Molecular Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Sheep (*Ovis aries*) Genes - BCKDHA, NAGA and HEXA

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ABSTRACT

The complete coding sequences of three sheep genes- BCKDHA, NAGA and HEXA were amplified using the reverse transcriptase polymerase chain reaction (RT-PCR), based on the conserved sequence information of the mouse or other mammals. The nucleotide sequences of these three genes revealed that the sheep BCKDHA gene encodes a protein of 313 amino acids which has high homology with the BCKDHA gene that encodes a protein of 447 amino acids that has high homology with the Branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA) of five species chimpanzee (93%), human (96%), crab-eating macaque (93%), bovine (98%) and mouse (91%). The sheep NAGA gene encodes a protein of 411 amino acids that has high homology with the alpha-N-acetylgalactosaminidase (NAGA) of five species human (85%), bovine (94%), mouse (91%), rat (83%) and chicken (74%). The sheep HEXA gene encodes a protein of 529 amino acids that has high homology with the hexosaminidase A (HEXA) of five species bovine (98%), human (84%), Bornean orangutan (84%), rat (80%) and mouse (81%). Finally these three novel sheep genes were assigned to GeneIDs: 100145857, 100145858 and 100145856. The phylogenetic tree analysis revealed that the sheep BCKDHA, NAGA, and HEXA all have closer genetic relationships to the BCKDHA, NAGA, and HEXA of bovine. Tissue expression profile analysis was also carried out and results revealed that sheep BCKDHA, NAGA and HEXA genes were differentially expressed in tissues including muscle, heart, liver, fat, kidney, lung, small and large intestine. Our experiment is the first to establish the primary foundation for further research on these three sheep genes.

Key terms: sheep, BCKDHA, NAGA, HEXA, tissue expression.

INTRODUCTION

Branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA) encodes the E1-alpha subunit of the branched-chain alpha-keto acid (BCAA) dehydrogenase complex (BCKD; EC 1.2.4.4), an inner-mitochondrial enzyme complex that catalyzes the oxidative decarboxylation of the branched-chain alpha-ketoacids derived from isoleucine, leucine, and valine. Mutations of this gene have also been reported to be associated with maple syrup urine disease (Flaschker et al., 2007; Naik et al., 2004; Chinsky et al., 2004).

The Alpha-N-acetylgalactosaminidase (NAGA) gene encodes the lysosomal

enzyme, which cleaves alpha-N-acetylgalactosaminyl moieties from glycoconjugates. Mutations in NAGA have been identified as the cause of Schindler disease types I and II (type II is also known as Kanzaki disease) (Chabás et al., 2007; Kanekura et al., 2005).

Hexosaminidase A (HEXA) is the alpha subunit of the lysosomal enzyme beta-hexosaminidase that, together with the cofactor GM2 activator protein, catalyzes the degradation of the ganglioside GM2, and other molecules containing terminal N-acetyl hexosamines. Beta-hexosaminidase is composed of two subunits, alpha and beta, which are encoded by separate genes. Both beta-hexosaminidase alpha and beta

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subunits are members of a family 20 of glycosyl hydrolases. Mutations in the alpha or beta subunit genes lead to an accumulation of GM2 ganglioside in neurons and neurodegenerative disorders termed the GM2 gangliosidosis. Alpha subunit gene mutations lead to Tay-Sachs disease (GM2-gangliosidosis type I) (Karpati et al., 2004; McGinniss et al., 2002).

Based on the above, these three genes are associated with three important mutation diseases of humans and these diseases had been all found in another kind of mammal, the mouse (Pessoa-Pureur et al., 2007; Herrmann et al., 1998; Miklyaeva et al., 2004). These inherited diseases may also occur in sheep. If this is true, it is essential to isolate these three genes from sheep for these diseases are highly related or potentially related to sheep production. On the other hand, if these diseases also occur in sheep, sheep would be another important model to study these diseases in humans. However, until today the sheep BCKDHA, NAGA and HEXA genes have not yet been reported.

In the present work, we isolated the coding sequences of sheep BCKDHA, NAGA and HEXA genes based on the conserved sequence information of humans, mice or other mammals and highly homologous sheep EST sequence information, and subsequently performed the necessary sequence analyses and tissue expression profile analyses for these genes. These established the primary foundation of understanding of these three sheep genes.

MATERIALS AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis

The tissue samples of liver, small intestine, large intestine, lung, muscle, fat, spleen, and kidney, were derived from three 120 days old Yunnan local sheep. Total RNA extraction and first-strand cDNA synthesis for these tissue samples were performed as the methods describe by Liu (2004).

Isolation of the sheep BCKDHA, NAGA and HEXA genes

The RT-PCR was performed to isolate these three sheep genes using the pooled cDNAs from different tissues above. The 25 μ l reaction system was: 2.0 μ l cDNA, 2.5 μ l 2 μ M mixed dNTPs, 2.5 μ l 10' Taq DNA polymerase buffer, 2.5 μ l 25 μ M MgCl₂, 2.0 μ l 10 μ M forward primer, 2.0 μ l 10 μ M reverse primer, 2.0 units of Taq DNA polymerase (1 U/1 μ l), and 9.5 μ l sterile water.

The primers for sheep BCKDHA gene isolation were designed based on the conserved CDS information from human, rat and mouse BCKDHA genes and the highly homologous sheep EST sequences: EE823419, EE827113 and EE770739. Similarly, the primers for sheep NAGA gene isolation were designed based on the conserved CDS information from human, rat and mouse NAGA genes and the highly homologous sheep EST sequences: DY493345, EE823664 and EE748803. The primers for sheep HEXA gene isolation were designed based on the conserved CDS information from human and mouse HEXA genes and the highly homologous sheep EST sequences: EE803104, EE829775, DY517999 and EE750801. These primer sequences and their annealing temperature for RT-PCR reaction are described in Table 1.

These PCR products for sheep BCKDHA, NAGA and HEXA cDNAs 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.

RT-PCR for tissue expression profile analysis

RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Liu et al., 2007). We selected the housekeeping gene β -actin (Accession no: NM_001009784) was performed as a positive control. The primers of sheep BCKDHA, NAGA and HEXA genes, which were used to perform the RT-PCR for tissue expression profile analysis,

were the same as the primers for isolation RT-PCR above. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The 25 μ L reaction system was: 1 μ L cDNA (100 ng/ μ L), 5 pmoles each oligonucleotide primer, 2.5 μ L 2mmol/L mixed dNTPs, 2.5 μ L 10 $\times$ Taq DNA polymerase buffer, 2.5 μ L 25 mmol/L MgCl₂, 1.0 units of Taq DNA polymerase, and finally added sterile water to a volume of 25 μ L. The PCR program initially started with a 94°C denaturation for 4min, followed by 25 cycles of 94°C/1 min, Ta °C /1 min, 72°C/1 min, 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 the Conserved Domain Architecture Retrieval Tool of BLAST 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 BCKDHA, NAGA, and HEXA genes

Through RT-PCR with pooled tissue cDNAs, for sheep BCKDHA, NAGA, and HEXA, the resulting PCR products were 1344 bp, 1236 bp and 1590bp.

Sequence analysis

The cDNA nucleotide sequence analysis using the BLAST software at NCBI server

TABLE 1

Primers for sheep BCKDHA, NAGA and HEXA isolation and annealing temperature.

Gene	Primer sequence	Ta / °C
BCKDHA	Forward 5'- ATGGCGTTGGCGGTGGCT -3 Reverse: 5'- TCACTTCTCAAAGTGGTCC -3'	53
NAGA	Forward :5'- ATGTTGCTGAAGACAGTGC -3 Reverse: 5'- TCACTGCGGGGGCGTCCCC -3	55
HEXA	Forward 5'- ATGGCAGCCTCCACGCTC -3' Reverse: 5'- TCAGGTTTGTTCAAACTCCAC -3'	54
β -actin	Forward: 5'- CTTGATGTCACGGACGATTT -3 Reverse: 5'- CACGGCATTGTCACCAACT -3'	56

Fig. 1: RT-PCR results for sheep BCKDHA, NAGA, and HEXA. M, DL2000 DNA markers; 1, PCR product for sheep BCKDHA gene; 2, PCR product for sheep NAGA gene; 3, PCR product for sheep HEXA gene.

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Sheep      -----MAVAAAAARVWRPSRGLGRAGLPLLRLLGARGLARSHTRWQQQRHFSSLLD
Bovine     MQGSAKMAMAVAVAVARVWRPSRGLGRTGLPLLRLLGARGLARSHPHRWQQQHFSSLLD
Chimpanzee -----MAVAIAAARVWRLNRGLSQAALLLLRRPGARGLARSHPRRQQQQ--FSSLLD
Human      -----MAVAIAAARVWRLNRGLSQAALLLLRQPGARGLARSHPRRQQQQ--FSSLLD
Crab-eating macaque -----MAVAIAAARVWRPNRGLSQAALLLLWRPGARGLARSHPRRQQQQ--FSSLLD
Mouse      -----MSAAKIWRPSRGLRQAALLLLGRSGVRLARSHPSRQQQQQ--FPSLLD
           :.:** .*** :.:* ** *.***** *. * *** *.***

Sheep      KPQFPGASAEFIDKLEFIQPNVISGPIYRVMDRQGGIINPSEDPHLPQEKVLKFKYSMT
Bovine     KPQFPGASAEFIDKLEFIQPNVISGPIYRVMDRQGGIINPSEDPHLPQEKVLKFKYSMT
Chimpanzee KPQFPGASAEFIDKLEFIQPNVISGPIYRVMDRQGGIINPSEDPHLPQEKVLKFKYSMT
Human      KPQFPGASAEFIDKLEFIQPNVISGPIYRVMDRQGGIINPSEDPHLPQEKVLKFKYSMT
Crab-eating macaque KPQFPGASAEFIDKLEFIQPNVISGPIYRVMDRQGGIINPSEDPHLPQEKVLKFKYSMT
Mouse      KPQFPGASAEFVDKLEFIQPNVISGPIYRVMDRQGGIINPSEDPHLPQEEVLKFKYSMT
           *****:*****:*****:*****:*****:*****:*****:*****:*****

Sheep      LLNTMDRILYESQRQGRISFYMTNYGEEGTHVGSAAALDDTDLVFGQYREAGVLMYRDYP
Bovine     LLNTMDRILYESQRQGRISFYMTNYGEEGTHVGSAAALDDTDLVFGQYREAGVLMYRDYP
Chimpanzee LLNTMDRILYESQRQGRISFYMTNYGEEGTHVGSAAALDDTDLVFGQYREAGVLMYRDYP
Human      LLNTMDRILYESQRQGRISFYMTNYGEEGTHVGSAAALDDTDLVFGQYREAGVLMYRDYP
Crab-eating macaque LLNTMDRILYESQRQGRISFYMTNYGEEGTHVGSAAALDDTDLVFGQYREAGVLMYRDYP
Mouse      LLNTMDRILYESQREGISFYMTNYGEEGTHVGSAAALERTDLVFGQYREAGVLMYRDYP
           *****:*****:*****:*****:*****:*****:*****:*****:*****

Sheep      LELFMAQCYGNVSDLGKGRQMPVHYGCRERHFVTISSPLATQIPQAVGAAYAANKRANANR
Bovine     LELFMAQCYGNVSDLGKGRQMPVHYGCRERHFVTISSPLATQIPQAVGAAYAANKRANANR
Chimpanzee LELFMAQCYGNISDLGKGRQMPVHYGCKERHFVTISSPLATQIPQAVGAAYAANKRANANR
Human      LELFMAQCYGNISDLGKGRQMPVHYGCKERHFVTISSPLATQIPQAVGAAYAANKRANANR
Crab-eating macaque LELFMAQCYGNISDLGKGRQMPVHYGCKERHFVTISSPLATQIPQAVGAAYAANKRANANR
Mouse      LELFMSQCYGNVNDPGKGRQMPVHYGCKERHFVTISSPLATQIPQAVGAAYAANKRANANR
           *****:*****:*****:*****:*****:*****:*****:*****:*****

Sheep      VVICYFGEGAASEGDAHAGFNFAATLECPPIIFCRNNGYAISTPTSEQYRGDGLAARGPG
Bovine     VVICYFGEGAASEGDAHAGFNFAATLECPPIIFCRNNGYAISTPTSEQYRGDGLAARGPG
Chimpanzee VVICYFGEGAASEGDAHAGFNFAATLECPPIIFCRNNGYAISTPTSEQYRGDGLAARGPG
Human      VVICYFGEGAASEGDAHAGFNFAATLECPPIIFCRNNGYAISTPTSEQYRGDGLAARGPG
Crab-eating macaque VVICYFGEGAASEGDAHAGFNFAATLECPPIIFCRNNGYAISTPTSEQYRGDGLAARGPG
Mouse      IVICYFGEGAASEGDAHAGFNFAATLECPPIIFCRNNGYAISTPTSEQYRGDGLAARGPG
           :*****:*****:*****:*****:*****:*****:*****:*****

Sheep      YGILSIRVDGNDVFAVYNATKEARRRAVAENQPFLIEAMTYRIGHSTSDSSAYRSVDE
Bovine     YGILSIRVDGNDVFAVYNATKEARRRAVAENQPFLIEAMTYRIGHSTSDSSAYRSVDE
Chimpanzee YGIMSIRVDGNDVFAVYNATKEARRRAVAENQPFLIEAMTYRIGHSTSDSSAYRSVDE
Human      YGIMSIRVDGNDVFAVYNATKEARRRAVAENQPFLIEAMTYRIGHSTSDSSAYRSVDE
Crab-eating macaque YGIMSIRVDGNDVFAVYNATKEARRRAVAENQPFLIEAMTYRIGHSTSDSSAYRSVDE
Mouse      YGIKSIRVDGNDVFAVYNATKEARRRAVAENQPFLIEAMTYRIGHSTSDSSAYRSVDE
           *** *****:*****:*****:*****:*****:*****:*****:*****:*****

Sheep      VNYWDKQDHPISRLRHHLSQGWWDDEQEKAWRKQSRKKVMEAFEQAERKPKPNPNLLFS
Bovine     VNYWDKQDHPISRLRHHLSQGWWDDEQEKAWRKQSRKKVMEAFEQAERKPKPNPNLLFS
Chimpanzee VNYWDKQDHPVSRRLRHYLLSQGWWDDEQEKAWRKQSRKKVMEAFEQAERKPKPNPNLLFS
Human      VNYWDKQDHPISRLRHYLLSQGWWDDEQEKAWRKQSRKKVMEAFEQAERKPKPNPNLLFS
Crab-eating macaque VNYWDKQDHPISRLRHYLLSQGWWDDEQEKAWRKQSRKKVMEAFEQAERKPKPNPNLLFS
Mouse      VNYWDKQDHPISRLRQYLLNQGWWDDEQEKAWRKQSRKKVMEAFEQAERKPKPNPNLLFS
           *****:*****:*****:*****:*****:*****:*****:*****:*****

Sheep      DVYQEMPAQLRKQESLARHLQTYGEHYPLDHFEK
Bovine     DVYQEMPAQLRKQESLARHLQTYGEHYPLDHFEK
Chimpanzee DVYQEMPAQLRKQESLARHLQTYGEHYPLDHFDK
Human      DVYQEMPAQLRKQESLARHLQTYGEHYPLDHFDK
Crab-eating macaque DVYQEMPAQLRKQESLARHLQTYGEHYPLEHFDK
Mouse      DVYQEMPAQLRRQQESLARHLQTYGEHYPLDHFEK
           *****:*****:*****:*****:*****:*****:*****:*****:*****

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Fig. 2: The alignment of the protein encoded by sheep BCKDHA gene and five other kindsof BCKDHA.


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Sheep      MLLKTVLLLLALVAQVLVLENGLLRKPPMGWLAWERFRCNIDCNEDPKSCISEELFMEMAD
Bovine     MLLKTVLLLLALASQVLVLENGLLRKPPMGWLAWERFRCNIDCSEDPKNCISEQLFMEMAD
Human      MLLKTVLLLLGHVAQVLMNDGLLQTPPMGWLAWERFRCNINCEDEPKNCISEQLFMEMAD
Mouse      MLQKTVLLLLALVAQVLMLENGLLRTPPMGWLAWERFRCNIDCVDPKNCISERLFMEMAD
Rat        MLQKTVLLLLALVAQVLMLENGLLRTPPMGWLAWERFRCNINCEDEPKNCISERLFMEMAD
Chicken    -----LENGLARTPPMGWLAWERFRCNVNCREDPKNCISEMLFMEMAD
           *:*** :.*****:;* ***:**** *****

Sheep      RLAQDGWRDLGYVYLNIDDCWIGG-RDAKGNLVPDRKRFPHGIAFLADYAHSLGKLGIIY
Bovine     RLAQDGWRDLGYVYLNIDDCWIGG-RDAKGNLVPDRKRFPHGIAFLADYAHSLGKLGIIY
Human      RMAQDGWRDMGYTTYLNIDDCWIGG-RDASGRIMPDPKRFPHGIPFLADYVHSLGKLGIIY
Mouse      RLAQDGWRDLGYVYLNIDDCWIGG-RDASGRILPDPKRFPHGIAFLADYAHSLGKLGIIY
Rat        RLAQDGWRDLGYVYLNIDDCWIGG-RDATGRILPDPKRFPHGIAFLADYAHSLGKLGIIY
Chicken    RIAEDGWRELGYKYINIDDCWAAKQDAEGRVLPDPERFPRGICALADYVHARGLKLDIY
           *:****:* *:.*****. *** *.*:** :***:** *****:*

Sheep      EDLGNFTCMGYPGTTLDKVVQDAQTFAEWKVDMLKLDGCYSSPQERAE GYPKMAAALNAT
Bovine     EDLGNFTCMGYPGTTLDKVVQDAQTFAEWKVDMLKLDGCYSTPQERAE GYPKMAAALNAT
Human      ADMGNFTCMGYPGTTLDKVVQDAQTFAEWKVDMLKLDGCFSTPEERAQGYPKMAAALNAT
Mouse      EDMGKMTCMGYPGTTLDKVELDAETFAEWKVDMLKLDGCFSSSREAE GYPKMAAALNAT
Rat        EDMGKMTCMGYPGTTLDKVELDAATFAEWKVDMLKLDGCYSTPKERAEGYPKMAAALNAT
Chicken    GDLGRITCGGYPGTTLDKVEQDAQTFAEWGVDMLKLDGCYSSGKEQAQGYPMARALNST
           *:*.:** *****:* ** ***** *****:*. :*.:**:** ***:**

Sheep      GRPIAFSCSWPAYEGGLPPKVNYTLLADICNLWRNFDDIQDSWRSVLSILDWVFAHQDVL
Bovine     GRPIAFSCSWPAYEGGLPPKVNYTLLADICNLWRNFDDIQDSWRSVLSILDWVFTHQDVL
Human      GRPIAFSCSWPAYEGGLPPRVNYSLADICNLWRNYDDIQDSWWSVLSILNWVFEHQDIL
Mouse      GRPIAFSCSWPAYEGGLPPKVNYTEVSRVCNLWRNYKDIQDSWKS VLSILDWVVRHQDVL
Rat        GRPIAFSCSWPAYEGGLPPKVNYTEVAGTCNLWRNYKDIQDSWKS VLSILDWVFKHQDIL
Chicken    GRPIVYSCSWPAYQGGLPPKVNYTLLGEICNLWRNYDDIQDSWDSVLSIVDWFFTNQDVL
           ****:.*****:*****:***: :. *****:***** *****:***:*. :***:*

Sheep      QPVAGPGHWNDPDVLLIGNFGLSFEQARAQMALWTVLAAPLLMSTD LRTISAQNVDILQN
Bovine     QPIAGPGHWNDPDMLLIGNFGLSFEQAQAQMALWTVLAAPLFMSTD LRTISAQNMDILQN
Human      QPVAGPGHWNDPDMLLIGNFGLSLEQSAQMALWTVLAAPLLMSTD LRTISAQNMDILQN
Mouse      QPVAGPGHWNDPDMLLIGNFGLSFDESRAQMALWTVLAAPLLMSTD LRTISPQNMDILQN
Rat        QPVSGPGHWNDPDMLLIGNFGLSFDESRAQMALWTVLAAPLFMSTD LRTISPQNIDILQN
Chicken    QPFAGPGHWNDPDMLIIGNFGLSFEQSRSAQMALWTIMAAPLLMSTD LRTISP SAKKILQN
           **:.*****:*.***** ::::*****:*****:*****.. .***

Sheep      PLMIKINQDPLGIQGRILKEKSYIEVYLRPLVNEASAIVFFSRRTDMPYHYHSSLARLN
Bovine     PLMIKINQDPLGIQGRILKEKSHIEVYLRPLASEASAIVFFSRRTDMPYHYHSSLARLN
Human      PLMIKINQDPLGIQGRIRIHEKSLIEVYMRPLSNKASALVFFSRTDMPYHYHSSLGQLN
Mouse      PLMIKINQDPLGIQGRILKSKSHIEVFKRYLSNQASALVFFSRTDMPFRFHC SLEELN
Rat        PLLIKINQDPLGIQGRILFKSKSHIEVFKRNLSDASALVFFSRTDMPYHFC SLEELN
Chicken    RLMIKINQDPLGIQGRRIKEGSHIEVFLRPLSQAASALVFFSRTDMPFRYTTSLAKLG
           *:***** ** * ***: * * . ***:**** * ****:: ** *.

Sheep      FSSSVVYEAQDVYTGDIISGLQDKTNFTVIINPSGVVMWYLYPI SKLGIPQ-----
Bovine     FSSSVVYEAQDVYTGDIISGLQDKTNFTVIINPSGVVMWYLYPIRKLEIPQ-----
Human      FTGSVIYEAQDVYSGDIISGLRDETNTFTVIINPSGVVMWYLYPIKNLEMSQ-----
Mouse      YPKGRVYEGQNVFTGDIISGLQTEVNTFTVIINPSGVVMWYLYPIKDLGISTMMSHW----
Rat        YPKGSVYEGQNVFTGDIISGLHPETNTFTVIINPSGVVMWYLYPVKGLGIYTMMSQL----
Chicken    FPMGAAYEVQDVYSGKIIISGLKTGDNFTVIINPSGVVMWYLCPKALLIQQAPGGPSRLP
           :. . ** *:***:*.***: ***** ***** * *

Sheep      --
Bovine     --
Human      --
Mouse      --
Rat        --
Chicken    LL

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Fig. 3: The alignment of the protein encoded by sheep NAGA gene and five other kinds of NAGA.

Fig. 4: The alignment of the protein encoded by sheep HEXA gene and five other kinds of HEXA.

(<http://www.ncbi.nlm.nih.gov/BLAST>) revealed that these genes were not homologous to any of the known sheep genes and they were then deposited into the GenBank database (Accession number: EU579456, EU579460 and EU579459). The sequence prediction was carried out using the GenScan software and results showed that the 1344bp, 1236bp and 1590bp cDNA sequences represent three single genes, which encoded 447,411,529 amino acids, respectively. Finally these three novel sheep genes were assigned to GeneIDs: 100145857, 100145858 and 100145856.

Further BLAST analysis of these proteins revealed that the sheep BCKDHA has high homology with the Branched chain keto acid dehydrogenase E1, alpha polypeptide BCKDHA of five species: chimpanzee (93%), human (96%), crab-eating macaque (93%), bovine (98%) and mouse (91%). The sheep NAGA gene has high homology with the alpha-N-acetylgalactosaminidase(NAGA) of five species-human (85%), bovine (94%), mouse (91%), rat (83%), and chicken (74%). The sheep HEXA has high homology with the hexosaminidase A HEXA of five species-cattle (98%), human (84%), Bornean orangutan (84%), rat (80%), and mouse (81%).

Based on the results of the alignment of BCKDHA, NAGA and HEXA, the phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software (<http://align.genome.jp/>), as shown in Fig. 5, Fig. 6, Fig. 7.

Fig. 5: The phylogenetic tree for seven kinds of BCKDHA.

Fig. 6: The phylogenetic tree for six kinds of NAGA.

Fig. 7: The phylogenetic tree for six kinds of HEXA.

The phylogenetic tree analysis revealed that the sheep BCKDHA, NAGA, and HEXA all have closer genetic relationships to the BCKDHA, NAGA, and HEXA of the bovine.

Tissue expression profile

Tissue expression profile analysis was carried out and results revealed that the sheep BCKDHA gene was moderately expressed in the liver, large intestine, lung and muscle, weakly expressed in the small intestine, kidney, fat and spleen. The sheep NAGA gene was highly expressed in the large intestine, moderately in muscle and the spleen, and weakly expressed in the liver, small intestine, lungs, fat, and the kidney. The sheep HEXA gene was highly expressed in fat, muscle and the kidney, moderately expressed in the liver, small intestine and spleen, and hardly expressed in the large intestine and lung (**Fig. 8**).

Chinsky (1998) reported the presentation of maple syrup urine disease associated with a nonsense mutation (R242X) in coding region of BCKDHA gene. To NAGA gene, mutation p.D217N

(c.649G>A) in exon 6, mutation p.E325K (c.973G>A) in exon 8, mutations of R329Q and R329W, may have roles in alpha-N-acetylgalactosaminidase deficiency with cardiomyopathy (Chabás et al., 2007; Kanekura et al., 2005). For HEXA gene, a substitution of cytosine 1351 by guanosine (C1351G) and other mutations have been also reported to be associated with Tay-Sachs disease (Karpati et al., 2004; McGinniss et al., 2002). Therefore, isolation of the encoding regions of the sheep BCKDHA, NAGA and HEXA genes is utmost important to detect these three kinds of diseases.

Fig. 8: Tissue expression distribution of sheep BCKDHA, NAGA, and HEXA genes.

From the alignment analyses of the BCKDHA, NAGA and HEXA proteins, there were no differences found at the above loci in humans, sheep, mice and other mammals, but many other amino acid differences were found in these three kinds of proteins. This implies that in future research of these three genes, attention should be given to whether these differences in amino acids and reported human mutations also occur in sheep and the potential associations of them to sheep diseases.

From the phylogenetic tree analysis, we found that sheep BCKDHA, NAGA and HEXA genes have much closer relationships to human than to mouse genes. This suggests that sheep would be more suitable as a model animal to study these three kinds of human mutation

diseases. In other words, theoretically, sheep are also animals susceptible to these diseases.

We also noted that human and mouse BCKDHA, NAGA and HEXA genes are expressed in most tissues (<http://www.ncbi.nlm.nih.gov/UniGene>). From the tissue distribution analysis in our experiment it can be seen that these genes were obviously differentially expressed in some tissues and there was no expression in some tissues. As we have not yet studied functions or protein levels, there might be many possible reasons for differential expression of these three sheep genes. The best explanation for this under current conditions is that the biological activities related to the mRNA expression of these genes were presented differently in different tissues.

The mutations of these three human or mouse genes at some loci are related to three types of important inherited diseases to which sheep are also susceptible. Thus, detection of the mutations of these three sheep genes and the association of these potential mutations with corresponding sheep diseases will be of interest beyond the importance to sheep production. Therefore, more research based on these preliminary foundations is needed.

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