

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), member 1 (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 relationship, 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; Guerinet, 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

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 MgCl₂, 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
<i>SLC39A1</i>	Forward: 5'-ATGGGGCCCTGGGGAGAG-3'	63
	Reverse: 5'-CTAGATTTGGATAAAGAGCAGG-3'	
<i>SLC39A2</i>	Forward: 5'-ATGGAACCACTACTAGGAG-3	58
	Reverse: 5'-TCAGGCCCAACAAGGCAAT-3	
<i>SLC39A7</i>	Forward: 5'-ATGGCCAGAGGCTGGGG-3	63
	Reverse: 5'-TCACTGGAGGTGGGCAATCA-3	
<i>β-actin</i>	Forward: 5'-CTTGATGTCACGGACGATTT-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). 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 MgCl₂, 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

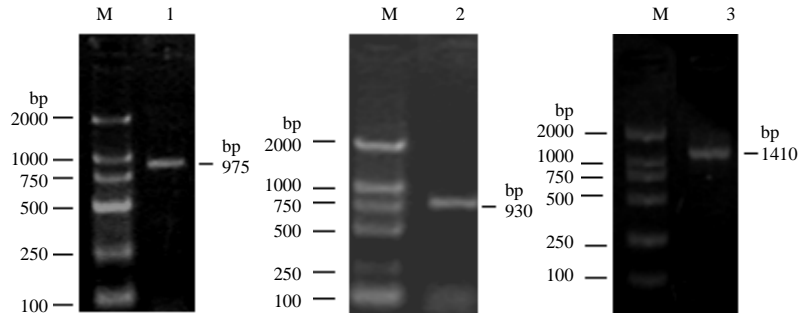


Fig. 1: RT-PCR results for sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes. M, DL2000 DNA markers; 1, PCR product for sheep *SLC39A1* gene; 2, PCR product for sheep *SLC39A2* gene; 3, PCR product for sheep *SLC39A7* gene

Chimpanzee	MGFWGEPPELLVWRPEAVASEPPVGVGLVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Human	MGFWGEPPELLVWRPEAVASEPPVGVGLVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Rhesus	MGFWGEPPELLVWRPEAVASEPPVGVGLVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Crab-eating	MGFWGEPPELLVWRPEAVASEPPVGVGLVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Rabbit	MGFWGEPPELLVWRPEAVASEPPVGVGLVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Mouse	MGFWGEPPELLVWRPEAVASEPPVGVGLVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Rat	MGFWGEPPELLVWRPEAVASEPPVGVGLVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Horse	MGFWGEPPELLVWRPEAVASEPPVGVGLVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Sheep	MGFWGEPPELLVWRPEAAASEAPVPMGLEVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Goat	MGFWGEPPELLVWRPEAAASEAPVPMGLEVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Cattle	MGFWGEPPELLVWRPEAAASEAPVPMGLEVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Pig	MGFWGEPPELLVWRPEAAASEAPVPMGLEVLEKLGALVLLVLTLLCSLVPICVLRPPGANHE
Chimpanzee	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Human	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Rhesus	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Crab-eating	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Rabbit	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Mouse	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Rat	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Horse	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Sheep	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Goat	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Cattle	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Pig	GSASRQKALSLVSCFAGGVFLATCLLDLDPDYLAIDEALAAHVTLQFFLQEFILAMGF
Chimpanzee	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGVQASGAPASPSALRA
Human	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGVQASGAPATPSALRA
Rhesus	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGIIPQASGAPASPSALRA
Crab-eating	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGIIPQASGAPASPSALRA
Rabbit	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGVQASGAPASPSALRA
Mouse	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGIIPQASGAPASPSALRA
Rat	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGIIPQASGAPASPSALRA
Horse	FLVLVMEQITLAYKEQSGPSPLEETRALLGTANGGQHWHDGSGVPTGASGAPATPSALRA
Sheep	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGVQASGAPASPSALRA
Goat	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGVQASGAPASPSALRA
Cattle	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGVQASGAPASPSALRA
Pig	FLVLVMEQITLAYKEQSGPSPLEETRALLGTVNGGQHWHDGPGVQASGAPASPSALRA
Chimpanzee	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Human	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Rhesus	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Crab-eating	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Rabbit	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Mouse	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Rat	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Horse	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Sheep	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Goat	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Cattle	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Pig	CVLVFSLALHSVFEGAVLQDRDRAMELCLALLHKGILAVLSLRLQLQSHLRAGVVA
Chimpanzee	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Human	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Rhesus	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Crab-eating	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Rabbit	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Mouse	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Rat	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Horse	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Sheep	GCGILFSCMTPLGIGLGTALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Goat	GCGILFSCMTPLGIGLGTALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Cattle	GCGILFSCMTPLGIGLGTALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Pig	GCGILFSCMTPLGIGLGTALAEASAGPLHQLAQSVEGMAAGTFLYITFLEILPQELATSE
Chimpanzee	QRILKVIILLAGFALLTGLLFIQI
Human	QRILKVIILLAGFALLTGLLFIQI
Rhesus	QRILKVIILLAGFALLTGLLFIQI
Crab-eating	QRILKVIILLAGFALLTGLLFIQI
Rabbit	QRILKVIILLAGFALLTGLLFIQI
Mouse	QRILKVIILLAGFALLTGLLFIQI
Rat	QRILKVIILLAGFALLTGLLFIQI
Horse	QRILKVIILLAGFALLTGLLFIQI
Sheep	QRILKVIILLAGFALLTGLLFIQI
Goat	QRILKVIILLAGFALLTGLLFIQI
Cattle	QRILKVIILLAGFALLTGLLFIQI
Pig	QRILKVIILLAGFALLTGLLFIQI

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

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Sheep      MEPLLGAIGKICL F ALLV L L V C G L I P I Y F K W F Q T A T A T G C H R R I L S F L G C T S A G V F L G A G
Goat       MEPLLGAIGKICL F ALLV L L V C G L I P I C F K W F Q T A T A T G C H R R I L S F L G C T S A G V F L G A G
Cattle     MEPLLGTIKIGCL F ALLV L L V C G L I P I C F K W F Q T T T A T G C H R R V L S F L G C T S A G V F L G A G
Giant      MEPLLGVKIGKICL F ALLV L L T L A C G L I P I C F K W F Q I D A A R G H H R R V L R L L G C I S A G V F L G A G
Horse      MEPLLGVKIGKICL F ALLT L L V C G L I P I C F K W F Q I N A A T G R H R R V L S L L G C T S A G V F L G A G
Chimpanzee MEQ L L S I K L G C L F A L L A L T I L G C G L T P I C F K W F Q I D A A R G H H R R V L R L L G C I S A G V F L G A G
Human      MEQ L L S I K L G C L F A L L A L T I L G C G L T P I C F K W F Q I D A A R G H H R R V L R L L G C I S A G V F L G A G
Northern   MEQ L L S I K L G C L F A L L A L T I L G C G L T P I C F K W F Q I D A A R G H H R R V L R L L G C I S A G V F L G A G
Rhesus     MEQ L L S I K L G C L F A L L A L T I L G C G L T P I C F K W F Q I D A A R G H H R R V L R L L G C I S A G V F L G A G
Rabbit     MEPLLGVKIGKICL F ALLV L L V C G L I P I C S K W F Q I E A A T G R H R R V L S L L G C A S A G V F L G A G
Mouse      MEV L L G V K I G C L L A L L V L T L G C G L T P I Y V K W F Q M D A A T G H H H R R V L S L L G C T S A G V F L G A G
Rat        MEV L P G V K I G C V L A L L V L T L G C G L T P I Y V K W F Q T D A A T G H H H R R V L S L L G C T S A G V F L G A G
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Sheep      FMHMTAEALEGIKSEI Q N L M I Q N R T K S E G H S D D D D A D S A Y M E Y P Y G E L V I S L G F F L V F L L E
Goat       FMHMTAEALEGIKSEI Q N L M I Q N R T K S E G H S D D D D A D S A Y M E Y P Y G E L I I S L G F F L V F L L E
Cattle     FMHMTAEALEGIKSEI Q N L V I Q N R T K S E G H S D D D D A D S A Y M E Y P Y G E L I I S L G F F L V F L L E
Giant      LMHMTAEALEGI D S E I Q K F M Q N R T E K E G N A S D D S E S A Q M E Y P Y G E L I I S L G F F V F F L E
Horse      FMHMTAEALEGI E S E I Q K F V Q N R T E S E G - S S D D A D S A Q T D Y P Y G E L I I S L G F F L V F F L E
Chimpanzee FMHMTAEALEE I E S Q I Q K F M V Q N R S A S E R N S S G D A D S A H M E Y P Y G E L I I S L G F F V F F L E
Human      FMHMTAEALEE I E S Q I Q K F M V Q N R S A S E R N S S G D A D S A H M E Y P Y G E L I I S L G F F L V F F L E
Northern   FMHMTAEALEE I E S Q I Q K F M V Q N R S A S E R N S S G V A D S V H M E Y P Y G E L I I S L G F F V F F L E
Rhesus     FMHMTAEALEE I E S Q I Q K F V Q N R S T S E R N S S G D A D S A H M E Y P Y G E L I I S L G F F V F F L E
Rabbit     FMHMTAEAL Q G T E S E I Q K F L V Q N R T P E R N S S H A S S A Q T E Y P Y G E L I I S L G F F V F F L E
Mouse      LMHMTAEALEGI E S E I Q K F V E Q N S T G S K G N S R D A A S S Y V E Y P Y G E L V I S L G F F V F F L E
Rat        LMHMTAEALEGI E S E I Q K F V V Q N S T G S K G N S R D A A S S Y V E Y P Y G E L V I S L G F F V F F L E
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Sheep      SLALQCCPGTAETPKVQEQELGTAHELEPHSHGLLPSPSRGPFPRAL I L L L S L S F H S V F E G
Goat       SLALQCCPGTAETPKVQEQELGTAHELEPHSHGLLPSPSRGPFPRAL I L L L S L S F H S V F E G
Cattle     SLALQCCPGAAGGTPVQEEWSGT-HVLELHSHGPLPLPSKRPLRALV L L L S L S F H S V F E G
Giant      SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Horse      SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Chimpanzee SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Human      SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Northern   SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Rhesus     SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Rabbit     SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Mouse      SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Rat        SLALQCCPGAAGGTPVQEEWGGAHVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
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Sheep      LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Goat       LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Cattle     LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Giant      LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Horse      LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Chimpanzee LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Human      LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Northern   LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Rhesus     LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Rabbit     LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Mouse      LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
Rat        LAVGLQITVAATVQLCLAVLAHKGIVVFGVGLRLVQIGTGSRWAVS I L L S L A L M S P L G L A
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Sheep      IGLAVPQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Goat       IGLAVPQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Cattle     IGLAVPQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Giant      LGLAVTQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Horse      LGLAVTQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Chimpanzee VGLAVTQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Human      VGLAVTQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Northern   VGMAVTQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Rhesus     VGLAVTQGDSEAGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Rabbit     LGLAVVGGDSEGGQGLVQALLEGVAAGTFLVTFLEIILPRELASEAPLAKWSCVAAGFVA
Mouse      LGLTVAGGASGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
Rat        LGLTVAGGASGQGLAQAVLEGMAGTFLYVTFLEIILPRELASEAPLAKWSCVAAGFVA
*****

Sheep      FMAVIALWA
Goat       FMAVIALWA
Cattle     FMAVIALWA
Giant      FMAFIALWA
Horse      FMAFIALWA
Chimpanzee FMAFIALWA
Human      FMAFIALWA
Northern   FMAFIALWA
Rhesus     FMAFIALWA
Rabbit     FMAFIALWA
Mouse      FMAFIALWA
Rat        FMAFIALWA
*****

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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%),


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Chimpanzee      MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Human           MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Northern       MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Sumatran       MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Rhesus         MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
White-tufted-ear MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Dog            MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Horse         MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Sheep         MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Cattle       MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Pig          MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Rabbit       MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Mouse       MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH
Rat         MARGLGAPHWVAVGLLTWAILGGLLVAGLGGHNDLHDDLQEDFHGHSRRHSHEDFHHGHSH

Chimpanzee      AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGHSHESLYHRGHGMDH-
Human           AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGYSHESLYHRGHGMDH-
Northern       AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGNSHESLYHRGHGMDH-
Sumatran       AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGMSHESLYHRGHGMDH-
Rhesus         AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGHSHESLYHRGHGMDH-
White-tufted-ear AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGHSHESLYHRGHGMDH-
Dog            AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGHSHESLYHRGHGMDH-
Horse         AHG-----HTHESIWWGHTHMDHMGHSHEDLHMG--HSMGHSHESLYHRGHGMDH-
Sheep         AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGHSHESLYHRGHGMDH-
Cattle       AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGHSHESLYHRGHGMDH-
Pig          AHGH-----GHTHESIWWGHTHMDHMGHSHEDLHMG--HSMGHSHESLYHRGHGMDH-
Rabbit       AHGHG--HGHTHESIWWGHTHMDHMGHSHEDLHMGHSHESLYHRGHGMDH-
Mouse       GHSHEDFHHGHGHTHESIWWGHANSMDHMGHSRELLHMG--HSMGHSHESLYHRGHGMDH-
Rat         G-----HGHTHESIWWGHANSMDHMGHSRELVHMG--HSMGHSHESLYHRGHGMDH-

Chimpanzee      EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Human           EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Northern       EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Sumatran       EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Rhesus         EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
White-tufted-ear EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Dog            EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Horse         EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Sheep         EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Cattle       EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Pig          EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Rabbit       EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Mouse       EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ
Rat         EMSGGGYGESGAPGIKQDLDVAVLWALGATVLSAAPPFFVLFLLIPVESNSPRHRSLLQ

Chimpanzee      ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Human           ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Northern       ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Sumatran       ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Rhesus         ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
White-tufted-ear ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Dog            ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Horse         ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Sheep         ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Cattle       ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Pig          ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Rabbit       ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Mouse       ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF
Rat         ILLSFASGGLLDGDAFLHLIPHALEPHSHHTLEQPHGHSHSGQGPILSVGLMVLVSGIVAF

Chimpanzee      LVVEKFRVHVKGKGGHSHGHG---HAHSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Human           LVVEKFRVHVKGKGGHSHGHG---HAHSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Northern       LVVEKFRVHVKGKGGHSHGHG---HPYSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Sumatran       LVVEKFRVHVKGKGGHSHGHG---HAHSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Rhesus         LVVEKFRVHVKGKGGHSHGHG---HAHSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
White-tufted-ear LVVEKFRVHVKGKGGHSHGHG---HAHSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Dog            LVVEKFRVHVKGKGGHSHGHG---HTHGHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Horse         LVVEKFRVHVKGKGGHSHGHG---HTHGHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Sheep         LVVEKFRVHVKGKGGHSHGHG---HAHSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Cattle       LVVEKFRVHVKGKGGHSHGHG---HAHSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Pig          LVVEKFRVHVKGKGGHSHGHG---HAHSHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Rabbit       LVVEKFRVHVKGKGGHSHGHG---HTHGHTRG-SHGHC-RQERSTKEKQSSSEEEKEKTR
Mouse       LVVEKFRVHVKGKGGHSHGHG---DRHAGDSHTGDRHECSKKEKPFSTEED-KEVG
Rat         LVVEKFRVHVKGKGGHSHGHG---DRHAGDSHTGDRHECSKKEKPFSTEED-KEVG

Chimpanzee      GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Human           GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Northern       GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Sumatran       GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Rhesus         GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
White-tufted-ear GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Dog            GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Horse         GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Sheep         GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Cattle       GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Pig          GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Rabbit       GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Mouse       GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG
Rat         GVEKRRGGSTVFKDGPVPRPQNAEEERKGLDLRVSGYLNLAADLAHNFTDGLAIGASFRRG

Chimpanzee      RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Human           RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Northern       RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Sumatran       RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Rhesus         RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
White-tufted-ear RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Dog            RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Horse         RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Sheep         RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Cattle       RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Pig          RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Rabbit       RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Mouse       RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG
Rat         RGLGILTTMTVLLHEVPEHEVDFAILVQSGCSKQKAMRLQLLTAVGALAGTACALLTEGG

Chimpanzee      AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Human           AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Northern       AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Sumatran       AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Rhesus         AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
White-tufted-ear AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Dog            AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Horse         AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Sheep         AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Cattle       AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Pig          AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Rabbit       AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Mouse       AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL
Rat         AVGSEIAGGAGPGWVLPFTAGGFIYVATVSVLPPELLREASPLQSLLEVLGLLGGVMMVVL

Chimpanzee      IAHLE
Human           IAHLE
Northern       IAHLE
Sumatran       IAHLE
Rhesus         IAHLE
White-tufted-ear IAHLE
Dog            IAHLE
Horse         IAHLE
Sheep         IAHLE
Cattle       IAHLE
Pig          IAHLE
Rabbit       IAHLE
Mouse       IAHLE
Rat         IAHLE

```

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

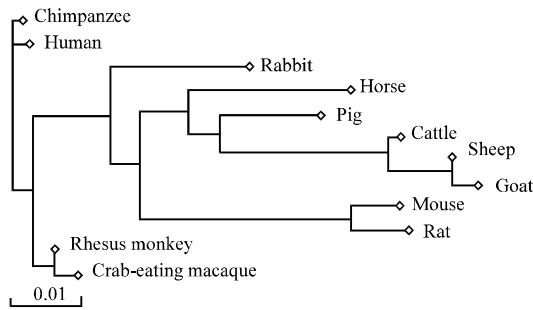


Fig. 5: The phylogenetic analysis for twelve kinds of *SLC39A1* genes

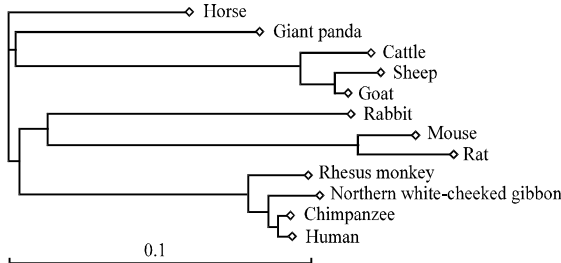


Fig. 6: The phylogenetic analysis for twelve kinds of *SLC39A2* genes

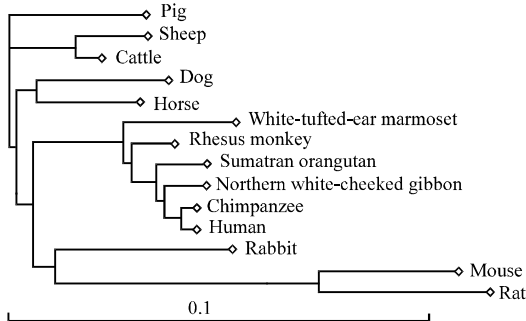


Fig. 7: The phylogenetic analysis for fourteen kinds of *SLC39A7* genes

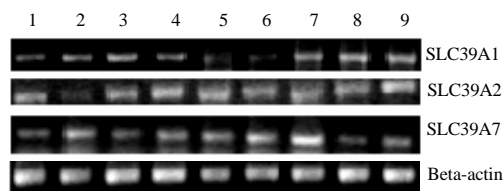


Fig. 8: Tissue expression distribution of sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes. The β -actin expression is the internal control. 1: Spleen; 2: Skin; 3: Lung; 4: Muscle; 5: Heart; 6: Fat; 7: Liver; 8: Kidney; 9: Ovary

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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