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Molecular cloning, sequence characterization, and gene expression profiling of a novel water buffalo (*Bubalus bubalis*) gene, *AGPAT6*

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ABSTRACT. Several 1-acylglycerol-3-phosphate-*O*-acyltransferases (AGPATs) can acylate lysophosphatidic acid to produce phosphatidic acid. Of the eight AGPAT isoforms, AGPAT6 is a crucial enzyme for glycerolipids and triacylglycerol biosynthesis in some mammalian tissues. We amplified and identified the complete coding sequence (CDS) of the water buffalo *AGPAT6* gene by using the reverse transcription-polymerase chain reaction, based on the conversed sequence information of the cattle or expressed sequence tags of other Bovidae species. This novel gene was deposited in the NCBI database (accession No. JX518941). Sequence analysis revealed that the CDS of this *AGPAT6* encodes a 456-amino acid enzyme (molecular mass = 52 kDa; pI = 9.34). Water buffalo AGPAT6 contains three hydrophobic transmembrane regions and a signal 37-amino acid peptide, localized

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in the cytoplasm. The deduced amino acid sequences share 99, 98, 98, 97, 98, 98, 97 and 95% identity with their homologous sequences from cattle, horse, human, mouse, orangutan, pig, rat, and chicken, respectively. The phylogenetic tree analysis based on the *AGPAT6* CDS showed that water buffalo has a closer genetic relationship with cattle than with other species. Tissue expression profile analysis shows that this gene is highly expressed in the mammary gland, moderately expressed in the heart, muscle, liver, and brain; weakly expressed in the pituitary gland, spleen, and lung; and almost silently expressed in the small intestine, skin, kidney, and adipose tissues. Four predicted microRNA target sites are found in the water buffalo *AGPAT6* CDS. These results will establish a foundation for further insights into this novel water buffalo gene.

Key words: Water buffalo; Isolation; Bioinformatic analysis; 1-Acylglycerol-3-phosphate-*O*-acyltransferase 6 (AGPAT6); Tissue expression profile

INTRODUCTION

The *sn*-1-acylglycerol-3-phosphate-*O*-acyltransferase (AGPAT) enzyme is crucial in *de novo* triacylglycerol synthesis in eukaryotes (Takeuchi and Reue, 2009). It catalyzes the second step by acylating lysophosphatidic acid to phosphatidic acid (Aguado and Campbell, 1998; Coleman and Lee, 2004; Ye et al., 2005; Agarwal et al., 2006, 2007; Nagle et al., 2008; Sukumaran et al., 2009). So far, eight members of the *AGPAT* gene family have been described in humans, which are *AGPAT1-8*, and each of them possesses a lysophosphatidic acid acyltransferase motif (Ye et al., 2005; Nagle et al., 2008). As a member of the AGPAT family, AGPAT6 appears to play a key role in lipid biosynthesis (Chen et al., 2008). After careful examination of the AGPAT enzyme activity by Chen et al. (2008) and Nagle et al. (2008), the enzyme was recognized as another endoplasmic reticulum-localized glycerol phosphate acyltransferase (GPAT) and renamed as *GPAT4*. The *AGPAT6* gene has recently been identified in humans, mice, cattle, and chickens (Li et al., 2003; Chen et al., 2008; Zimin et al., 2009). In both human and mouse, the *AGPAT6* gene consists of 13 exons, whereas that in cattle and chicken contains 12 exons. The AGPAT6 protein contains 456 amino acids (aa) in the species mentioned above, except chicken, in which it is composed of 455 aa (Li et al., 2003; Beigneux et al., 2006; Chen et al., 2008; Nagle et al., 2008).

Study using *AGPAT6*-deficient mice showed that the milk produced from them was markedly depleted in diacyglycerols and triacylglycerols, and *AGPAT6* is crucial for the production of milk fat by the mammary gland (Beigneux et al., 2006). The polymorphisms of *AGPAT6* were highly significantly associated with the milk fat percentage estimated breeding values in the German Holstein-Friesian population, as shown in a recent research (Wang et al., 2012). The result of a study by Nafikov (2010) showed that *AGPAT6* was associated significantly with large differences in the compositions of milk fat, such as concentrations of saturated fatty acids, unsaturated fatty acids, monounsaturated fatty acids, and so on.

AGPAT6, which was identified on *Bos taurus* autosome 27, is a pivotal gene related to catalytic biosynthesis of glycerolipids. It coordinately regulates the channeling of fatty acids toward copious milk fat synthesis in bovine mammary (Bionaz and Loor, 2008a). Therefore, *AGPAT6* can be considered as a potential gene involved in regulating the milk fat composition in dairy cattle. Water buffalo contributes significantly to the agricultural economy and dairy industry in the tropical and subtropical countries (Singh et al., 2000; Khan et al., 2011; Perera, 2011). It is the second largest source of milk supply in the world, and buffalo milk contains less water and more fat, lactose, protein, and minerals than cow milk (Vijh et al., 2008; Mahmood and Usman, 2010; Yindee et al., 2010). However, the *AGPAT6* gene in water buffalo has not yet been isolated and characterized, and its tissue expression has not been clear to date. In the current study, we isolated the full-length coding sequence of the water buffalo *AGPAT6* gene, based on the reverse transcription-polymerase chain reaction (RT-PCR). We also analyzed its primary structure, and displayed the tissue distribution of its expression. The data obtained will serve as a basis for understanding the water buffalo *AGPAT6* gene.

MATERIAL AND METHODS

Sample collection, RNA extraction, and first-strand complementary DNA (cDNA) synthesis

The fresh tissue samples, which included the heart, pituitary gland, small intestine, muscle, spleen, liver, mammary gland, skin, lung, brain, kidney, and fat, were collected from three Binglangjiang water buffalo after they had been slaughtered. The samples were snap-frozen immediately in liquid nitrogen and then stored at -80°C before processing for RNA isolation. Total RNA was extracted using RNAiso Plus (TaKaRa, Dalian, China) according to the manufacturer instructions. To remove genomic DNA contamination, total RNA was digested with RNase-free DNase I (TaKaRa). The total RNA (1 µL) was checked by electrophoresis on a 2.0% agarose gel containing ethidium bromine. The RNA (3 µg) was reverse-transcribed with the oligo $(dT)_{18}$ primer and M-MLV reverse transcriptase (Invitrogen, USA).

Isolation of the water buffalo *AGPAT6* gene

The *AGPAT6* message RNA (mRNA) sequence for *B. taurus* (accession No. NM_001083669), available in the National Center for Biotechnology Information (NCBI) database, and its highly homologous expressed sequence tags were used to design a pair of primers to obtain the full-length coding regions of the *AGPAT6* gene. The primers amplified for *AGPAT6* are listed in Table 1. The PCR was performed to isolate the water buffalo *AGPAT6* gene using the pooled cDNAs from the different tissues mentioned above. The 25-µL reaction system contained 2.0 µL 50 ng/µL cDNA, 2.0 µL 2.5 mM dNTPs mixed (TaKaRa), 2.5 µL 10X *Taq* DNA polymerase buffer (Mg^{2+} Plus), 0.5 µL 10 µM forward primer, 0.5 µL 10 µM reverse primer, 0.25 µL 5 U/µL *Ex Taq* DNA polymerase (TaKaRa), and 17.5 µL sterile water. The amplification conditions were as follows: 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 2 min, and then a final extension at 72°C for 5 min. The amplified fragment was subcloned into the pMD18-T vector (TaKaRa) and then sequenced bidirectionally using the commercial fluorometric method. At least eight independent clones were sequenced.

Table 1. Primer set information for semi-quantitative RT-PCR.

Gene (GenBank No.)	Primers (5' to 3')[1]	Amplicon length (bp)	Annealing temperature (°C)	Usage
AGPAT6 (NM_001083669)	F: GGGATGCGAACTTGGGAATG R: ACTCAGCGGAAAGGGACACA	1585	55	CDS cloning
AGPAT6 (NM_001083669)	F: AACCTGCATCAATAATACATCA R: GGTAGGTCACCATTCCGTA	146	55	Expression
18S rRNA (JN412502)	F: GGACATCTAAGGGCATCACAG R: AATTCCGATAACGAACGAGACT	145	55	Expression

[1]Primer direction (F = forward; R = reverse).

Software for bioinformatic analysis

To predict the physical and chemical properties of the putative AGPAT6 protein, the software on the ExPASy server (http://au.expasy.org/) was used. The protein prediction and analysis were performed using the Conserved Domain Architecture Retrieval Tool of BLAST at the NCBI server (http://www.ncbi.nlm.nih.gov/BLAST). The ClustalW software (http://align.genome.jp/) was used for alignment of multiple sequences. The theoretical molecular weight (Mw) and isoelectric point (pI) of these deduced aa of AGPAT6 were computed using the Compute pI/Mw (http://us.expasy.org/tools/pi_tool.html) tool. Signal peptides were predicted using the ProP 1.0 server (http://www.cbs.dtu.dk/services/ProP/) and SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP-3.0/). PSort II (http://psort.hgc.jp/) was employed to predict protein sorting signals and intracellular localization. Secondary structures of deduced aa sequences were predicted by SOPMA (http://npsa-pbil.ibcp.fr/). Web-based microRNA (MicroRNA) predicting programs were used to locate conversed potential microRNA targets: miRBase (http://www.mirbase.org/). TMHMM Server version 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) was used to predict transmembrane helices in the proteins. A phylogenetic tree was generated based on *AGPAT6* nucleotide sequences by applying the neighbor-joining method in the ClustalX version 2.0 program, which subsequently subjects to be edited manually. Statistical significance of groups within phylogenetic trees was evaluated using the bootstrap method with 1000 replications.

Semiquantitative RT-PCR

To characterize the *AGPAT6* further, semiquantitative RT-PCR was conducted to determine its expression in 12 water buffalo tissues. To eliminate the effect of cDNAs concentration, we repeated the RT-PCR five times using 1, 2, 3, 4, and 5 μL cDNAs as templates, respectively. We tested the housekeeping gene 18S ribosomal RNA (JN412502) as a positive control. The details of semiquantitative RT-PCRs for *AGPAT6* and 18S rRNA amplification are listed in Table 1. PCR analyses were optimized for a number of cycles to ensure product identity within the linear phase of amplification.

RESULTS

Cloning and identification of water buffalo *AGPAT6* complete coding sequence

The PCR product was a 1585-bp-long fragment (Figure 1), which was consistent with

expectations. Homology analysis for the sequence obtained in this study was carried out using the BLAST software at the NCBI server. The results showed that the sequence was homologous to the known sequences of the *AGPAT6* gene in some species reported. The sequence was then submitted to the NCBI database (accession No. JX518941). The sequence prediction results from the GenScan software analysis showed that a 1371 bp coding sequence represented one single gene, which encoded 456 aa. The complete coding sequence (CDS) of the gene and the deduced aa are presented in Figure 2.

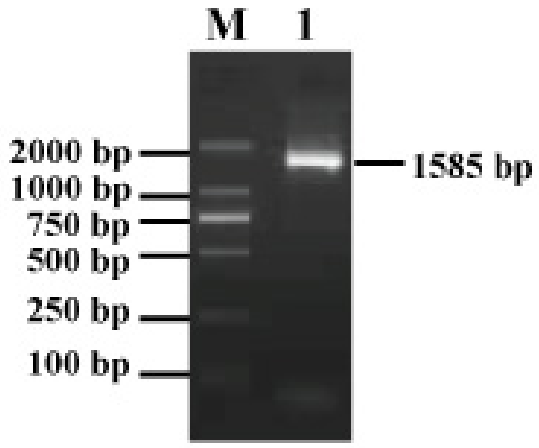

Figure 1. RT-PCR results for water buffalo *AGPAT6* gene. *Lane 1* = PCR product for water buffalo *AGPAT6* gene and *lane M* = DL2000 DNA markers.

```
1     ATGTTCCTGCTGCTGCCTTTCGACAGCCTGATTGTCAGCCTCCTGGGCATCTCCCTGACGGTCCTCTTCACCCTCCTGCTGGTTTTCATCATCGTCCCCGCCATT
1      M  F  L  L  L  P  F  D  S  L  I  V  S  L  L  G  I  S  L  T  V  L  F  T  L  L  L  V  F  I  I  V  P  A  I
106   TTCGGAGTCTCCTTTGGTATCCGAAAGCTCTACATGAAAACTCTGTTAAAGATCTTTGCGTGGGCTACCTTGAGGATGGAGAGAGGAGCCAAGGAGAAGAACCAC
36    F  G  V  S  F  G  I  R  K  L  Y  M  K  T  L  L  K  I  F  A  W  A  T  L  R  M  E  R  G  A  K  E  K  N  H
211   CAGCTTTACAAGCCCTACACCAATGGAATCATTGCAAAAGACCCCACGTCACTAGAGGAGGAGATCAAAGAAATCCGCCGGAGCGGGAGCAGTAAGGCCCTGGAC
71     Q  L  Y  K  P  Y  T  N  G  I  I  A  K  D  P  T  S  L  E  E  E  I  K  E  I  R  R  S  G  S  S  K  A  L  D
316   AACACTCCCGAGTTTGAGCTCTCGGACATTTTCTATTTCTGCCGGAAAGGAATGGAGACCATCATGGACGACGAGGTGACCAAGAGGTTCTCGGCAGAGGAGCTG
106    N  T  P  E  F  E  L  S  D  I  F  Y  F  C  R  K  G  M  E  T  I  M  D  D  E  V  T  K  R  F  S  A  E  E  L
421   GAGTCCTGGAACCTACTGAGCAGGACCAATTATAACTTCCAGTACATCAGCCTGCGGCTCACCGTGCTGTGGGGCTTGGGCGTACTCATCCGCTACTGCTTCCTG
141    E  S  W  N  L  L  S  R  T  N  Y  N  F  Q  Y  I  S  L  R  L  T  V  L  W  G  L  G  V  L  I  R  Y  C  F  L
526   CTGCCGCTCAGGATAGCTCTCGCTTTCACGGGGATCAGCCTCCTGGTGGTGGGCACAACGATGGTGGGGTACCTGCCAAACGGGAGGTTCAAGGAGTTCCTGAGC
176    L  P  L  R  I  A  L  A  F  T  G  I  S  L  L  V  V  G  T  T  M  V  G  Y  L  P  N  G  R  F  K  E  F  L  S
631   AAGCACGTTCACCTCATGTGCTACCGGATTTGCGTGCGCGCTCTGACGGCCATCATCACCTACCACGACAGGAAGAACCGGCCTAGAAACGGCGGCATCTGCGTG
211    K  H  V  H  L  M  C  Y  R  I  C  V  R  A  L  T  A  I  I  T  Y  H  D  R  K  N  R  P  R  N  G  G  I  C  V
736   GCTAACCACACGTCTCCCATTGACGTCATCATCCTGGCCAGCGACGGCTACTACGCCATGGTGGGGCAGGTGCACGGCGGCCTCATGGGAGTCATCCAGAGAGCC
246    A  N  H  T  S  P  I  D  V  I  I  L  A  S  D  G  Y  Y  A  M  V  G  Q  V  H  G  G  L  M  G  V  I  Q  R  A
841   ATGGTCAAGGCCTGCCCCCACGTCTGGTTCGAGCGCTCCGAAGTGAAGGATCGCCACCTGGTGGCCAGAAGGCTGACCGAGCACGTGCAGGATAAAAGCAAGTTG
281    M  V  K  A  C  P  H  V  W  F  E  R  S  E  V  K  D  R  H  L  V  A  R  R  L  T  E  H  V  Q  D  K  S  K  L
946   CCCATCCTCATCTTTCCGGAGGGAACCTGCATCAATAATACATCAGTGATGATGTTCAAAAAGGGAAGTTTCGAAATTGGAGCCACAGTTTACCCTGTTGCTATC
316    P  I  L  I  F  P  E  G  T  C  I  N  N  T  S  V  M  M  F  K  K  G  S  F  E  I  G  A  T  V  Y  P  V  A  I
1051  AAGTATGACCCGCAGTTCGGGGACGCCTTCTGGAACAGCAGCAAGTACGGAATGGTGACCTACCTGCTTAGGATGATGACCAGCTGGGCCATCGTCTGCAGCGTG
351    K  Y  D  P  Q  F  G  D  A  F  W  N  S  S  K  Y  G  M  V  T  Y  L  L  R  M  M  T  S  W  A  I  V  C  S  V
1156  TGGTACCTGCCCCCGATGACCAGACAGGCAGAGGAGGATGCAGTCCAGTTTGCCAACAGGGTGAAGTCTGCCATCGCCAGGCAGGGCGGCCTGGTGGACCTGCTG
386    W  Y  L  P  P  M  T  R  Q  A  E  E  D  A  V  Q  F  A  N  R  V  K  S  A  I  A  R  Q  G  G  L  V  D  L  L
1261  TGGGACGGCGGCCTGAAGCGGGAGAAGGTGAAGGACACGTTCAAGGAGGAGCAGCAGAAGCTGTACAGCAAGATGATTGTCGGCAACCACGAGGACCGGAGCCGG
421    W  D  G  G  L  K  R  E  K  V  K  D  T  F  K  E  E  Q  Q  K  L  Y  S  K  M  I  V  G  N  H  E  D  R  S  R
1366  TCGTGA
456    S  *
```

Figure 2. CDS of water buffalo *AGPAT6* (accession No. JX518941) and its encoding amino acid sequences. *Stop codon. Conserved domain sequences of LPLAT_LPCAT1-like are underlined. The red characters donate nucleotide difference sites of complete *AGPAT6* CDS between water buffalo and cattle. Transmembrane sequences are boxed. MicroRNA target sites predicted are shaded.

Sequence analysis

The theoretical pI and Mw for the deduced amino acid sequence of water buffalo AGPAT6 were 9.34 and 52 kDa, respectively. Hydrophobicity analysis showed water buffalo AGPAT6 to have three putative transmembrane domains (Figure 3). The SignalP 3.0 server analysis showed that AGPAT6 includes an N-terminal signal peptide of 37 aa. The results of cytoplasmic/nuclear discrimination suggested with high reliability (94.1%) that the water buffalo AGPAT6 function was in the cytoplasm. The conserved domain (LPLAT LPCAT1-like protein domain: 228-IITYHDRGICVANHTSPIDVIILYAMVGQVHGVIQRAMKAHVWFE RSVARRLTEHVPILIFPEGTCINNTSVMMFKKGSFEIGATVYPVAIKYDAFWNSSKYLL RMMTSWAIVCSVWYLPPEDAVQFANRVKSAIARQ-413) was found using BLAST and ClustalW (Figures 2 and 4). Five kinds of modification sites were also found in water buffalo AGPAT6, which include 7 *N*-myristoylation sites (16-GislTV-21, 37-GvsfGI-42, 99-GsskAL-104,242-GIcvAN-247,267-GQvhGG-272,271-GGlmGV-276,323-GtciNN-328), 6 protein kinase C phosphorylation sites (58-TlR-60, 100-SsK-102, 132-TkR-134, 157-SlR-159, 363-SsK-365, 433-TfK-435), 7 casein kinase II phosphorylation sites (86-TslE-89, 87-SleE-90, 125-TimD-128, 136-AaeE-139, 230-TyhD-233, 250-SpiD-253, 433-TfkE-436), 3 cAMP- and cGMP-dependent protein kinase phosphorylation sites (133-KRfS-136, 303-RRlT-306, 335-KKgS-338), and 4 *N*-glycosylation sites (247-NHTS-250, 327-NNTS-330, 328-NTSV-331, 362-NSSK-365). The results of secondary structure prediction indicated that the deduced water buffalo AGPAT6 contains 230 aa alpha helices, 70 aa extend strands, 13 aa beta turns, and 143 aa random coils (Figure 5). Three transmembrane regions were predicted in water buffalo AGPAT6 (Möller et al., 2001) (Figure 6).

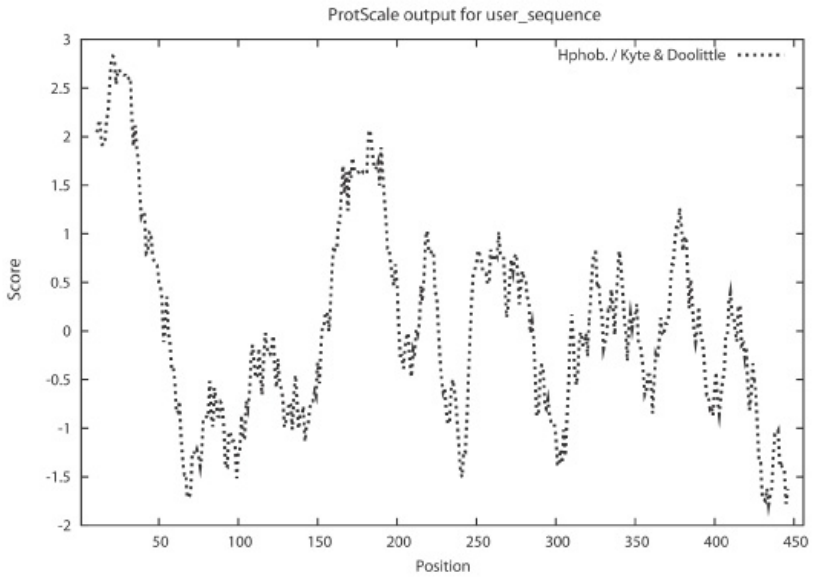

Figure 3. Hydrophobicity structure prediction of water buffalo AGPAT6 by ProtScale. Score >0 means hydrophobic; score <0 means hydrophilic.

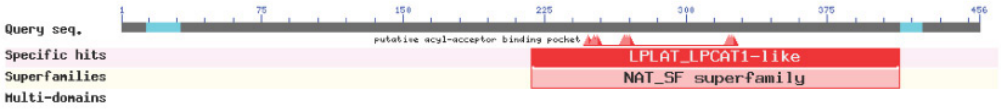

Figure 4. Putative conversed domain of the protein encoded by water buffalo *AGPAT6*.

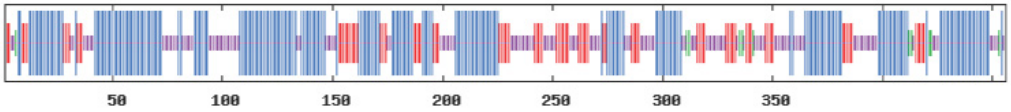

Figure 5. Secondary structure predicted of the water buffalo AGPAT6 protein by SOPMA. Alpha helices, extended strands, beta turn, random coils are indicated with the longest, the second longest, the third longest, and the shortest vertical lines, respectively.

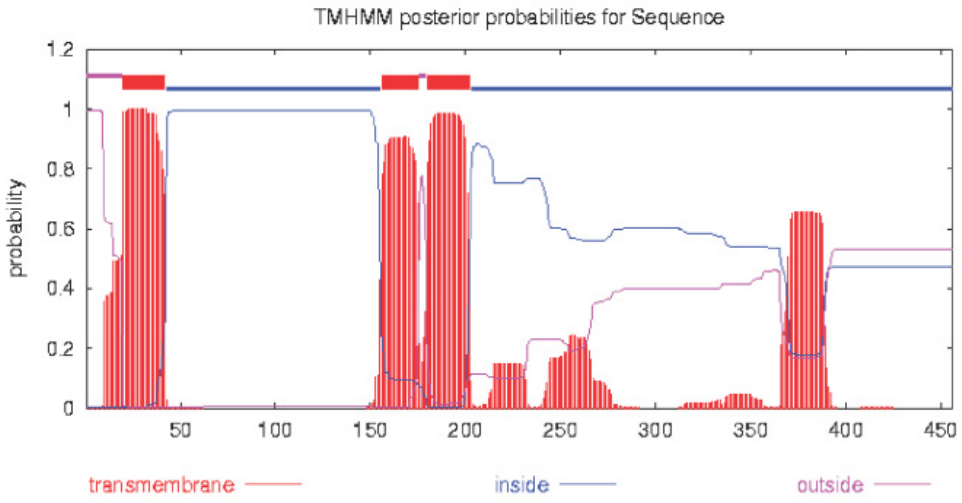

Figure 6. Prediction of transmembrane regions of water buffalo AGPAT6.

Sequence identity and evolutionary relationships of AGPAT6

The results of similarity comparison revealed that the water buffalo *AGPAT6* coding sequence in this study had 99% identity with that of cattle (NM_001083669). To evaluate the evolutionary relationships of water buffalo *AGPAT6* with other species, we constructed a phylogenetic tree using the neighbor-joining method on the basis of the *AGPAT6* nucleotide sequences of horse, human, mouse, orangutan, pig, rat, and chicken. Phylogenetic tree analysis showed that the water buffalo *AGPAT6* gene has a closer genetic relationship with the *AGPAT6* gene of cattle than with those of other species (Figure 7).

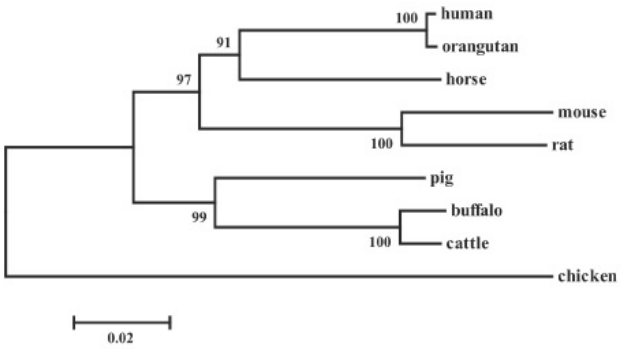

Figure 7. Neighbor-joining phylogenetic tree based on *AGPAT6* gene among some species.

The complete CDS of the *AGPAT6* gene and its deduced aa are presented in Figure 2. The deduced aa sequence of the water buffalo *AGPAT6* gene shares 99, 98, 98, 97, 98, 98, 97, and 95% homology with that of cattle, horse, human, mouse, orangutan, pig, rat, and chicken, respectively. There are 24 nucleotide differences for the *AGPAT6* coding region between water buffalo and cattle, of which three nonsynonymous ones were identified (viz., c.103 A>G, c.520 T>C, and c.529 T>C). The c.103 A>G and c.529T>C cause the 35th encoded amino acid of AGPAT6 to change from isoleucine to valine acid (p. I35V), and the 177th residue to change from proline to serine (p. P177S), respectively. Both involve a change from a nonpolar hydrophobic amino acid to a charged acidic amino acid. Another substitution is c. 520 T>C, which brings about the corresponding deduced amino acid p.174 F>L change. The homology trees for the deduced amino acid sequences of *AGPAT6* revealed that water buffalo *AGPAT6* has the highest identity to the cattle *AGPAT6* than to those of other species in our study (Figure 8).

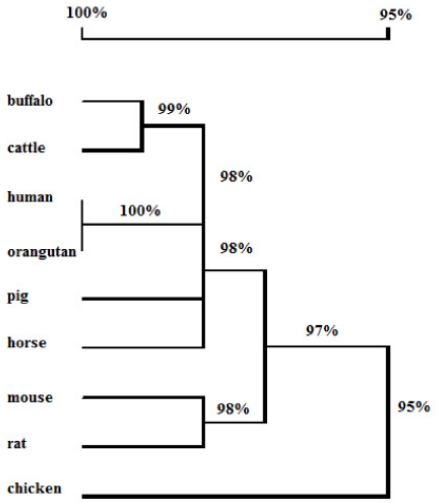

Figure 8. Homology tree based on the AGPAT6 amino acid sequences in some species. Water buffalo (accession No. AFV46336; this study), cattle (accession No. NP_001077138), human (accession No. NP_848934), orangutan (accession No. NP_001126531), pig (accession No. NP_001138491), horse (accession No. XP_001490154), mouse (accession No. NP_061213), rat (accession No. NP_001041314), chicken (accession No. XP_424400).

Potential microRNAs targets

In this study, four microRNAs of *B. taurus* (viz., bta-miR-2439-5p, bta-miR-3431, bta-miR-182, and bta-miR-16a) were found to have their target sites in the water buffalo *AGPAT6* coding sequence. These are 776-ugacgucaucauccuggccag-756, 921-ccagaaggcugaccgag-905, 1213-agggugaagucugccaucgcca-1234, and 1087-agcagcaaguacggaauggug-1107, respectively.

mRNA tissue expression profile

To characterize the *AGPAT6* gene further, we conducted RT-PCR to determine its expression level in various tissues. The ratio of the target band intensity of the *AGPAT6* gene to the 18S ribosomal RNA band intensity was used to represent the relative expression level of the target gene. The results revealed that the *AGPAT6* gene was expressed in 12 of the water buffalo tissues tested with varying degrees. Among them, mammary gland had a high expression level, whereas heart, muscle, liver, and brain had moderate expressions; pituitary gland, spleen, and lung had lower expressions; and small intestine, skin, kidney, and adipose tissue had almost no expression (Figure 9).

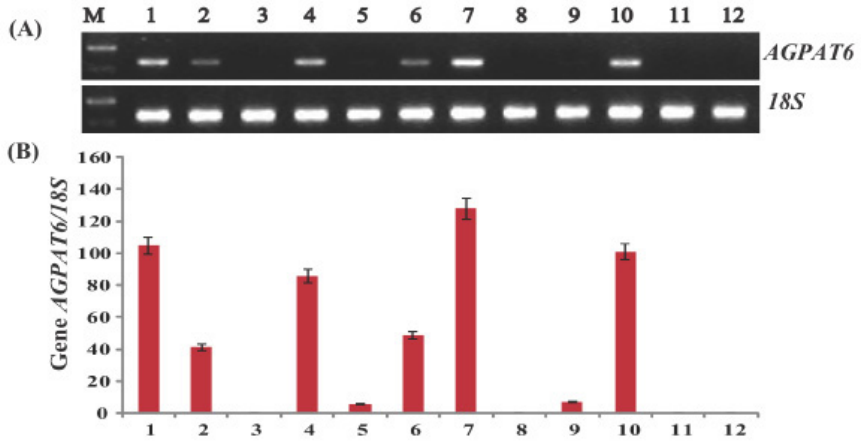

Figure 9. Tissue expression profile of water buffalo *AGPAT6* gene. The 18S ribosomal RNA expression level was used as the internal control. **A.** *Lane 1* = heart; *lane 2* = pituitary gland; *lane 3* = small intestine; *lane 4* = muscle; *lane 5* = spleen; *lane 6* = liver; *lane 7* = mammary gland; *lane 8* = skin; *lane 9* = lung; *lane 10* = brain; *lane 11* = kidney; *lane 12* = adipose tissue, and *lane M* = DNA marker (DL2000). **B.** Columns related to the lanes above.

DISCUSSION

In this study, the full-length coding sequence of the *AGPAT6* gene was cloned and characterized in water buffalo. It contains 1371 nucleotides encoding a putative protein of 456 aa, 52 kDa in size, with a pI of 9.34. As in previous reports of human and mouse, the AGPAT6 protein in water buffalo also contains three hydrophobic transmembrane regions and a signal peptide of 37 aa, which implies that it is a transmembrane protein (Beigneux et al., 2006; Chen et al., 2008; Nagle et al., 2008). Generally, most protein functions are regulated by phosphorylation/dephosphorylation. In this study, several kinds of phosphorylation sites were found in

the water buffalo AGPAT6, such as the protein kinase C phosphorylation site, casein kinase II phosphorylation site, and cAMP- and cGMP-dependent protein kinase phosphorylation sites. This indicates that AGPAT6 may play an important functional role through these sites and domains in water buffalo.

The results of homology analysis showed that water buffalo AGPAT6 has high identity to that of other mammals at the amino acid level, suggesting that the AGPAT6 protein is highly conserved among different species and has fundamental and critical effects on cell function. The phylogenetic tree analysis revealed that the water buffalo *AGPAT6* gene has closer genetic relationships with the *AGPAT6* gene in cattle. This implies that the *AGPAT6* gene in water buffalo is more similar functionally to cattle.

In our study, four cattle microRNAs were found to have the corresponding target sites in the coding regions of water buffalo *AGPAT6*. MicroRNAs are noncoding single-stranded RNA molecules of 17 to 24 nucleotides that can regulate gene expression by binding to or regulating the translation process of some specific mRNAs (Zeng and Cullen, 2003; Bartel, 2004, 2009; Agarwal et al., 2006; Sukumaran et al., 2009). Whether these microRNA molecules predicted in this study can regulate the *AGPAT6* gene expression in water buffalo requires further investigation.

AGPAT6 is broadly expressed and can be detected at the mRNA level in multiple tissues examined (Vergnes et al., 2006; Agarwal et al., 2007; Chen et al., 2008). In mouse, *AGPAT6* is expressed at a high level in brown adipose tissue, white adipose tissue, liver, and mammary epithelium of breast tissue (Vergnes et al., 2006; Bionaz and Loor, 2008a). In our experiment, the *AGPAT6* gene was obviously differentially expressed in the tissues detected, being especially highly expressed in mammary gland tissue. AGPAT6 has been found to be a crucial enzyme for the biosynthesis of glycerolipids and triacylglycerol in some mammalian tissues in recent years (Takeuchi and Reue, 2009). This implies that the *AGPAT6* gene may play important roles for milk fat synthesis in water buffalo. The expression of *AGPAT6* also changes with various physiological status in cattle. Previous studies in cattle showed that the mRNA abundance at 60 days postpartum for *AGPAT6* increased by 15-fold relative to 15 days antepartum (Bionaz and Loor, 2008a,b). As we have not yet studied AGPAT6 functions at protein levels, there may be many possible reasons for the differential expression of the *AGPAT6* gene in water buffalo. The suitable explanation is that the biological activities associated with the functions of the *AGPAT6* gene are presented diversely in different tissues and under different physiological states.

In conclusion, we first isolated the water buffalo *AGPAT6* gene and then performed the necessary bioinformatics analysis and tissue transcription profile analysis. Furthermore, several microRNAs were found to have the corresponding target sites in the coding sequence of water buffalo *AGPAT6* by theoretical prediction. This will establish the primary foundation for further insight into the structure and function of the *AGPAT6* gene.

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