Molecular identification, sequence analyses, tissue expression profile of a novel cattle gene-GBE1 in Yunnan cattle

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ABSTRACT

The complete coding sequences of a cattle gene - the glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme), GBE1, was amplified using the reverse transcriptase polymerase chain reaction (RT-PCR) based on the conserved coding sequence information of the human, mouse and highly homologous cattle ESTs. Sequence analysis revealed that cattle GBE1 has high homology with the GBE1 from six species: dog (92%), cat (91%), horse (91%), human (89%), mouse (88%) and rat (88%). The phylogenetic tree analysis revealed that the cattle GBE1 has closer genetic relationships with the GBE1 of dog and cat. Tissue expression profile analysis revealed that the cattle GBE1 gene was highly expressed in liver, moderately expressed in muscle and fat, weakly expressed in kidney, small intestine, large intestine, heart and lung. Our experiment established the primary foundation for further research on the cattle GBE1 gene.

KEY WORDS: cattle, GBE1, tissue expression

INTRODUCTION

The glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme), GBE1, is one important monomeric enzyme functions in glycogen synthesis by catalyzing the formation of alpha 1,6-glucosidic linkages. It is most highly expressed in liver and muscle. Mutations in the gene coding GBE1 enzyme can result in glycogen storage disease IV (Andersen's disease) (Chan et al., 1999;

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Ziemssen et al., 2000; McCarthy et al., 2003; Bruno et al., 2007; Fyfe et al., 2007). Andersen's disease always leaded to the infants death, muscle hypotonia, retarded growth, splenomegaly, and progressive hepatomegaly (Gómez-Garre et al., 2007; Miyahara et al., 2007).

Based on above described, GBE1 gene is associated with glycogen synthesis, mutation disease of human and animals such as horse (Ward et al., 2003). As same as mammalian, this inherited, recessive disease also maybe occur in cattle. If this is a fact, it is essential to isolate this gene from cattle for this disease, potentially, is related to the cattle production (glycogen synthesis, mutation disease). If this disease was found in cattle, cattle would be another important kind of animal model of human to study this disease. Althrough a partial encoding sequence of the cattle GBE1 gene had been deposited into the NCBI database (GeneBank number: XM 867634), until today, the cattle GBE1 has not been reported yet.

In this study we isolated the coding sequence of cattle GBE1 gene, subsequently performed some necessary sequence analyses and tissue expression profile analysis for this gene. This will establish the primary foundation of understanding this cattle gene.

MATERIAL AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis

The tissue samples of muscle, heart, liver, fat, kidney, lung, small intestine, large intestine, were derived from five adult Yunnan local cattles (all about 3 years old, female, healthy, housed in one cote and freely graze). Total RNA extraction and first-strand cDNA synthesis for these tissue samples were performed as the methods described by Liu et al. (2004).

Isolation of the cattle GBE1 gene

The RT-PCR was performed to isolate this cattle gene using the pooled cDNAs from different tissues mentioned above. The 25 μ l reaction system was: 2.0 μ l cDNA(100 ng/ μ l), 2.5 μ l 2 mM mixed dNTPs, 2.5 μ l 10×Taq DNA polymerase buffer, 2.5 μ l 25 mM 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 (1U/1 μ l), and 9.5 μ l sterile water. The primers for cattle GBE1 gene isolation was designed based on the conserved CDS sequences information from human and mouse GBE1 gene (GeneBank numbers: L07956.1 and BC017541) and their highly homologous cattle EST sequences (GeneBank numbers: EE348093, DV917642, DV775425 and

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DY458736). The primer sequences and their annealing temperature for RT-PCR reaction were described in Table 1. The PCR program initially started with a 94°C denaturation for 4 min, followed by 35 cycles of 94°C/1 min, Ta°C/1 min, 72°C/1 min, 72°C/1 min, then 72°C extension for 10 min, finally 4°C to terminate the reaction.

Gene	Primer sequence	Ta ∕°C
GBE1	Forward: 5' - ATGGCGGCTCCGGCAGGT-3' Reverse: 5' - TCAGTTGGGCAGGTCCAC-3'	57
G3PDH	Forward: 5' - ACCACAGTCCATGCCATCAC-3 Reverse: 5' - AAGAAGGTGGTGAAGCAGG-3	55

This PCR product for cattle GBE1 was then cloned into PMD18-T vector and sequenced bidirectionally with the commercial fluorometric method. At least five independent clones was sequenced.

RT-PCR for tissue expression profile analysis

RT-PCR tissue expression profile analyses was performed as previously described elsewhere (Liu et al., 2007). We selected the cattle housekeeping gene G3PDH (glyceraldehyde-3-phosphate dehydrogenase) (Accession number: AF077815) as the internal control. The control primers used are presented in Table 1. The PCR product is 148 bp in length. The primers of cattle GBE1 gene 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 reaction was optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The 25 µl reaction system was: 2 µl pooled cDNA of each tissue (100 ng/µl), 5 pM each oligonucleotide primer, 2.5 µl 2 mM mixed dNTPs, 2.5 µl 10×Taq DNA polymerase buffer, 2.5 µl 25 mM MgCl₂, 1.0 units of Taq DNA polymerase, and sterile water to the volume of 25 µl. The PCR program initially started with a 94°C denaturation for 4 min, 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://gene.mit.edu/GENSCAN.html). The protein prediction and analysis were performed using the Conservedd Domain Architecture Retrieval Tool of BLAST at the National Center for Biotechnology Information (NCBI) server

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(http://www.ncbi.nlm.nih.gov/BLAST) and the ClustalW software (http://www.ebi. ac.uk/clustalw).

RESULTS

cDNA amplification of cattle GBE1 gene. Through RT-PCR with pooled tissue cDNAs from muscle, heart, liver, backfat, kidney, lung, small intestine, large intestine, for cattle GBE1, the resulting PCR product was 2118 bp in length (Figure 1).



Figure 1. RT-PCR result for cattle GBE1. M, DL2000 DNA marker; 1, PCR product for cattle GBE1 gene

Sequence analysis. The cDNA nucleotide sequence analysis using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) revealed that this gene was not homologous to any of the known cattle gene and it was then deposited into the GenBank database (Accession number: EU442575). The sequence prediction was carried out using the GenScan software and results showed that the 2118bp cDNA sequence represented a single gene which encoded 705 amino acids. The complete CDS of this gene and the encoded amino acids was presented in Figure 2.

Further BLAST analysis of this protein revealed that cattle GBE1 has high homology with the glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme), GBE1, from six species - dog (92%), cat (91%), horse (91%), human (89%), mouse (88%) and rat (88%) (Figure 3).

Based on the result of the alignment of GBE1 the phylogenetic tree was constructed using the ClustalW software (http://www.ebi.ac.uk/clustalw), as shown in Figure 4. The phylogenetic tree analysis revealed that the cattle GBE1 has closer genetic relationship with the GBE1 of dog and cat.

Tissue expression profile. Tissue expression profile analysis was carried out and results revealed that, compared to G3PDH expression, the cattle GBE1 gene was highly expressed in liver, moderately expressed in muscle and fat, weakly expressed in kidney, small intestine, large intestine, heart and lung (Figure 5).

ATGGCGGCTCCGGCAGGTCTCCCGGCTGAGGCGGCTGGGGCCGAATGCTCTGAGGAGGCGCTAGCC G L Р E А G Α E C GCTCCGGĂCTŤCCĂGCGCĂGGTĂTAĂGCĜGTŤTAĂCCĂGAČTTTGAČCGĂCATTGGĂGAGAATGAA D F 0 R R K R F N т I т D 0 G **GGTGGTATTGATAGGTTTTCCAGAGGTTATGAATCATTTGGCGTCCACAGATGCGCTGATGGTGGTTTA** IDR F S R G Y E S F G V H R C G Α D G G TACTGCAAAGAATGGGCCCCAGGAGCTGAAGGAGTTTTTCTTACTGGAGACTTCAATGATTGGAAC w A P FND K F G Α EGVEL TGD w CCATTTTCATACCCATATAAAAAACTGGATTATGGAAAATGGGAGCTGTATATCCCACCAAAGCAAAAT F D G K W E Y L 1 K AGATCTGTCTTAGTACCTCATGGATCCAAACTAAAGCTTGTTATCAGGAGTAAAAGTGGAGAAATCTTG Р НG v L. v S KI K 1 I R S К S G F TACCGTATTTCACCATGGGCGAAGTATGTGACTCGTGAAGGCAGTAATGTGAACTATGATTGGATACAG W S V TREGSN R Р K V N Y D w 1 TGGGATCCAGAATACTCGTATAAAATTTAAACATTCCAAAACCAAAAGAAGCCAAAAGGTCTACGAATTTAT Р E F P ĸ K H S к K K P K G Ι. R I GAATCTCACGTGGGAATTTCTTCCTATGAAGGAAAAATAGCTTCTTATAAACATTTTACATGCAATGTA S H V GIS S Y E GKIASYKH FΤ N C CTACCAAGAATCAAAGACCTTGGATATAATTGCATTCAGTTGATGGCGATCATGGAACATGCTTACTAT L P R I K D L G Y N C I Q L M A I M E H A Y Y GCCAGTTTTGGTTACCAAATCACAAGCTTCTTTGCAGCTTCAAGCCGTTATGGAACTCCTGAAGAG F 0 т G I SFFA Α S S R Y G Р F CTGAAAGAACTGGTTGATACAGCCCATTCCATGGGTATTACAGTGCTCTTAGATGTGGTACACAGCCAT L K E L V D T A H S M G I T V L L D V V H S H GCCTCAAAAAATTCAGAAGATGGACTAAATATGTTTGACGGGACTGAATCCTGTTACTTTCATTATGGA F D G L N Μ D G E C $\begin{array}{c} CCTAGAGGGACTCATCTTCTATGGGATAGTCGGTTATTTGCCTACGCCAGCTGGGAAGTTTTAAGATTT\\ P & G & T & H & L & W & D & S & R & L & F & A & Y & A & S & W & E & V & L & R & F \\ \end{array}$ CTTCTGTCAAACATAAGATGGTGGTTGGAAGAGTATGGCTTTGATGGATTTCGTTTTGACGGTGTTACA W W N R L E E Y G F D G F R D G TCAATGCTCTATCATCACCATGGAATAGGTGAGAATTTTTCAGGTGATTACCATGAGTATTTTGGACTA M н Ι. н н G 1 G E N F S G D v н F CAAGTAGATGAAGATGCTTTGACTTACATCATGTTGGCAAATCATTTGGTTCACACACTGTATCCAGAT V D E ΥI D LT Μ LANHL V Т н L Y Р D E D V S G M Р I C S р S A 0 G G GGTTTTGACTATCGATTGGCTATGGCAATTCCAGATAAATGGATCCAGTTACTGAAAGÀATATAAAGAT Р R G F D Y L A M A D K W I 0 L - L. F GAAGATTGGAACÄTGĞGGAATÄTAGTGTATACACTCÄCAÄACCGĂCGČTAČCTŤGAĂAAGTĞCATT E D W N M G N I V Y T L T N R R Y L E K C I GCCTACGCAGAGAGCCATGATCAGGCACTTGTTGGGGATAAGACGTTGGCATTTTGGTTGATGGAT Н 0 L v D Α G K L Α F W M I... GCTGAAATGTATACCAACATGAGCGTTCTGACCCCATTTACTCCCGTTATTGATCGTGGAATACAGCTT A E M Y T N M S V L T P F T P V I D R G I Q L CATAAGATGATTCGACTCATTACTCACGCACTCGGTGGAGAGGGGGTATCTCAATTTCATGGGTAATGAA H Μ ІТНА LGGEGYLNF M G E TTTGGGCATCCTGAATGGTTAGACTTCCCCAGAAAAGGAAATAATGAGAGCTACCATTATGCACGA G н F w L. D F R ĸ N N E S AAGCAGTTTCATTTAACTGATGATGACCTTCTTCGCTATAAGTTCCTAAATAACTTTGACAGGGATATG Κ L E E R G W L S Р 0 Н C Α Α v E Α FΕ R A S L LF I F N F н P TATACTGATTACCGAGTTGGAACAACATTGCCAGGAAAGTACAAAATTGTGCTCGATTCAGATGCA Υ R V G T T L P G K Y K I V L Т D D S D ġĊĂĠĂĂŤĂĊĠĠĂĠĠĂĊĂĊĂĂĠĂĠĂĊŦĠĠĂĊĊĂĊĂĂŤĂĊĊĠĂĂŤŤĊŦŤĊŦĊŦĠĂĂĊČŦŦŤŦĠĂĂĊĂŤ G н K R D Н N F G L. T E F F F F н AATAACTGTCCCTGTTCTCTTTTGGTGTACATTCCAAACCGAGTGGCCCTCATCCTTCGCAATGTGGAC N C Р С S LL V Y I P NR V Α L I L R CTGCCCAACTGA LPN*

Figure 2. The complete coding sequence of cattle GBE1 gene and its encoding amino acids * indicates the stop codon

Dog	
Cat	
Cat	RAAPVARGECSEARLAAALADVPELARLLELDPYLKPFALDFQRRYKKFNEILN
Cattle	MAAPAGLPAEAAGAECSEEALAAALADVPELARLLETDPYLKPYAPDFQRRYKRFNQTLT
Horse	MAAPAARADGSDAALAAALADVPDLGRLLEVDPYLKPYAPDFQRRYNRFSQTLD
Human	MAAPMTPAARPEDYEAALNAALADVPELARLLEIDPYLKPYAVDFORRYKOFSOILK
Mouse	MAAPAAPAAGETGPDARLEAALADVPELARLLETDPYLKPFAADFORRYKKFSOVLH
Det	
Ruc	
	· · · · · · · · · · · · · · · · · · ·
Dog	NIGENEGGIDKFSRGYESFGVHRCADGGLYCKEWAPGAEGVFLTGDFNDWNPFSYPYKKL
Cat	NIGENE GGIDKFSRGYESFGVHRCADGGLYCKEWAPGAE GVFLTGD FNDUNPFSYPYKKL
Cattle	DIGENFEGIDDESDEVESEGVHDCADEELVCVFMADEAFEVELTEDENDINDESVDVVVI
Horse	MICHAEGEDUESDOVESEGUNDCADGELVCVENADCAEGUETCOEMDMIDESSUDVIC
Numer	NIGENEGGIDET SKOLEST GVIRCAD GOLI CREWAT GALGVIT LIGDTNDWNTT SITTIKL
	NIGENEGGIDKFSRGTESFGVIRCADGGLICKEWAPGALGVFLIGDFNGWNPFSIPIKKL
nouse	DIGENEGGIDKFSRGYESFGIHRCSDGGIYCKEWAPGAEGVFLTGEFSGWNPFSHPYKKL
Rat	DIGENEGGIDKFSRGYESFGIHRCSDGGIYCKEWAPGAEGVFLTGEFSGWNPFSHPYKKL
	: **: *****: ********: ***: ***: ***: ******
Dog	DYGKWELYIPPKQNKSLLVPHGSKLKVVIRSKSGEILYRISPWAKYVTREGDNVNYDWIH
Cat	DYGKWELYI PPKONKSOLYPHGSKLKVY I BSKSGEI LYBI SPMAKYVTBEGENVNYDHTH
Cattle	DYGKWFLYT PPKONDSYLYPHGSKLKLYT PSKSGFTLYD T SPWAKYYT DFGSNYNYDWT O
Horse	DECEMPT AT DEPARTY I ADDRESS INVESTIGATION OF THE DEPARTMENT OF THE DEPARTMENT. THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT. THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT. THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT. THE DEPARTMENT OF THE DEPARTMENT OF THE DEPARTMENT. THE DEPARTMENT. THE DEPARTMENT. THE DEPARTMENT.
IIOLSE IIonse	DIGKWDLIIFFKFNKSLLVFNGSKLKVVIKSKSGEILIKISFWAKIVVRESGNVNIDWIN
Human	DYGKWELYIPPKUNKSVLVPHGSKLKVVITSKSGEILYRISPWAKYVVREGDNVNYDWIH
Mouse	EYGKWELYIPPKQNKSPLIPHGSKLKVVITSKSGEILYRISPWAKYVVRENNNVNYDWIH
Rat	EYGKWELYIPPKQNKSPPIPHGSKLKVVITSKSGEILYRISPWAKYVVRENNNVNYDWIH

Dog	
Con	
Lac .	WDPEHPYKFKHSKPKKPKGVRI IESHVGISSIEGKIASIKHFTINVLPRIKDLGINCIQM
Cattle	WDPEYSYKFKHSKPKKPKGLRIYESHVGISSYEGKIASYKHFTCNVLPRIKDLGYNCIQL
Horse	WDPEQPYKFKHSRPKKPRSLRIYESHVGISSHEGKIASYKHFTCNVLPRIKGLGYNCIQM
Human	WDPEHSYEFKHSRPKKPRSLRIYESHVGISSHEGKVASYKHFTCNVLPRIKGLGYNCIQL
Mouse	WAPED PYKFKHSRPKKPRSLRIYE SHVGISSHEGKIASYKHFTSNVL PRIKDLGYNCIOL
Rat	WD PENPYK FRHSR PKK PRSLRTYFSHVGTSSHFGKTASYKHFTSNVL PRTKDLGVNCTOL
pog	MAIMEHAYYASFGYQITSFFAASSRYGTPEELKELIDTAHSMGITVLLDVVHSHASKNSE
Cat	MAIMEHAYYASFGYQITSFFAASSRYGTPEELKELVDTAHSMGITVLLDVVHSHASKNSE
Cattle	MAIMEHAYYASFGYQITSFFAASSRYGTPEELKELVDTAHSMGITVLLDVVHSHASKNSE
Horse	MAIMEHAYYASFGYQITSFFAASSRYGTPEELKELVDTAHSMGITVLLDVVHSHASKNSE
Human	MAIMEHAYYASFGYOITSFFAASSRYGTPEELOELVDTAHSMGIIVLLDVVHSHASKNSA
Mouse	MAINFHAVVASFGVOITSFFAASSDVGTDFFLEFLEDINGHSMGTWULLDWUNSF
Pat	MATMENAVVASECVOVISERAASSOVCIDERIVEI UDIALISUUVI JED VIIMIRANAS
Nac	ARTICLARI INSTOLUCIST FARSSKIDT FEELKE LVD TATLAGI VVLDVVISAASKNSE
	······································
pog	DGLNMFDGIDSCYFHSGPRGNHDLWDSRLFAYSSWEVLRFLLSNIRWWLEEYYFDGFRFD
Cat	DGLNMFDGTDSCYFHSGPRGNHDLWDSRLFIYSSWEVLRFLLSNIRWWLEEYGFDGFRFD
Cattle	DGLNMFDGTESCYFHYGPRGTHLLWDSRLFAYASWEVLRFLLSNIRWWLEEYGFDGFRFD
Horse	DGLNMFDGTDSCYFHSGPRGTHDLWDSRLFIYSSWEVLRFLLSNIRWWLEEYGFDGFRFD
Human	DGLNMFDGTDSCYFHSGPRGTHDLWDSRLFAYSSWEVLRFLLSNIRWWLEEYRFDGFRFD
Mouse	DGLNMFDGTDSCYFHSGPRGTHDLWDSRLFTYSSWFYLRFLLSNTRWULFFYCFDGFPFD
Pet	DGI MMEDCTDSCVENSCHOCTADI MDSCALL TYSSUEVI DEL I SWIDDUI EFYCEDCEDCED
nuc	Velantrolid Sciringer Chindre Scirit 153 Control Lantrow Lee I Crograd
Dog	
bog	GVISMLYHHHGMGEGFSGDYHEYFGLUVDEDALVYLMLANHLVHTLYPDSITVAEDVSGM
Cat	GVTSMLYHHHGMGQAFSGDYHEYFGLQVDEDALIYLMLANHLVHTLYPNSITIAEDVSGM
Cattle	GVTSMLYHHHGIGENFSGDYHEYFGLQVDEDALTYIMLANHLVHTLYPDSITIAEDVSGM
Horse	GVTSMLYHHHGIGASFSGDYHEYFGLQVDEDALTYLMLANHLVHTLYPDSITIAEDVSGM
Human	GVTSMLYHHHGVG0GFSGDYSEYFGL0VDEDALTYLMLANHLVHTLCPDSITIAEDVSGM
Mouse	GYTSMI, VHHHGWGGGEFSGDVNFVFGI, GYDFDAL, TVI MI, ANHI, AHTI VDDSTTI AFDVSGW
Det	CVTSWI WHNIGWGGESGDVSFVEGI OVDEDA I VVI WIANNA TITAVDSTTI AEDVSGN
Rac	GVISHLINNGGGFSGDISEIFGLUVDEDALVILHLANNLININIPDSIIIAEDVSGM
Dog	
500	FALCSFISGOGGED YKLAMAI PDKWIQLLKEFKDEDWNMGNI VYTLTNRRYLEKCIAYA
Lat	PALUSPISUGGVGFDYRLAMAIPDKWIQLLKEFKDEDWNMGNIVYTLTNRRYLEKCIAYA
Cattle	PALCSPISQGGGGFDYRLAMAIPDKWIQLLKEYKDEDWNMGNIVYTLTNRRYLEKCIAYA
Horse	PALCSPISQGGGGFDYRLAMAIPDKWIQLVKEFKDEDWNMGNIVYTLTNRRHLEKCIAYA
Human	PALCSPISQGGGGFDYRLAMAIPDKWIQLLKEFKDEDWNMGDIVYTLTNRRYLEKCIAYA
Mouse	PALCSPTSOGGGGFDYRLAMAIPDKWIOLLKEFKDEDWNMGNIVVTLTNRRVLFKCVAVA
Mouse Rat	PALCSPTSQGGGGFDYRLAMAIPDKUIQLLKEFKDEDUNMGNIVYTLTNRRYLEKCVAYA PALCSPTSQGGGGFDYRLAMAIPDKUIQLLKEFKDEDUNMGNIVYTITNPBHIFKCVAYA
Mouse Rat	PALCSPTSQGGGGFDYRLAMAIPDKWIQLLKEFKDEDWNMGNIVYTLTNRRYLEKCVAYA PALCSPTSQGGGFDYRLAMAIPDKWIQLLKEFKDEDWNMGNIVYTLTNRRHLEKCVAYA

Figure 3. The alignment of the proteins encoded by GBE1 genes of cattle and other six species (continued on the page 497)

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Dog	ESHDQALVGDKTLAFWLMDAEMYTNMSVLTPFTPVIDRGIQLHKMIRLITHALGGEGYLN
Cat	ESHDQALVGDKTLAFWLMDAEMYTNMSVLTPFTPVIDRGIQLHKMIRLITHALGGEGYLN
Catle	ESHDQALVGDKTLAFWLMDAEMYTNMSVLTPFTPVIDRGIQLHKMIRLITHALGGEGYLN
Horse	ESHDQALVGDKSLAFWLMDAEMYTNMSVLTPFTPVIDRGIQLHKMIRLITHALGGEGYLN
Human	ESHDQALVGDKSLAFWLMDAEMYTNMSVLTPFTPVIDRGIQLHKMIRLITHGLGGEGYLN
Mouse	ESHDQALVGDKTLAFWLMDAEMYTNMSVLAPFTPVIDRGIQLHKMIRLITHGLGGEGYLN
Rat	ESHDQALVGDKTLAFWLMDAEMYTNMSVLAPFTPVIDRGIQLHKMIRLITHGLGGEGYLN
Dog	FMGNE FGHPEWLD FPRKGNNE SYHYARROFHLTDDD LLRYKFLNN FDRDMNKLEERC GWL
Cat	FMGNE FGHPEWLD FPRKGNNE SYHYARROFHLTDDD LLRYKFLNN FDRDMNKLEERC GWL
Cattle	FMGNE FGHPEWLD FPRKGNNE SYHYARROFHLTDDD LLRYKFLNN FDRDMNKLEERC GWL
Horse	FMGNE FGHPEWLD FPRKGNNE SYHYARROFHLTDDD LLRYKFLNN FDRDMNRLEERC GWL
Human	FMGNE FGHPEWLD FPRKGNNE SYHYARROFHLTDDD LLRYKFLNN FDRDMNRLEERC GWL
Mouse	FMGNE FGHPEWLD FPRKGNNE SYHYARROFNLTDDD LLRYKFLNN FDRDMNRLEERC GWL
Rat	FMGNE FGHPEWLD FPRKGNNE SYHYARROFNLTDDD LLRYKFLNN FDRDMNRLEERC GWL
Dog Cat Catle Horse Human Mouse Rat	SAPQAYVSEKHEGNKIIAFERAGLLFIFNFHPSKSYTDYRVGTTLPGKYPFCCCRIIVLD SAPQAFVSEKHEGNKIIAFERAGLVFIFNFHPSKSYTDYRVGTTLPGKYF
Dog	TDAAEYGGHQRLDHNTDFFSEDFKHNERPFSLLVYIPSRVGLILQNVDMPN
Cat	TDAAEYGGHQRLDHSTEFFSQPFKHNERPCSLLVYIPNRVGLILQNVDMPN
Catle	SDAAEYGGHKRLDHNTFFFSEPFEHNNCPSLLVYIPNRVALILQNVDLPN
Horse	SDAAEYGGHQRLDHNTDFFSEPFEHNRCPSSLLVYIPSRVALILQNVDLPN
Human	SDAAEYGGHQRLDHNTNYFAEAFEHNGRPYSLLVYIPSRVALILQNVDLPN
Mouse	SDAAEYGGHQRLDHNTNYFAEAFEHNGRPYSLLVYIPSRVALILQNVDLPN
Rat	SDAAEYGGHQRLDHNTNYFAEAFEHNGRPYSLLVYIPSRVALILQNVDLPN

Figure 3. continued



Figure 4. The phylogenetic tree for seven kinds of GBE1



Figure 5. Tissue expression of cattle GBE1 gene. The G3PDH expression is the internal control

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DISCUSSION

Tay et al. (2004) had reported two single deletions (one in exon 10 and another in exon 12) in the CDS of GBE1 gene were associated with the human Andersen's disease. Wagner et al. (2006) also had reported a nonsense mutation in codon 34 of the GBE1 gene was associated with the horse Andersen's disease. Therefore, isolation of the coding regions of the GBE1 gene is utmost important to study this kind of disease.

Comparative genomics is the analysis and comparison of genomes from different species. Researchers have learned a great deal about the function of human gene by examining their counterparts in simpler model organisms such as the mouse and some results has revealed that virtually all (99%) of the protein-coding gene in humans align with homologues in mouse, and over 80% are clear 1:1 orthologs (Hardison et al., 2003). This extensive conservation in protein-coding regions implied that this conservation of protein-coding sequences may be expected in different mammals including cattle. From the isolation of cattle GBE1 gene, we can find that cattle GBE1 is highly homologous with GBE1 of human, mouse and other mammals. This further validated that comparative genomics method is one useful tool to isolate the unknown gene especially the conserved coding region of gene for cattle.

From the alignment analyses for GBE1 proteins we found that no differences were detected at above mentioned loci associated with the glycogen storage disease IV in human, cattle, mouse and other mammals. This implied that the polymorphism of GBE1 gene at above mentioned loci may not depend on species but on individuals. These also implied that in the future research of this new gene, we should pay more attentions to that if above reported mutations would also happen in different cattle individuals and the potential associations of them with the cattle glycogen storage disease IV. For only five cattles were studied in this experiment, we think, more cattles are needed to study for the further detection.

The phylogenetic tree analysis revealed that the cattle GBE1 has closer genetic relationship with the GBE1 of dog and cat. This implied that dog and cat should be better animal models to study this gene of cattle than others.

From the tissue expression profile analysis in our experiment it can be seen that this gene was obviously differentially expressed in some tissues and highly expressed in liver, moderately expressed in muscle and fat. These agree to the results obtained by Fyfe et al. (2007).

CONCLUSIONS

In conclusion, we first isolated the cattle GBE1 gene and performed some necessary analyses. This established the primary foundation for further research on this gene in cattle.

REFERENCES

- Bruno C., Cassandrini D., Assereto S., Akman H. O., Minetti C., Di Mauro S., 2007. Neuromuscular forms of glycogen branching enzyme deficiency. Acta Myol. 26, 75-78
- Chan Y.J., Lin S.P., Chen B.F., 1999. Glycogen storage disease type IV: a case report. Zhonghua Yi Xue Za Zhi (Taipei) 62, 743-747
- Fyfe J.C., Kurzhals R.L., Hawkins M.G., Wang P., Yuhki N., Giger U., Van Winkle T.J., Haskins M.E., Patterson D.F., Henthorn P.S., 2007. A complex rearrangement in GBE1 causes both perinatal hypoglycemic collapse and late-juvenile-onset neuromuscular degeneration in glycogen storage disease type IV of Norwegian forest cats. Mol. Genet. Metab. 90, 383-392
- Gómez-Garre P., Gutiérrez-Delicado E., Gómez-Abad C., Morales-Corraliza J., Villanueva V.E., Rodríguez de Córdoba S., Larrauri J., Gutiérrez M., Berciano J., Serratosa J.M., 2007. Hepatic disease as the first manifestation of progressive myoclonus epilepsy of Lafora. Neurology 68, 1369-1373
- Hardison R.C., 2003. Comparative genomics. PLoS Biol. 1, E58 Vol. 1, No. 2, e58 doi:10.1371/ journal.pbio.0000058
- Liu G.Y., Xiong Y.Z., 2007. Isolation, sequence analysis and expression profile of a novel cattle gene, NIP7, differentially expressed in the Longissimus dorsi muscle tissues from Meishan, Meishan x Large White cross and Large White cattles. Mol. Biol. Rep. 34, 213-219
- Liu Y.G., Xiong Y.Z., Deng C.Y., Zuo B., Zhang J.H., 2004. Comparison of gene expression patterns in Longissimus dorsi of cattles between the high-parent heterosis cross combination Landrace×Large White and the mid-parent heterosis cross combination Large White×Meishan. Asian-Austr. J. Anim. Sci. 17, 1192-1196
- McCarthy J.J., Meyer J., Moliterno D.J., Newby L.K., Rogers W.J., Topol E.J., 2003. GenQuest multicenter study. Evidence for substantial effect modification by gender in a large-scale genetic association study of the metabolic syndrome among coronary heart disease patients. Hum. Genet. 114, 87-98
- Miyahara A., Sugie H., 2007. Anderson disease/chylomicron retention disease. Nippon Rinsho 65, 597-599
- Tay S.K., Akman H.O., Chung W.K., Pike M.G., Muntoni F., Hays A.P., Shanske S., Valberg S.J., Mickelson J.R., Tanji K., DiMauro S., 2004. Fatal infantile neuromuscular presentation of glycogen storage disease type IV. Neuromuscular Disord. 14, 253-260
- Wagner M.L., Valberg S.J., Ames E.G., Bauer M.M., Wiseman J.A., Penedo M.C., Kinde H., Abbitt B., Mickelson J. R., 2006. Allele frequency and likely impact of the glycogen branching enzyme deficiency gene in Quarter Horse and Paint Horse populations. J. Vet. Intern. Med. 20, 1207-1211
- Ward T.L., Valberg S.J., Lear T.L., Guérin G., Milenkovic D., Swinburne J.E., Binns M.M., Raudsepp T., Skow L., Chowdhary B.P., Mickelson J.R., 2003. Genetic mapping of GBE1 and its association with glycogen storage disease IV in American Quarter horses. Cytogenet. Genome Res. 102, 201-206
- Ziemssen F., Sindern E., Schröder J.M., Shin Y.S., Zange J., Kilimann M.W., Malin J.P., Vorgerd M., 2000. Novel missense mutations in the glycogen-branching enzyme gene in adult polyglucosan body disease. Ann. Neurol. 47, 536-540