

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'-ATGGCCAGAGGCCTGGGG-3	63
	Reverse: 5'-TCACTGGAGGTGGGCAATCA-3	
β -actin	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

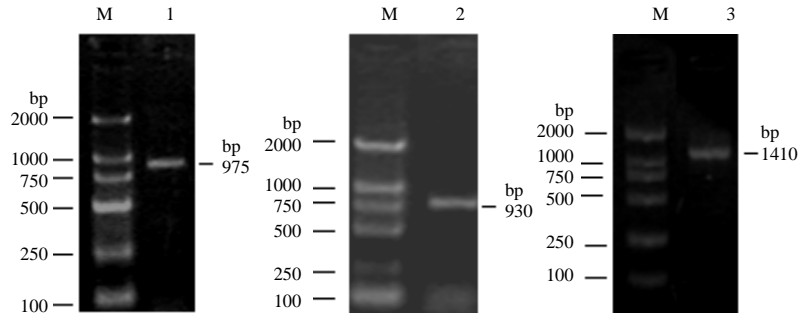


Fig. 1: RT-PCR results for sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes. M, DL2000 DNA markers; 1, PCR product for sheep *SLC39A1* gene; 2, PCR product for sheep *SLC39A2* gene; 3, PCR product for sheep *SLC39A7* gene

Chimpanzee	MGFWGEPPELLVWRPEAVASEPPVGVGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Human	MGFWGEPPELLVWRPEAVASEPPVGVGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Rhesus	MGFWGEPPELLVWRPEAVASEPPVGVGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Crab-eating	MGFWGEPPELLVWRPEAVASEPPVGVGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Rabbit	MGFWGEPPELLVWRPEAVASEPPVGVGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Mouse	MGFWGEPPELLVWRPEAVASEPPVGVGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Rat	MGFWGEPPELLVWRPEAVASEPPVGVGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Horse	MGFWGEPPELLVWRPEAVASEPPVGVGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Sheep	MGFWGEPPELLVWRPEAAASEAPVPMGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Goat	MGFWGEPPELLVWRPEAAASEAPVPMGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Cattle	MGFWGEPPELLVWRPEAAASEAPVPMGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Pig	MGFWGEPPELLVWRPEAAASEAPVPMGLVGLGALVLLVLTLLCSLVFICVLRPPGANHE
Chimpanzee	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Human	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Rhesus	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Crab-eating	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Rabbit	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Mouse	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Rat	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Horse	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Sheep	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Goat	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Cattle	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Pig	GSASRQKALSILVSCFAGGVFLATCLLDLLPDYLAIDEALAAHVTLOFFLQEFILAMGF
Chimpanzee	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Human	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPATPSALRA
Rhesus	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Crab-eating	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Rabbit	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Mouse	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Rat	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Horse	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Sheep	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Goat	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Cattle	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Pig	FLVLVMEQITLAYKEQSGSPPLEETRALLTGTVNGGPGQHWHDGPGVQASGAPASPSALRA
Chimpanzee	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Human	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Rhesus	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Crab-eating	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Rabbit	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Mouse	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Rat	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Horse	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Sheep	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Goat	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Cattle	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Pig	CVLVFSLALHSVFEGGLAVGLQDRARAMELCALLLHKGILAVSLSLRLLQSHLRAGVVA
Chimpanzee	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Human	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Rhesus	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Crab-eating	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Rabbit	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Mouse	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Rat	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Horse	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Sheep	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Goat	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Cattle	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Pig	GCGILFSCMTPLGIGLGAALAEASAGPLHQLAQSVLEGMAGTFLYITFLEILPQELATSE
Chimpanzee	QRILKVILLLAGFALLTGLLFIQI
Human	QRILKVILLLAGFALLTGLLFIQI
Rhesus	QRILKVILLLAGFALLTGLLFIQI
Crab-eating	QRILKVILLLAGFALLTGLLFIQI
Rabbit	QRILKVILLLAGFALLTGLLFIQI
Mouse	QRILKVILLLAGFALLTGLLFIQI
Rat	QRILKVILLLAGFALLTGLLFIQI
Horse	QRILKVILLLAGFALLTGLLFIQI
Sheep	QRILKVILLLAGFALLTGLLFIQI
Goat	QRILKVILLLAGFALLTGLLFIQI
Cattle	QRILKVILLLAGFALLTGLLFIQI
Pig	QRILKVILLLAGFALLTGLLFIQI

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      MEPLLGAIGCLFALLVLTLCGLIPIYFKWFQTATATGCHRRILSLGCTSAGVFLGAG
Goat       MEPLLGAIGCLFALLVLTLCGLIPIYFKWFQTATATGCHRRILSLGCTSAGVFLGAG
Cattle     MEPLLGTIKIGCLFALLVLTLCGLIPIYFKWFQTATATGCHRRILSLGCTSAGVFLGAG
Giant      MEPLLGVKIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
Horse      MEPLLGVKIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
Chimpanzee MEQLLSIKLIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
Human      MEQLLSIKLIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
Northern   MEQLLSIKLIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
Rhesus     MEQLLSIKLIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
Rabbit     MEPLLGVKIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
Mouse      MEVLGVKIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
Rat        MEVLGVKIGCLFALLVLTLCGLIPIYFKWFQIDAATGRHRRILSLGCTSAGVFLGAG
*****

Sheep      FMHMTAEALEGKSEIQNLMIQNRTKSEHSDDDADSAIMEYYPYGLVLSLGGFFLVFLE
Goat       FMHMTAEALEGKSEIQNLMIQNRTKSEHSDDDADSAIMEYYPYGLVLSLGGFFLVFLE
Cattle     FMHMTAEALEGKSEIQNLMIQNRTKSEHSDDDADSAIMEYYPYGLVLSLGGFFLVFLE
Giant      LMHMTAEALEGIDSEIQKFMQNRTEKEGNASDDSESAQMEYYPYGLVLSLGGFFLVFLE
Horse      FMHMTAEALEGIESEIQKFMQNRTESEG-SSDDADSAQTDYPYGLVLSLGGFFLVFLE
Chimpanzee FMHMTAEALEEIESIQKFMQNRTESEG-SSDDADSAQTDYPYGLVLSLGGFFLVFLE
Human      FMHMTAEALEEIESIQKFMQNRTESEG-SSDDADSAQTDYPYGLVLSLGGFFLVFLE
Northern   FMHMTAEALEEIESIQKFMQNRTESEG-SSDDADSAQTDYPYGLVLSLGGFFLVFLE
Rhesus     FMHMTAEALEEIESIQKFMQNRTESEG-SSDDADSAQTDYPYGLVLSLGGFFLVFLE
Rabbit     FMHMTAEALEEIESIQKFMQNRTESEG-SSDDADSAQTDYPYGLVLSLGGFFLVFLE
Mouse      LMHMTAEALEGIESEIQKFMQNRTESEG-SSDDADSAQTDYPYGLVLSLGGFFLVFLE
Rat        LMHMTAEALEGIESEIQKFMQNRTESEG-SSDDADSAQTDYPYGLVLSLGGFFLVFLE
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Sheep      SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Goat       SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Cattle     SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Giant      SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Horse      SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Chimpanzee SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Human      SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Northern   SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Rhesus     SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Rabbit     SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Mouse      SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
Rat        SLALQCCPGTAETPKVQEQELGTAHELEPHSHGGLLPSPSRGPFPRALILLLSLFSHVFEG
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Sheep      LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Goat       LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Cattle     LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Giant      LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Horse      LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Chimpanzee LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Human      LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Northern   LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Rhesus     LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Rabbit     LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Mouse      LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
Rat        LAVGLQITVASTVQLCLAVLAHKGIVVFGVGLRLVQVGTESRWAVLSILSLALMSPGLA
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Sheep      IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Goat       IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Cattle     IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Giant      IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Horse      IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Chimpanzee IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Human      IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Northern   IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Rhesus     IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Rabbit     IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Mouse      IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
Rat        IGLAVPQGDSEAGQGLAQVLEGMAGTFLYVTFLEILPRELASEAPLAKWSCVAAGFA
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Sheep      FMAVIALWA
Goat       FMAVIALWA
Cattle     FMAVIALWA
Giant      FMAVIALWA
Horse      FMAVIALWA
Chimpanzee FMAVIALWA
Human      FMAVIALWA
Northern   FMAVIALWA
Rhesus     FMAVIALWA
Rabbit     FMAVIALWA
Mouse      FMAVIALWA
Rat        FMAVIALWA
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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   MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Human        MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Northern     MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Sumatran     MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Rhesus       MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
White-tufted-ear MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Dog          MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Horse        MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Sheep        MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Cattle       MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Pig          MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Rabbit       MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Mouse        MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS
Rat          MARGLGAPHWVAVGLLTWATLGLLVAGLGGMNDLHDDLQEDFHGHSRRSHSDEDFHHGHS

Chimpanzee   AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Human        AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGYSSESILYHRGHGMDH-
Northern     AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Sumatran     AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Rhesus       AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
White-tufted-ear AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Dog          AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Horse        AHG-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Sheep        AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Cattle       AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Pig          AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Rabbit       AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Mouse        AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-
Rat          AHGH-----GHTHESIWNHGHHTHMDHGHSHSDEDLHNG--NSHGHSSESILYHRGHGMDH-

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

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

Chimpanzee   LVVEKFVRHVKGHGHSHGHG---HAHSHTRG-SHGNG-RQERSTKEKQSSEEEKEKTR
Human        LVVEKFVRHVKGHGHSHGHG---HAHSHTRG-SHGNG-RQERSTKEKQSSEEEKEKTR
Northern     LVVEKFVRHVKGHGHSHGHG---HPYSHTRG-SHGNG-RQERSTKEKQSSEEEKEKTR
Sumatran     LVVEKFVRHVKGHGHSHGHG---HAHSHTRG-SHGNG-RQERSTKEKQSSEEEKEKTR
Rhesus       LVVEKFVRHVKGHGHSHGHG---HAHSHTRG-SHGNG-RQERSTKEKQSSEEEKEKTR
White-tufted-ear LVVEKFVRHVKGHGHSHGHG---HAHSHTRG-SHGNG-RQERSTKEKQSSEEEKEKTR
Dog          LVVEKFVRHVKGHGHSHGHG---HTHGHTQG-SHGNG-TQKPSKEKQSSEEEKEKTR
Horse        LVVEKFVRHVKGHGHSHGHG---HTHGHTQG-SHGNG-RQERSTKEKQSSEEEKEKTR
Sheep        LVVEKFVRHVKGHGHSHGHG---HTHGHTQG-SHGNG-RQERSTKEKQSSEEEKEKTR
Cattle       LVVEKFVRHVKGHGHSHGHG---HAHSHTRG-SHGNG-RQERSTKEKQSSEEEKEKTR
Pig          LVVEKFVRHVKGHGHSHGHG---HAHSHTRG-SHGNG-RQERSTKEKQSSEEEKEKTR
Rabbit       LVVEKFVRHVKGHGHSHGHG---HTHGHTQG-SHGNG-RQERSTKEKQSSEEEKEKTR
Mouse        LVVEKFVRHVKGHGHSHGHG---HTHGHTQG-SHGNG-RQERSTKEKQSSEEEKEKTR
Rat          LVVEKFVRHVKGHGHSHGHG---HTHGHTQG-SHGNG-RQERSTKEKQSSEEEKEKTR

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

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

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

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

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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

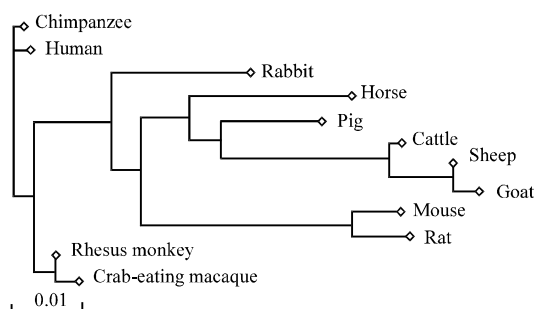


Fig. 5: The phylogenetic analysis for twelve kinds of *SLC39A1* genes

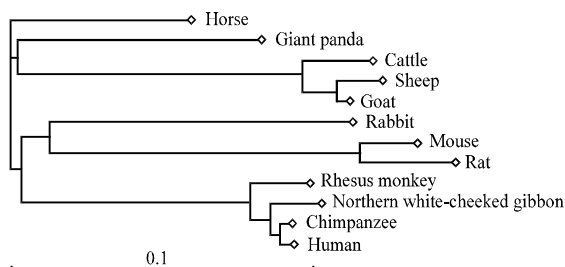


Fig. 6: The phylogenetic analysis for twelve kinds of *SLC39A2* genes

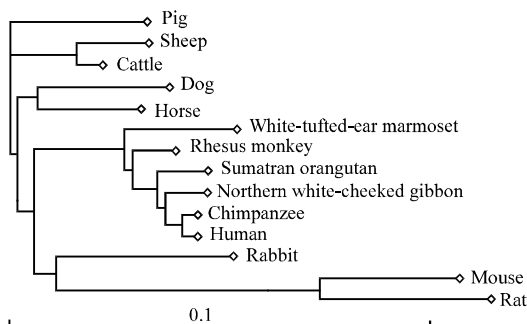


Fig. 7: The phylogenetic analysis for fourteen kinds of *SLC39A7* genes

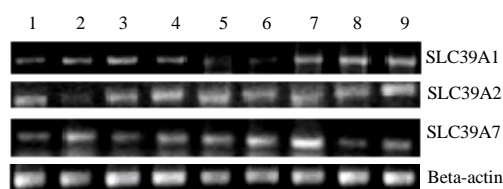


Fig. 8: Tissue expression distribution of sheep *SLC39A1*, *SLC39A2* and *SLC39A7* genes. The β -actin expression is the internal control. 1: Spleen; 2: Skin; 3: Lung; 4: Muscle; 5: Heart; 6: Fat; 7: Liver; 8: Kidney; 9: Ovary

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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