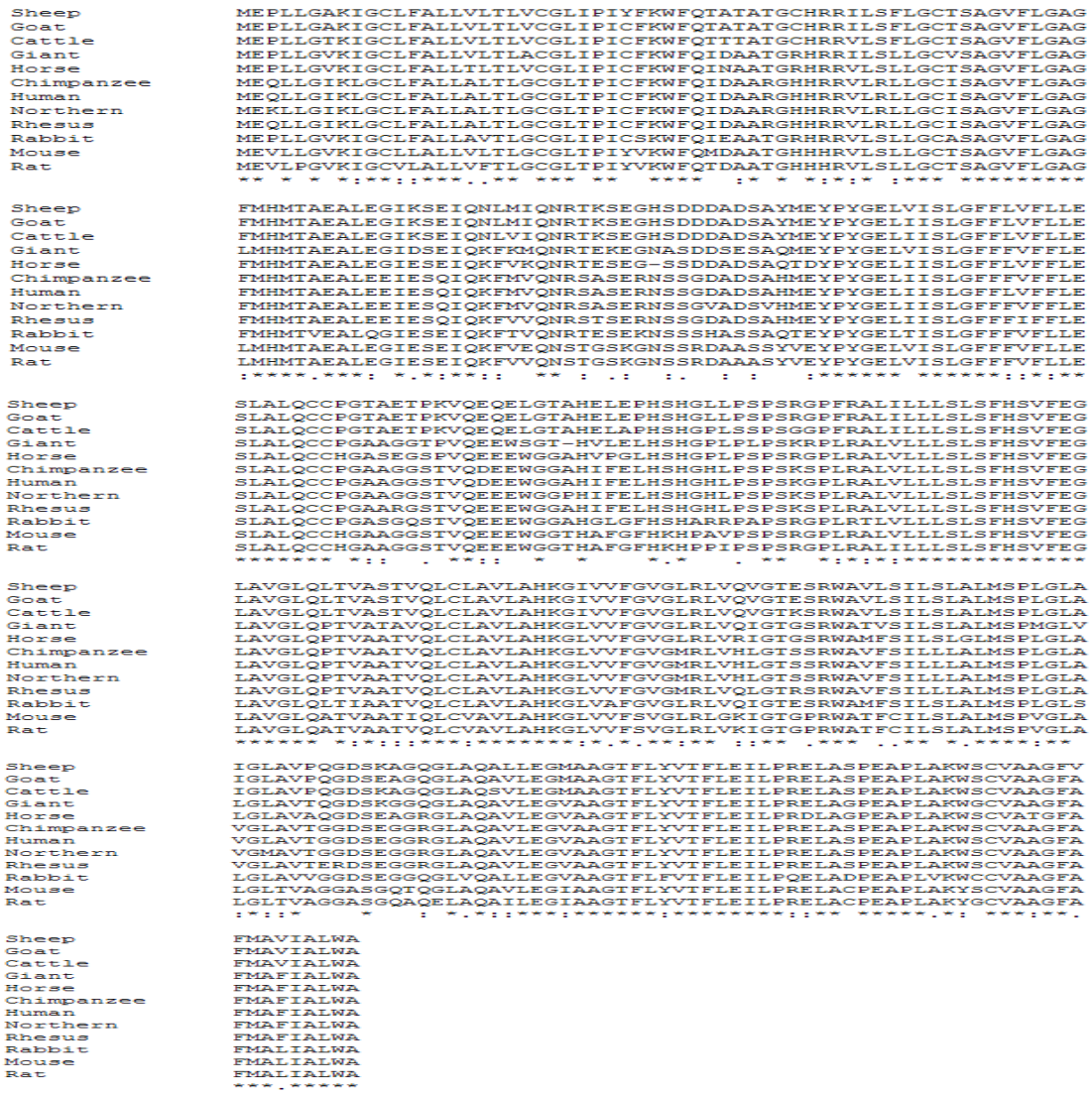

Fig. 3: The alignment of the protein encoded by sheep *SLC39A2* gene and eleven other kinds of SLC39A2 proteins. Rhesus, rhesus monkey; Northern, Northern white-cheeked gibbon; Giant, giant panda

No.: NP_055252; 94%), rabbit (Accession No.: XP_002715529; 94%), crab-eating macaque (Accession No.: BAE01945; 93%), mouse (Accession No.: Q9QZ03; 93%) and rat (Accession No.: NP_001128049; 92%) (Fig. 2).

The sheep SLC39A2 protein has high homology with the solute carrier family 39 (zinc transporter), member 2 (SLC39A2) proteins of eleven species goat (Accession No.: ADU18525; 98%), cattle (Accession No.: NP_001192577; 95%), horse (Accession No.: XP_001505193; 81%), human (Accession No.: AAF35832; 79%), giant panda (Accession No.: XP_002927868; 80%), rhesus monkey (Accession No.: XP_001093488; 78%), chimpanzee (Accession No.: XP_520676; 78%), rabbit (Accession No.: XP_002718088; 77%), Northern white-cheeked gibbon (Accession No.: XP_003260612; 77%), rat (Accession No.: NP_001100730; 74%) and mouse (Accession No.: NP_001034765; 75%) (Fig. 3). The sheep SLC39A7 protein has high homology with the solute carrier family 39 (zinc transporter), member (SLC39A7) proteins of thirteen species cattle (Accession No.: NP_001069705; 98%), dog (Accession No.: NP_001041565; 93%), white-tufted-ear marmoset (Accession No.: XP_002746472; 92%), pig (Accession No.: NP_001124517; 94%), chimpanzee (Accession No.: XP_003311256; 93%), human (Accession No.: NP_008910; 93%), rat (Accession No.: NP_001008885; 93%), horse (Accession No.: XP_001496865; 93%),

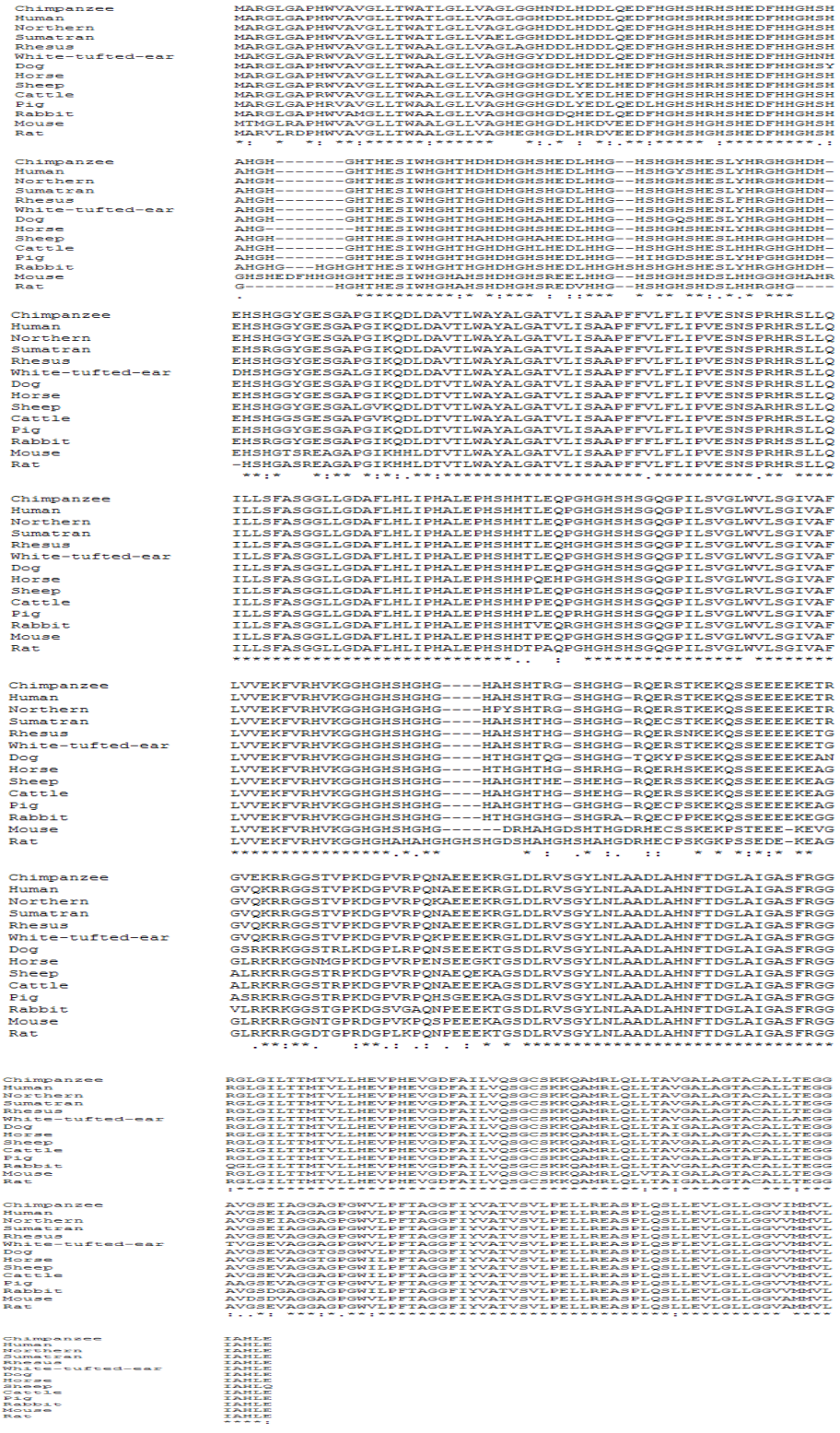

Fig. 4: The alignment of the protein encoded by sheep *SLC39A7* gene and thirteen other kinds of SLC39A7 proteins. White-tufted-ear, white-tufted-ear marmoset; Northern, Northern white-cheeked gibbon; Sumatran, sumatran orangutan; Rhesus and rhesus monkey

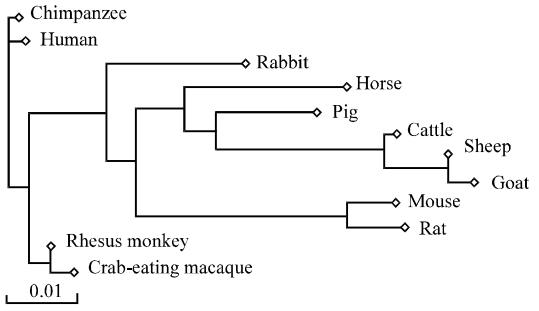

Fig. 5: The phylogenetic analysis for twelve kinds of *SLC39A1* genes

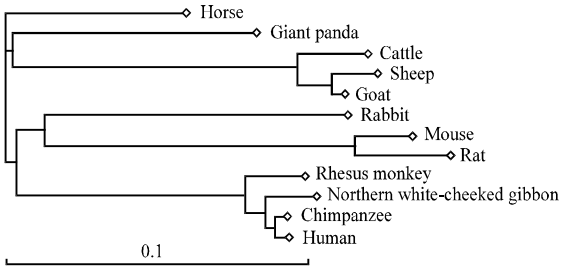

Fig. 6: The phylogenetic analysis for twelve kinds of *SLC39A2* genes

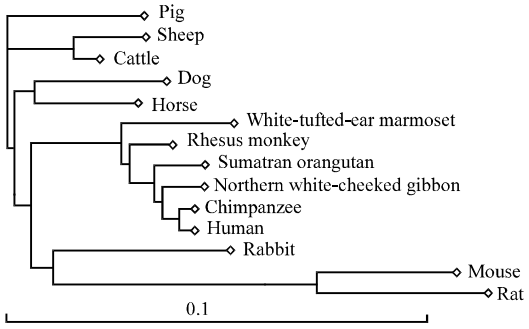

Fig. 7: The phylogenetic analysis for fourteen kinds of *SLC39A7* genes

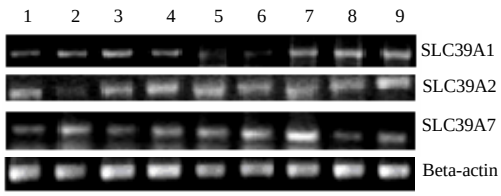

Fig. 8: Tissue expression distribution of sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes. The β-actin expression is the internal control. 1: Spleen; 2: Skin; 3: Lung; 4: Muscle; 5: Heart; 6: Fat; 7: Liver; 8: Kidney; 9: Ovary

Northern white-cheeked gibbon (Accession No.: XP_003271937; 92%), sumatran orangutan (Accession No.: NP_001127161; 92%), rhesus monkey (Accession No.: XP_002803736; 93%), rabbit (Accession No.: XP_002714615; 89%) and mouse (Accession No.: BAE35522; 86%) (Fig. 4).

Based on the results of the alignment of SLC39A1, SLC39A2 and SLC39A7 proteins, three phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software as shown in Fig. 5-7.

The phylogenetic analysis revealed that the sheep *SLC39A1* and *SLC39A2* genes both have closer genetic relationships with the *SLC39A1* and *SLC39A2* genes of goat. The sheep *SLC39A7* gene has a closer genetic relationship with the *SLC39A7* gene of cattle.

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes are all generally but differentially expressed in tissues including spleen, lung, muscle, kidney, ovary, skin, liver, heart and fat (Fig. 8).

DISCUSSION

In the current study, researchers firstly get the coding sequences of sheep *SLC39A1*, *SLC39A2* and *SLC39A7* 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, there 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 *SLC39A1*, *SLC39A2* and *SLC39A7* 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 *SLC39A1*, *SLC39A2* and *SLC39A7* genes are highly homologous with SLC39A1, SLC39A2 and SLC39A7 proteins of human and some other animals. This implied that the *SLC39A1*, *SLC39A2* and *SLC39A7* genes were highly conserved in some species and the sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes will have similar functions as the *SLC39A1*, *SLC39A2* and *SLC39A7* genes of human and other animals. Researchers also found that the sheep SLC39A1, SLC39A2 and SLC39A7 proteins do not show complete identity to human or other animals. This implied that the sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes

will have some differences in functions to those of human or other mammals. The phylogenetic analysis revealed that the sheep *SLC39A1* and *SLC39A2* genes both have closer genetic relationships with the *SLC39A1* and *SLC39A2* genes of goat. This implied that we can use goat as a model organism to study the sheep *SLC39A1* and *SLC39A2* genes or use sheep as a model organism to study the goat *SLC39A1* and *SLC39A2* genes. The sheep *SLC39A7* gene has a closer genetic relationship with the *SLC39A7* gene of cattle so that there can use cattle as a model organism to study the sheep *SLC39A7* gene or use sheep as a model organism to study the cattle *SLC39A7* gene. From the tissue distribution analysis in the experiment it can be seen that the sheep *SLC39A1*, *SLC39A2* and *SLC39A7* 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 *SLC39A1*, *SLC39A2* and *SLC39A7* 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 *SLC39A1*, *SLC39A2* and *SLC39A7* genes were presented diversely in different tissues.

CONCLUSION

In this study, the researchers first isolated the sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes and performed necessary sequence analysis and tissue transcription profile analysis. This established the primary foundation for further insight into these novel sheep genes.

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