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Molecular Cloning, Tissue Distribution and Segmental Ontogenetic Regulation of $b^{0,+}$ Amino Acid Transporter in Lantang Pigs*

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ABSTRACT : Cationic amino acid transporter $b^{0,+}$AT (HGMW-approved gene symbol SLC7A9, solute carrier family 7, member 9) plays a crucial role in amino acid nutrition. In the present study, we describe the cloning and sequencing of porcine $b^{0,+}$AT. Based on the sequence of porcine $b^{0,+}$AT deposited in the NCBI (National Center for Biotechnological Information), we identified a putative porcine homologue. Using rapid amplification of cDNA ends (RACE), the full-length cDNA encoding porcine $b^{0,+}$AT was isolated. The porcine $b^{0,+}$AT cDNA was 1,680 bp long, encoding a 487 amino acid trans-membrane protein. The predicted amino acid sequence was found to have 88.9% and 87.1% identity with human and mouse $b^{0,+}$AT, respectively. Real-time RT-PCR indicated porcine $b^{0,+}$AT transcripts expressed in heart, kidney, muscle and small intestine. The small intestine had the highest $b^{0,+}$AT mRNA abundance while the muscle had the lowest ($p<0.05$). Along the longitudinal axis, the ileum had the highest $b^{0,+}$AT mRNA abundance while the colon had the lowest ($p<0.05$). The $b^{0,+}$AT mRNA level was highest on day 7 and 90 in the duodenum ($p<0.05$). It increased from day 1 to day 26 in the jejunum ($p>0.05$) and had the highest abundance on day 60 ($p<0.05$). There was, however, no difference between day 1, 7, 26, 30, 90 and 150 ($p>0.05$). The strongest $b^{0,+}$AT expression appeared on day 7 in the ileum before weaning, and then decreased till day 30 but rose gradually again from day 60 to 150 ($p<0.05$). (**Key Words :** Cationic Amino Acid Transporter, $b^{0,+}$AT, SLC7A9, Ontogenetic Regulation)

INTRODUCTION

Free amino acids need to be transported from the lumen of the intestine into the intracellular space by different amino acid transporters. Several distinct transport systems, including system $b^{0,+}$, have been identified based on their ion dependence (i.e. Na^+ and/or Cl^- dependence) as well as their profile of amino acids (Palacin et al., 1998). System $b^{0,+}$, one of the heterodimeric amino acid transporters (HATs), is composed of a heavy subunit (related to $b^{0,+}$ amino acid transport, rBAT) and a light subunit ($b^{0,+}$ amino acid transport, $b^{0,+}$AT), which mediates high-affinity transport of cystine and cationic amino acids in a tightly coupled equimolar exchange with neutral amino acids in the plasma membrane as antiporters (Chillaron et al., 2001; Kanai1 et al., 2001; Wagner et al., 2001). The rBAT is a type II membrane glycoprotein, whereas the $b^{0,+}$AT is an unglycosylated membrane protein bearing 12 putative transmembrane domains. Two conserved cysteines form a disulfide-linked heterodimer between the heavy and the light subunit (Pfeiffer et al., 1998). System $b^{0,+}$ uses a tertiary active mechanism of renal reabsorption and intestinal absorption of cationic amino acids and cystine in the apical plasma membrane. It mediates the electrogenic exchange of cationic amino acids (influx) for neutral amino acids (efflux). It is favored by the membrane potential (negative inside the cell) and the high intracellular concentration of neutral amino acids, which is the result of the activity of sodium-dependent transport systems for neutral amino acids in the apical domains of the epithelial cells (Palacin et al., 1998). Knowledge about HATs has dramatically increased in the past few years since the human, mouse and rat cDNA of $b^{0,+}$AT have been cloned (Chairoungdua et al., 1999; Feliubadalo et al., 1999; Pfeiffer et al., 1999). Research shows that mutations in system $b^{0,+}$ (rBAT-$b^{0,+}$AT) cause the primary inherited amino acidurias (PIAs) and cystinuria (Chillaron et al., 1996; Font et al., 2001; Font-Llitjos et al., 2007). Therefore, it is attracting more attention nowadays. Moreover, works

* Sequence data from this article have been deposited with the GenBank Data Library under Accession No. EU047704.

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done so far indicate that the specificity and the characteristics of system $b^{0,+}$ are mainly determined by the light chains. Reconstitution in liposomes have shown that the light subunit $b^{0,+}$AT is fully functional in the absence of the heavy subunit rBAT (Reig et al., 2002). As such, the $b^{0,+}$AT plays a very important role in the nutrition of cationic amino acids.

Most of the studies conducted earlier on system $b^{0,+}$ focused on human and mouse. In contrast to the detailed information available on the structure and function of human and mouse $b^{0,+}$AT, there is dearth of information on porcine $b^{0,+}$AT. Therefore, the goal of the present study was to clone the $b^{0,+}$AT gene of pigs and investigate the segmental distribution and the developmental regulation of $b^{0,+}$AT mRNA abundance along the intestinal tract, which will enrich our understanding of the relationship that exists between age, system $b^{0,+}$ gene expression and amino acid absorption. Furthermore, knowing the sequence of the porcine SLC7A9 (Solute carrier family 7, member 9) gene would facilitate the further elucidation of structure-function relationship of the gene that can not be offered in other species.

Chinese indigenous pig breeds have been provisionally grouped into north-China type, south-China type, central-China type, lower-Changjiang River Basin type, South-west type and Plateau type. The animal selected by our study is a domesticated pig type of China (South China) which has been ranked in "The pig resource protection list of China". The common colours of Lantang pigs are black or a mixture of black. Lantang pigs are perceived to have high longevity, high fertility, high drought tolerance, high heat tolerance, high disease tolerance. These traits are all of economic importance especially for sustainable agriculture. The results of ontogenetic regulation of amino acid transporter in Lantang pigs should be helpful to the crossbreeding for improving the economic importance.

MATERIALS AND METHODS

Animals

A total of 35 littermate purebred Lantang gilts were divided into seven groups at the ages of d 1, 7, 26 (5 days post-weaning), 30, 60, 90 and 150, respectively, and provided with food and water *ad libitum* throughout the duration of the experiment. The handing of the animals strictly followed the procedure and approved by the Animal Care Committee of South China Agricultural University. Intestinal tissue samples were collected from a total of 70 pigs at different ages: namely suckling (1 and 7 days) and post-weaning (26, 30, 60, 90 and 150 days).

Intestinal tissue sample collection

Pigs were euthanized with an overdose injection of 10% sodium pentobarbital before sampling. The entire small intestine was then removed and dissected free of mesenteric attachments and placed on a smooth, cold surface. The duodenum, jejunum, ileum and colon were separated. The isolated intestinal segments were immediately opened lengthwise following the mesentery line and flushed with ice-cold saline (154 mM NaCl, 0.1 mM PMSF, pH 7.4) and divided into 15-cm segments and deposited in marked tubes. Each segment were separated one centimeter segments as a mix sample to detect the expression of tissue distribution. Each tube, which contained approximately 15 g of tissue, was tightly capped and stored at -80°C until further analysis.

RNA extraction and cDNA synthesis

Total RNA was isolated from 100 mg of intestinal tissue samples using TRIZOL reagent (Invitrogen) and treated with DNase I (Invitrogen) according to the manufacturer's instructions. The RNA quality was checked by 1% agarose gel electrophoresis and stained with 10 μg/ml ethidium bromide. The RNA had an OD_{260}:OD_{280} ratio between 1.8 to 2.0. Synthesis of the first strand cDNA was performed with oligo (dt) 20 and Superscript II reverse transcriptase (Invitrogen).

cDNA cloning strategy

A partial sequence of SLC7A9 (GenBank Accession No. AF141289) was screened from the NCBI and on the basis of this sequence, porcine SLC7A7 gene-specific primers were synthesized. 3'/5' RACE was carried out according to the manufacturer's instructions (BD Biosciences Clontech). Briefly, the first strand cDNA was generated from 1 μg total RNA using 3' RACE CDS primer A (3' CDS) and 5'-CDS/SMART II A (Clontech) for 3' RACE and 5' RACE, respectively. For 3' RACE, the amplification reaction was performed first touch down PCR for 40 cycles (94°C for 5 min, 94°C for 30 s, 70°C for 30 s, 72°C for 2 min, 5 cycles, 94°C for 30 s, 65°C for 30 s, 72°C for 2 min, 5 cycles, 94°C for 30 s, 61°C for 30 s, 72°C for 2 min, 30 cycles) using the GSP2 and the reverse primer UPM. After the first PCR, the second (nest) PCR was performed under similar condition using the nest primer NGSP2 and the reverse primer NUP. For 5' RACE, a similar amplification reaction but a 3-min elongation time was carried out using the forward primer (UPM and NUP) and reverse primer GSP1and NGSP1. The RACE products were gel-purified and cloned into the pGMT vector (Invitrogen). After transformation into *Escherichia coli*, the plasmid purifications from the overnight-grown colonies were done and the cloned cDNA sequenced. Based on the newly obtained sequence for the full-length Cdna, a pair of PCR primers, forward primer ZY1 and reverse primer ZY2 were designed to amplify the sequence covering the ORF (open reading frame) of porcine SLC7A9. All the primers except for those provided by

Table 1. Primers for smart RACE cDNA and ORF amplification

Primer	Application	Sequence
GSP1	1st PCR	5' GCCTGCGTAGAAGGGCGAAGAAACATA3'
NGSP1	2nd PCR	5'TTCTGGAGATTTGTGGTCTTGGGTTCA3'
GSP2	1st PCR	5' TCCGAGTATGTTTCTTCGCCCTTCTA3'
NGSP2	2nd PCR	5' GCTGTAGCTGTGACATTTGGTGACCG3'
ZY1	ORF clone	5' ATGCAAGAGACAAGCCTGAG 3'
ZY2	ORF clone	5' TTACTCTGGCGGTTCC TCC 3'

Clontech RACE kit are shown in Table 1.

Sequence and structural analysis

Nucleotide and amino acid sequence alignment were analyzed with DNAMAN software package. Homology searches were performed using BLAST and FASTA at the National Center for Biotechnological Information (NCBI) and DNA Data Bank of Japan (DDBJ).

Detection of tissue distribution and ontogenetic regulation of porcine SLC7A7 by real-time RT-PCR analysis

Real-time RT-PCR was performed using one-step SYBR Green PCR Mix (Takara, Dalian, China), containing $MgCl_2$, dNTP, and Hotstar Taq polymerase. Two microlitres cDNA template was added to make a total volume of 25 μl containing 12.5 μl SYBR Green mix, 0.25 μl RT mix and 1 μM each of forward ($b^{0,+}$AT: 5' ATCGGTCTGGCGTT TTAT 3', 18S: 5' GGACATCTAAGGGCATCACAG 3') and reverse primers ($b^{0,+}$AT: 5' GGATATAGCACCCTGTCA 3', 18S: 5' AATTCCGATAACGA ACGAGACT 3'). Primers for 18S were design with Primer 5.0 based on porcine sequence (Accession No. AY265350) in order to produce an amplification product that spanned at least two exons. The following protocol: (i) denaturation program (15 min at 95°C); (ii) amplification and quantification program, repeated 40 cycles (15 s at 95°C, 15 s at 58°C, 15 s at 72°C); (iii) melting curve program (60-99°C with heating rate of 0.1°C s-1 and fluorescence measurement). An abundantly expressed gene, 18S, was used as the internal control to normalize the amount of starting RNA used for RT-PCR for all the samples. Amplification and melting curve analysis were performed in ABI 7500 (Applied BioSystems). Melting curve analysis was conducted to confirm the specificity of each product, and the size of products were verified on ethidium bromide-stained 2% agarose gels in Tris acetate-EDTA buffer. The identity of each product was confirmed by dideoxy-mediated chain termination sequencing at Takara Biotechnology, Inc. The relative expression ratio (R) of mRNA was calculated by $2^{-\Delta Ct}$ (Livak et al., 2001). Real-time PCR efficiencies were acquired by amplification of dilution series of RNA according to the equation 10 (-1/slope) and were consistent between target mRNA and 18S. Negative controls were performed in which water was substituted for RNA.

Statistical analysis

Developmental data of mRNA abundance were subjected to analysis of variance of mRNA abundance among day 1, 7, 26, 30, 60, 90 and 150 using Tukey test by SAS (The SAS Institute, Cary, NC). Multiple comparisons of mRNA abundance among duodenum, jejunum, ileum and colon at day 60 were made using Tukey test by SAS (The SAS Institute, Cary, NC). Data are presented as means±SE. Significance was determined using the $p<0.05$ levels.

RESULTS

Cloning of porcine $b^{0,+}$AT cDNA sequence

3' RACE (~0.8 kb) and 5' RACE (~0.2 kb) products were cloned into the pGMT vector and sequenced. Finally, 1,680 bp of the cDNA was assembled from the overlapping 3' (741 bp), known sequence and 5' RACE (197 bp). The full-length cDNA encoding porcine $b^{0,+}$AT from porcine was isolated. Sequence analysis of the porcine SLC7A9 cDNA revealed an ORF of 1,464 bp that would encode a protein of 487 amino acid residues. 90 bp of 5' untranslated region (UTR) and 126 bp of 3' UTR with a consensus AATAAA polyadenylation signal at 7-12 nt upstream of a poly(A) stretch. BLASTn or BLASTp analysis demonstrated that the porcine sequence shared a high degree of sequence identity, both in the nucleotide sequences, especially in coding sequence (CDS) regions (86.1 and 83%, respectively, as shown in Figure 1), and in the deduced amino acid sequences (88.9 and 87.1%, respectively), with those of human (Accession No. NM_014270), and mouse (Accession No. NM_021291). The alignment of amino acid sequence are shown in Figure 2. Hydrophobicity prediction suggested 12 putative membrane-spanning domains within porcine $b^{0,+}$AT (Krogh et al., 2001), similar to other mammalian $b^{0,+}$ATs. Analysis of the amino acid sequence by ScanProsite (de Castro et al., 2006) revealed several consensus sites for post-translational modification. Three consensus sites for protein kinase C phosphorylation were located at 5-7, 169-171 and 399-401. The two 169-171 and 399-401 sites were also present in the amino acid transporters of human $b^{0,+}$AT (Figure 2).

Tissue distribution of porcine $b^{0,+}$AT mRNA

The tissue distribution of $b^{0,+}$AT mRNA at day 60 are presented in Figure 3. Real-time RT-PCR results indicated

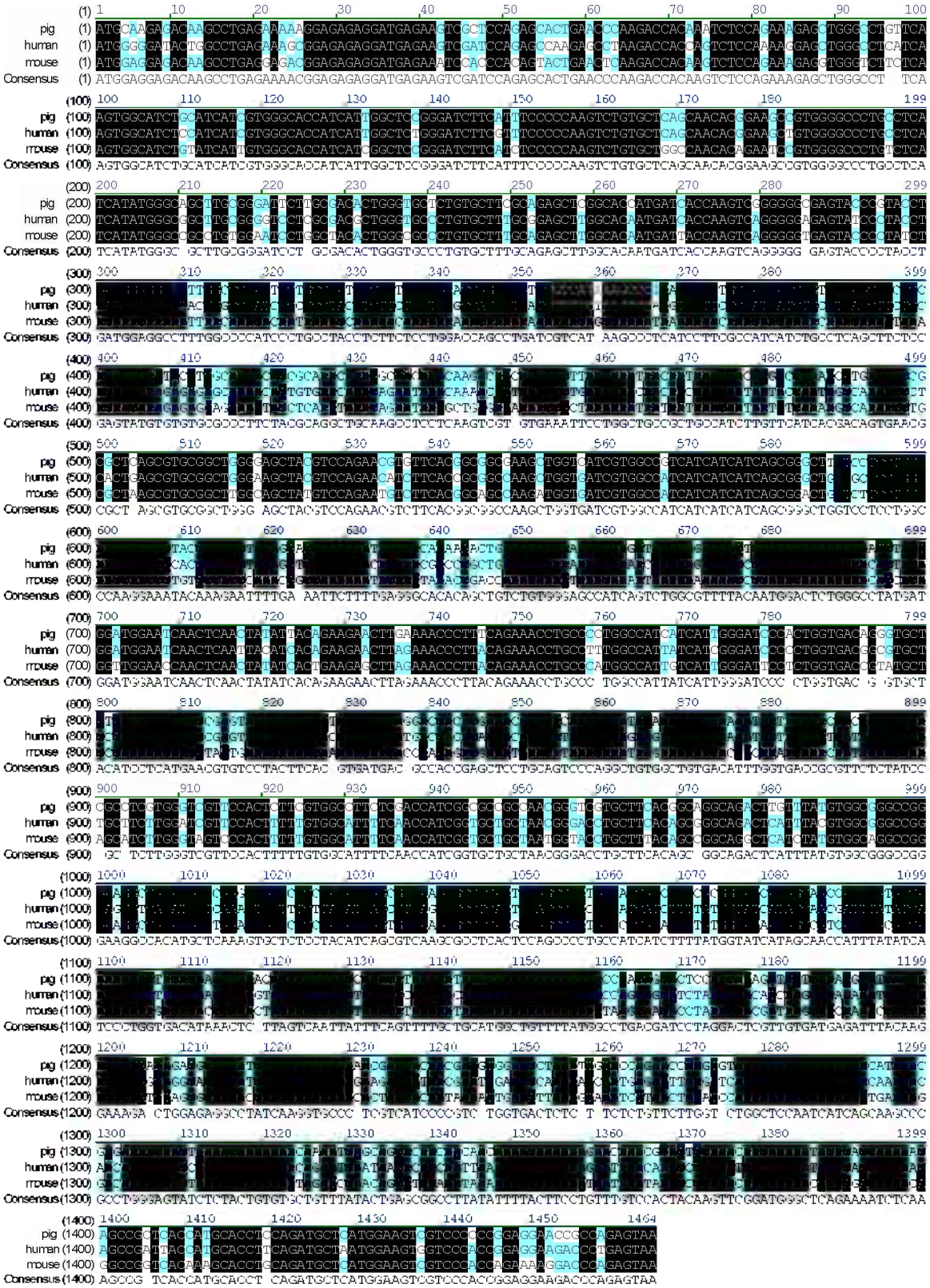

Figure 1. Comparison of coding sequence of $b^{0,+}$AT from pig, human and mouse. Identical nucleic acids were shown in black background. The porcine coding sequence of $b^{0,+}$AT (Accession No. EU047704) shows 86.1 and 83% homology with the human (Accession No. NM_014270) and mouse (Accession No. NM_021291) $b^{0,+}$AT, respectively.

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pig     MQETSLRKRREDEKSLQSTEPKTTNLQKELGLFSGICIIVGTIIGSGIFISPKSVLSNTE   60
human   MgdTgLRKRREDEKSiQSqEPKTTsLQKELGLiSGIsIIVGTIIGSGIFvSPKSVLSNTE   60
mouse   MeETSLRrRREDEKSthSTElKTTsLQKEvGLlSGICIIVGTIIGSGIFISPKSVLaNTE   60

pig     AVGPCLIIWAACGILATLGALCFAELGTMITKSGGEYPYLMEAFGPIPAYLFSWTSLFVI   120
human   AVGPCLIIWAACGvLATLGALCFAELGTMITKSGGEYPYLMEAyGPIPAYLFSWaSLiVI   120
mouse   sVGPCLIIWAACGILATLGALCFAELGTMITKSGGEYPYLMEAFGPIPAYLFSWTSLiVm   120

pig     KPSSFAIICLSFSEYVSSPFYAGCSPPQVVVKFLAAAAILVITMVNALSVRLGSYVQNVF   180
human   KPtSFAIICLSFSEYVcaPFYvGCkPPQiVVKcLAAAAILfIstVNsLSVRLGSYVQNiF   180
mouse   KPSSFAIICLSFSEYVcaaFYsGCkPPaVVVKlLAAAAILfITtVNALSVRLGSYVQNVF   180

pig     TAAKLVIVAVIIISGLVLLAQGNTKNFENSFEGTKLSVGAIGLAFYNGLWAYDGWNQLNY   240
human   TAAKLVIVAiIIISGLVLLAQGNTKNFdNSFEGaqLSVGAIsLAFYNGLWAYDGWNQLNY   240
mouse   TAAKmVIVAiIIISGLVfLAQGNvKNFqNSFEGTqtSVGAIsLAFYNGLWAYDGWNQLNY   240

pig     ITEELENPFRNLPLAIIIGIPLVTGCYILMNVSYFTVMTATELLQPQAVAVTFGDRVLYP   300
human   ITEELrNPyRNLPLAIIIGIPLVTaCYILMNVSYFTVMTATELLQsQAVAVTFGDRVLYP   300
mouse   ITEELrNPyRNLPmAIvIGIPLVTvCYILMNiaYFTVMTpTELLQsQAVAVTFGDRVLYP   300

pig     ASWVVPLFVAFSTIGAANGSCFTAGRLVYVAGREGHMLKVLSYISVKRLTPAPAIIFHGI   360
human   ASWiVPLFVAFSTIGAANGtCFTAGRLiYVAGREGHMLKVLSYISVrRLTPAPAIIFyGI   360
mouse   ASWVVPLFVAFSTIGAANGtCFTAGRLiYVAGREGHMLKVLSYISVKRLTPAPAlIFyGI   360

pig     IATIYIIPGDINSLVNYFSFAAWLFYGLTISGLVVMRFTRKELKRPIKVPIVIPVVVTLL   420
human   IATIYIIPGDINSLVNYFSFAAWLFYGLTIlGLiVMRFTRKELeRPIKVPvVIPVlmTLi   420
mouse   IAiIYIIPGDINSLVNYFSFAAWLFYGmTIlGLVVMRFTRKdLeRPIKVPlfIPiiViLv   420

pig     SLFLVLAPIISMPAWEYLYCVLFMLSGLIFYFLFVRYKFGWAQKISKPLTMHLQMLMEVV   480
human   SvFLVLAPIISkPtWEYLYCVLFiLSGLlFYFLFVhYKFGWAQKISKPiTMHLQMLMEVV   480
mouse   SLFLiLAPIISePAWEYLYCVLFiLSGLIFYFLFVyYKFGWAQrISrPvTkHLQMLMEVV   480

pig     PPEEPPE                                                        487
human   PPEEdPE                                                        487
mouse   PPEkdPE                                                        487
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Figure 2. Comparison of amino acid sequence of porcine $b^{0,+}$AT from human and mouse. Amino acid sequence determined from the porcine $b^{0,+}$AT cDNA (Accession No. EU047704) was shown on the top line. Amino acid sequence for human $b^{0,+}$AT (Accession No. NM_014270) and mouse $b^{0,+}$AT (Accession No. NM_021291) were shown below. Putative membrane-spanning domains were indicated by open box and in bold. Potential sites for protein kinase C phosphorylation were stressed by black dots and putative intracellular domains underlined. Lowercase indicate difference of amino acid to porcine $b^{0,+}$AT.

porcine $b^{0,+}$AT transcripts expressed in the heart, kidney, muscle and small intestine. The small intestine had the highest $b^{0,+}$AT mRNA abundance while the muscle had the lowest ($p<0.05$). However, undetectable levels of $b^{0,+}$AT mRNA expression were observed in the brain, lung and liver.

Expression of Lantang porcine $b^{0,+}$AT mRNA along the longitudinal axis

The intestinal distribution of $b^{0,+}$AT mRNA at day 60 is

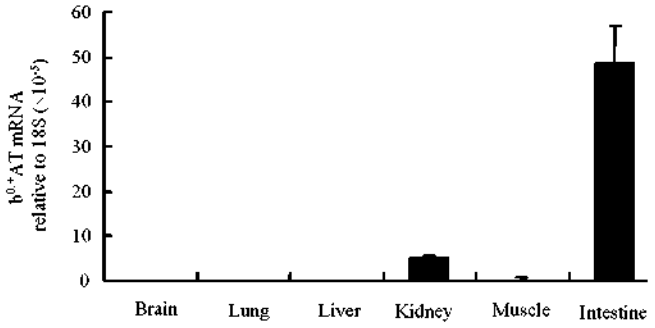

Figure 3. Tissue distribution of porcine $b^{0,+}$AT in the heart, liver, lung, kidney, brain, muscle and intestine. The small intestine had the highest $b^{0,+}$AT mRNA abundance while the muscle had the lowest ($p<0.05$). All samples were normalized using 18S expression as an internal control in each real-time PCR. Relative level of $b^{0,+}$AT mRNA were analyzed by the 2(-Delta Ct) method. Data are presented as means±SE (n = 5), in arbitrary units.

shown in Figure 3. The ileum had the highest $b^{0,+}$AT mRNA abundance while the colon had the lowest ($p<0.05$). The $b^{0,+}$AT mRNA level was significantly higher in the duodenum and jejunum than in colon ($p<0.05$), no difference was observed between duodenum and jejunum ($p>0.05$).

Ontogenetic regulation of $b^{0,+}$AT mRNA expression

Developmental changes in $b^{0,+}$AT mRNA expression along the small intestine is shown on Figure 5. The highest level of $b^{0,+}$AT mRNA in the duodenum was observed on days 7 and 90 ($p<0.05$). The expression of $b^{0,+}$AT mRNA in jejunum increased gradually with age from 1 to 26 days of age, with the peak level at 60 days of age ($p<0.05$). In the case of ileum, the strongest $b^{0,+}$AT expression appeared on day 7 before weaning, and then decreased till day 30 but rose gradually again from day 60 to 150 ($p<0.05$).

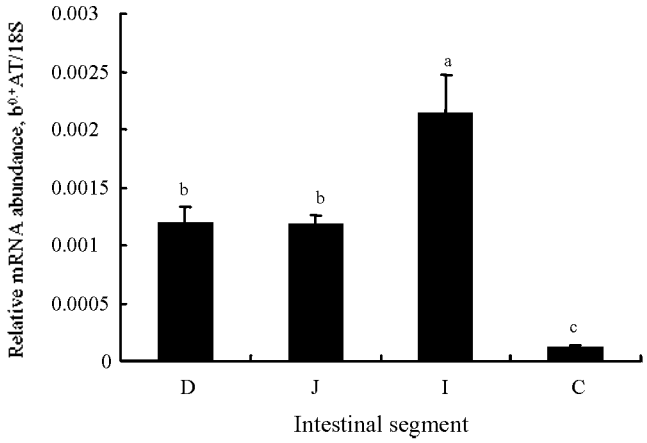

Figure 4. Relative mRNA expression of porcine $b^{0,+}$AT along longitudinal axis of intestine on day 60. All samples were normalized using 18S expression as an internal control in each real-time PCR. Relative level of $b^{0,+}$AT mRNA were analyzed by the 2 (-Delta Ct) method. Bars without common letters differ significantly ($p<0.05$). Data are expressed as means±SE (n = 5), in arbitrary units. D = Duodenum; J = Jejunum; I = Ileum; C = colon.

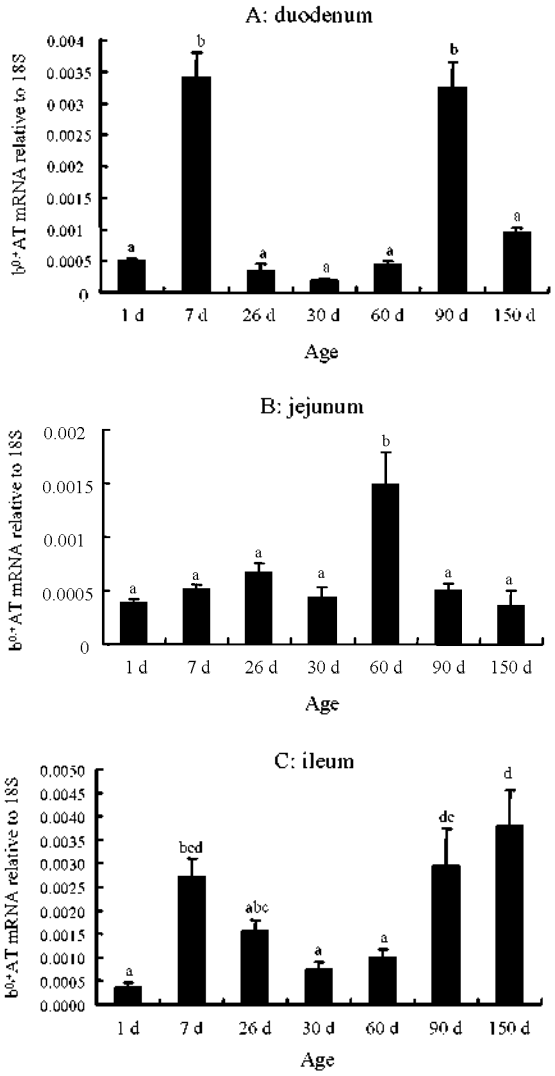

Figure 5. Relative mRNA expression of porcine $b^{0,+}$AT in pig duodenum, jejunum and ileum during postnatal development. All samples were normalized using 18S expression as an internal control in each real-time PCR. Relative level of $b^{0,+}$AT mRNA were analyzed by the 2 (-Delta Ct) method. Bars without common letters differ significantly ($p<0.05$). Data are presented as means±SE (n = 5), in arbitrary units.

DISCUSSION

Feed proteins are absorbed in the small intestine in the form of small peptides and free amino acids (AA) (Johnson, 1997). Absorption of amino acid involves participation of several transporters, which differ in their substrate specificity and driving force. Unlike glucose, amino acid does not have a large storage pool in the body. The amino acids are metabolized quickly. Their requirements are so critical that the feed must have well balanced content of them especially the essential amino acids. A number of HATs amino acid transporters have been isolated since the end of last century, which are the Na^+-dependent system ASCT1 (Arriza et al., 1993), the Na^+-dependent system B^{0+} (Kekuda et al., 1996; Kekuda et al., 1997), the Na^+-independent system L (Mastroberardino et al., 1998), the system N (Chaudhry et al., 1999) as well as to the system A (Varoqui et al., 2000). HATs play a very important role in the absorption of cationic amino acid, including lysine, which is a very important essential amino acid in pigs. In the course of transport studies in mouse blastocysts, Van Winkle and co-workers identified a novel transport Na^+-independent system $b^{0,+}$ carrying both neutral and cationic amino acids (Van Winkle et al., 1988). cDNA of human and mouse $b^{0,+}$AT was cloned in 1996 by Feliubadaló, Chairoungdua and their co-workers, respectively. In this study, porcine complete mRNA sequence of the $b^{0,+}$AT gene was cloned. Sequence analysis of the porcine SLC7A9 cDNA revealed an ORF of 1,464 bp that would encode a protein of 488 amino acid residues. BLASTn or BLASTp analysis demonstrated that the porcine sequence shared a high degree of sequence identity with the human and mouse $b^{0,+}$AT gene. Hydrophobicity prediction suggested 12 putative membrane-spanning domains within porcine $b^{0,+}$AT which were similar to those of other mammalian $b^{0,+}$ATs. The two 169-171 and 399-401 sites of porcine $b^{0,+}$AT were also present in the amino acid transporters of human $b^{0,+}$AT. Therefore, we consider it to represent the porcine homology of human $b^{0,+}$AT (SLC7A9). Isolation of this porcine cDNA could lead to the study of the possible involvement of $b^{0,+}$AT function.

The tissue distribution was likely to suggest the primary function of this gene. The porcine $b^{0,+}$AT mRNA detected in different tissues showed the small intestine to have the highest $b^{0,+}$AT mRNA abundance while the muscle had the lowest. As far as the intestines were concerned, the ileum had the highest $b^{0,+}$AT mRNA abundance while the colon had the lowest. These results are in agreement with the work done by Munck and co-workers, which showed the presence of system $b^{0,+}$ in the swine small intestine at the molecular level (Munck et al., 2000). It is not surprising that the colon had the lowest abundance of $b^{0,+}$AT because of the fact few amino acids are usually absorbed in the colon. The different distribution of transporters along the intestinal axis from proximal to distal, and from the crypt to villous may be due to the unique morphological characteristics of the intestine and substrates, even though further research is needed to confirm this. Human and rabbit give positive response only in the kidney and small intestine by way of Northern analysis (Chairoungdua et al., 1999; Rajan et al., 1999). Another aspect of the study that is of interest is that even though the small intestine is physiologically divided into four segments, namely duodenum, jejunum, ileum and colon, there is still the possibility that the mRNA levels are differently expressed within each segment. More knowledge of the function of each gene is needed to further understand the precise pattern of their distribution.

The intestine undergoes dramatic structural and functional changes after birth such as increased dry mass and absorptive surface area changes in membrane permeability as well as fluidity (Buddington et al., 2001). In addition to these nonspecific changes, the absorptive capability per cell and the expression of transporters also alters with aging. In this study, the results revealed that the $b^{0,+}$AT mRNA abundance increased with age in jejunum and ileum. However, no distinct pattern in relative to earlier the whole intestine could be established. Many aspects of this work are sharp contrast to the studies conducted by a colleague in our laboratory on segmental ontogenetic regulation of the heterodimeric $b^{0,+}$AT in Landrace pigs (unpublished data). The difference in expression response could be ascribed to the species difference. After the sucking period in day 30, the patterns of porcine $b^{0,+}$AT mRNA expression in the three segments were decreased distinctly. This could be due to the weaning stress since weaning stress decreases the performance of piglets around weaning time (Yuan et al., 2007; Wang et al., 2005). Ontogenic changes of porcine amino acid transporter expression have not been examined before. Both the species difference and diet reportedly can possibly regulate the intestinal amino acid transporter expression in the developmental stage. However, embedded research need be done to explore exact reason. The next goal of our research is to investigate the $b^{0,+}$AT mRNA expression profile at different amino acid levels, especially lysine, in growing pigs via not only *in vivo* but *in vitro* experiments using primary swine intestinal epithelia cells.

In conclusion, we have cloned a cationic amino acid transporter $b^{0,+}$AT from pig. This cationic amino acid transporter revealed significant homology with human and murine $b^{0,+}$AT. The mRNA of heterodimeric amino acid transporter $b^{0,+}$AT was not only developmentally expressed but also segment-specifically distributed along the small intestine of pigs at early as well as growing stages of life. This may be related to luminal substrate concentration,

amino acid requirement as well as hormonal status. Further studies are needed to elucidate which cationic amino acid is transported by $b^{0,+}$AT as well as its function in the porcine nutrition and physiology. Further research is also necessary on developmental changes in protein production of $b^{0,+}$AT in the whole small intestine in order to comprehensively understand ontogenetic regulation.

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