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Identification of a Membrane Protein, LAT-2, That Co-expresses with 4F2 Heavy Chain, an L-type Amino Acid Transport Activity with Broad Specificity for Small and Large Zwitterionic Amino Acids*

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Marta Pineda‡§, Esperanza Fernández‡¶, David Torrents¶, Raúl Estévez§, Carmen López‖, Marta Camps, Jorge Lloberas, Antonio Zorzano, and Manuel Palacín‡‡**

*From the Departament de Bioquímica i Biologia Molecular and **Departament de Fisiologia, Facultat de Biologia, and the ‖Serveis Científico-Tècnics, Universitat de Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain*

We have identified a new human cDNA, L-amino acid transporter-2 (LAT-2), that induces a system L transport activity with 4F2hc (the heavy chain of the surface antigen 4F2, also named CD98) in oocytes. Human LAT-2 is the fourth member of the family of amino acid transporters that are subunits of 4F2hc. The amino acid transport activity induced by the co-expression of 4F2hc and LAT-2 was sodium-independent and showed broad specificity for small and large zwitterionic amino acids, as well as bulky analogs (*e.g.* BCH (2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid)). This transport activity was highly *trans*-stimulated, suggesting an exchanger mechanism of transport. Expression of tagged N-*myc*-LAT-2 alone in oocytes did not induce amino acid transport, and the protein had an intracellular location. Co-expression of N-*myc*-LAT-2 and 4F2hc gave amino acid transport induction and expression of N-*myc*-LAT-2 at the plasma membrane of the oocytes. These data suggest that LAT-2 is an additional member of the family of 4F2 light chain subunits, which associates with 4F2hc to express a system L transport activity with broad specificity for zwitterionic amino acids. Human LAT-2 mRNA is expressed in kidney >>> placenta ≫ brain, liver > spleen, skeletal muscle, heart, small intestine, and lung. Human *LAT-2* gene localizes at chromosome 14q11.2–13 (13 cR or ~286 kb from marker D14S1349). The high expression of LAT-2 mRNA in epithelial cells of proximal tubules, the basolateral location of 4F2hc in these cells, and the amino acid transport activity of LAT-2 suggest that this transporter contributes to the renal reabsorption of neutral amino acids in the basolateral domain of epithelial proximal tubule cells.

Last year, three amino acid transporter cDNAs (LAT-1, y^+LAT-1, and y^+LAT-2)[1] were identified as subunits of the heavy chain of the cell surface antigen 4F2 (4F2hc, also named CD98) (1–3). These subunits co-express amino acid transport activity with 4F2hc in oocytes (*i.e.* system L for LAT-1, and system y^+L for y^+LAT-1 and y^+LAT-2) (1–4). The role of this family of proteins in amino acid transport has recently been demonstrated by the fact that mutations in the *y^+LAT-1* gene cause lysinuric protein intolerance, an inherited amino aciduria due to a defective renal reabsorption mechanism of dibasic amino acids (5, 6). The structural and functional similarities between 4F2hc and its homologous protein rBAT suggest that a member of this family of subunits might be the subunit of rBAT needed to fully express the amino acid transport system $b^{o,+}$ activity (reviewed in Refs. 7 and 8). After the identification of *rBAT* as the Type I cystinuria gene (9), this subunit is a good candidate for non-Type I cystinuria (7). A search throughout gene data bases suggests that there may be as many as four new human members of the family of subunits of 4F2hc and rBAT.

Kanai and co-workers (1) identified rat LAT-1 (also known as TA1) by co-expression cloning with 4F2hc in oocytes. The co-expressed transport activity shows clear characteristics of the amino acid transport system L: high affinity (K_m in the low μM range), sodium-independent, and *trans*-stimulated transport for large zwitterionic amino acids. Some of these characteristics have also been demonstrated for the human (E16, Ref. 2) and *Xenopus laevis* orthologs of LAT-1 (ASUR4, Ref. 2; IU12, Ref. 3). System L is almost ubiquitous (10), and variants of system L have been described (11, 12). The expression of rat LAT-1 is not ubiquitous, and it is not present in tissues such as kidney and liver (1), which suggests that homologs of LAT-1 might encode system L amino acid transporter variants.

In this study we have identified the fourth human member (LAT-2) of this family of amino acid transporters. LAT-2 does not induce transport of amino acids in oocytes when it is injected alone, but a variant of system L transport activity (*i.e.* with broad specificity for small and large zwitterionic amino acids) is co-expressed when LAT-2 is injected with 4F2hc. We demonstrate here that co-expression of LAT-2 with 4F2hc brings the former to the oocyte plasma membrane. Its expres-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF135828, AF135829, AF135830, and AF135831.

‡ These two authors contributed equally to this study.

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‡‡ To whom correspondence should be addressed. Tel.: 34-93-4021543; Fax: 34-93-4021559; E-mail: mpalacin@porthos. bio.ub.es.

[1] The abbreviations used are: LAT, L-amino acid transporter; y^+LAT, y^+ L-amino acid transporter; 4F2hc, heavy chain of the cell surface antigen 4F2; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid; rBAT, related to $b^{o,+}$ amino acid transporter; PCR, polymerase chain reaction; ORF, open reading frame; nt, nucleotide; bp, base pair(s); kb, kilobase or kilobase pair(s); TBS, Tris-buffered saline; SHGC, Stanford Human Genome Center; EST, expressed sequence tag.

sion in the epithelial cells of the proximal tubule suggests a role of LAT-2/4F2hc in the renal reabsorption of neutral amino acids.

EXPERIMENTAL PROCEDURES

PCR Amplification, Sequencing, and LAT-2 cDNA Construction—For PCR amplification, first-strand cDNA was synthesized from 5 μg of total RNA purified from opossum kidney (13) cells using SuperScript II kit (Life Technologies, Inc.). Two degenerate forward and reverse primers were designed based on two highly conserved regions among the first known members of this family of amino acid transporters. PCR amplification, subcloning into pGEM-T easy vector (Promega), and sequencing were carried out as described elsewhere (3).

The open reading frame (ORF) of LAT-2 was obtained from two partial human LAT-2 cDNA clones (IMAGE No. 322502 and No. 267204). Clone 322502 was cut with *Not*I and *Avr*II to create the 5′-end fragment of LAT-2 (from nt 1 to 1152 of the LAT-2 cDNA). Clone 267204 was digested with *Eco*RI and *Avr*II to create the 3′-end fragment of the LAT-2 coding sequence (nt 1152 to 3′-end of the LAT-2 cDNA) ligated to the pT7T3D vector. Both fragments were ligated to create a LAT-2 cDNA fragment covering the ORF (5′-end to nt 2050; see Fig. 1) in pT7T3D vector. To improve expression in oocytes, an *Ssp*I-*Not*I fragment of LAT-2 was cloned into pNKS2-*myc Not*I vector (a gift from G. Schmalzing; Ref. 14). To create an N-*myc*-tagged LAT-2 cDNA, pT7T3D-LAT-2 was PCR-amplified with primers M13 forward (16-mer) and 5′-ACGTCTAGTCGACATGGAAGAAGGAGCCAGGCAC-3′ (containing a *Sal*I site and the first 21 nt of the ORF of LAT-2). The PCR product was digested with *Sal*I and *Not*I. The resulting fragment of LAT-2 was cloned into pNKS2-*myc Not*I. The N-*myc*-tagged LAT-2 cDNA was tested by sequencing. All sequences carried out in this work were performed in both directions with d-rhodamine dye terminator cycle sequencing ready reaction kit (Perkin-Elmer). The sequence reactions were analyzed with an Abi Prism 377 DNA sequencer.

Oocytes, Injections, and Uptake Measurements—Oocyte origin, management, and injections were as described elsewhere (15, 16). Defolliculated stage VI *X. laevis* oocytes were injected with 10 ng/oocyte human 4F2hc, human LAT-2, N-*myc*-LAT-2, or *X. laevis* IU12 cRNA. Synthesis of human 4F2hc cRNA (17) was as described (18). *X. laevis* LAT-1 (*i.e.* IU12) was a gift from Y. B. Shi (19), and the cRNA was synthesized as described elsewhere (3). Human LAT-2 cRNA was obtained by cutting the cDNA with *Not*I and using T7 polymerase.

Influx rates of L-[^{3}H]arginine, L-[^{3}H]leucine, L-[^{3}H]alanine, and L-[^{3}H]glutamine (Amersham Pharmacia Biotech) were measured in 100 mM NaCl or 100 mM CholineCl medium at the indicated number of days after injection and under linear conditions as described (15). Amino acid transport rates obtained with oocytes injected with water (50 nl) were similar to those of noninjected oocytes (data not shown). For L-[^{3}H]isoleucine efflux measurements, groups of five cRNA-injected or noninjected oocytes were incubated with 50 μM L-[^{3}H]isoleucine (3 μCi/μl) for the indicated period of time (see legend to Fig. 7). Efflux was measured as described elsewhere (20).

Computer Analysis—Amino acid or nucleotide sequence homology search and the prediction of transmembrane segments of LAT-2 were performed as indicated elsewhere (3).

Northern Blot Analysis—A human adult poly(A$^+$) membrane from CLONTECH (Palo Alto, CA) was used. The insert of clone 267204 was separated from the pT7T3D vector by *Not*I-*Eco*RI digestion. This ~1-kb DNA fragment was purified, labeled with [α-^{32}P]dCTP (Amersham Pharmacia Biotech) using a random oligonucleotide-priming labeling kit (Amersham Pharmacia Biotech), and used as a probe. Hybridization and washing conditions were as recommended by CLONTECH. In these conditions, y+LAT-1 and y+LAT-2 cRNAs were not detected (data not shown).

In Situ Hybridization—Sense and antisense cRNA probes were labeled with digoxigenin-11-UTP (Roche Molecular Biochemicals) by transcription of a LAT2 fragment (1–310 nt of the contig shown in Fig. 1) contained in the pT7T3D vector. The transcription reactions were set up at room temperature by mixing 7.5 μl of double-distilled water treated with diethyl pyrocarbonate, 1 μl of linearized template cDNA (1 μg), 4 μl of 5× transcription buffer (Promega), 2 μl of NTPmix (10 mM ATP, CTP, GTP, 6.5 mM UTP, 3.5 mM digoxigenin-11-UTP, Roche Molecular Biochemicals), 1 μl of RNAsin (30.6 units/μl, Amersham Pharmacia Biotech), and 2 μl of RNA polymerase (T7 or T3, 15 units/μl, Promega). Labeling reactions were performed at 37 °C for 2 h and stopped by incubation with 2 μl of RNase-free DNase (10 units/μl, Stratagene) for 15 min at 37 °C. cRNA fragments were precipitated overnight with 1/10 vol of 4 M LiCl and 2.5 volumes of ethanol at −80 °C. The precipitated cRNA was recovered in 10 μl of double-distilled water treated with diethyl pyrocarbonate.

Fresh human kidney was fixed in 4% paraformaldheyde, 0.1 M phosphate buffer and kept at 4 °C before use. Thereafter, the sections were washed in 0.1 M phosphate buffer (2 h, room temperature) and dehydrated with 70, 90, and 100% alcohol, alcohol/xylene (v/v), and xylene (2 h for each). Pieces were embedded in paraffin. 5-μm sections were cut on a Leica RM 2135 microtome and mounted on silenized slides (Perkin-Elmer). Sections were deparaffined with xylene and hydrated with 100, 90, and 70% ethanol and double-distilled water treated with diethyl pyrocarbonate, permeabilized with proteinase K (Roche Molecular Biochemicals) (1 μg/ml) in Tris-EDTA buffer, pH 8 (3 min, 37 °C), 0.2 N HCl (20 min, room temperature), and washed twice in 2× SSC solution (30 min, room temperature). The hybridization step was carried out with a solution containing 50% formamide, 10% dextran sulfate, 2× SSC solution, 1× Denhardt's solution, 400 ng/μl denatured salmon sperm DNA, and denatured (4 min, 70 °C) sense or antisense probes (5 ng/μl) for 16 h at 42 °C in a moist chamber. The sections were then washed in 4× SSC solution containing 45% formamide (2 min, room temperature), 0.1× SSC (1 h, 37 °C), digested with 20 μg/ml RNase A (Roche Molecular Biochemicals) (30 min, room temperature), and washed in 0.1× SSC solution (5 min, room temperature). Sections were rinsed twice in Tris-buffered saline (TBS), pH 7.5 (10 min, room temperature), blocked with 1% bovine serum albumin in TBS (30 min, room temperature), and incubated overnight with an alkaline phosphatase-conjugated anti-digoxigenin antibody (1:500) (Biocell, Cardiff, UK). They were then washed in TBS, pH 7.5 (10 min, room temperature), and TBS, pH 9.5, containing 50 mM $MgCl_2$ (10 min, room temperature) and developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Roche Molecular Biochemicals). Slides were examined on an Olympus microscope.

Chromosome Mapping—Chromosome mapping was done using the Stanford Human Genome Center G3 radiation hybrid panel (medium resolution). DNA samples of this panel, along with total genomic DNA and pT7T3-249835 (used as a positive control), were PCR screened for the presence of the genomic sequences flanked by the primers 12D (5′-GGCATCTCTCTTCCTAATG-3′) and 7R (5′-GCCAATGCTCTCCTCAGT-3′), which are located in the 3′-untranslated region of the cDNA. PCR amplifications were carried out in a Perkin-Elmer 9600 thermocycler as described elsewhere (3). Amplification conditions were as follows: 35 cycles of denaturing (94 °C, 30 s), annealing (58 °C, 40 s), and extension (74 °C, 30 s). PCR results were transformed into zeros (for no amplification) and ones (for positive amplification) and submitted to the radiation hybrid mapping e-mail server at the Stanford Human Genome Center (SHGC). The resulting chromosomal location, referred to a SHGC marker, was obtained automatically via e-mail from this server.

Localization of LAT-2 Expression by Confocal Microscopy—Groups of five oocytes were prepared for immunofluorescence 2 days after injection with 10 ng/oocyte human 4F2hc or N-*myc*-LAT-2 cRNA, alone or in combination. Oocytes were placed in 500 mm^3 cryomolds (Tissue-Tek, Miles Inc., Elkhart, IN), sliced, fixed, and permeabilized as described elsewhere (18). Slices were incubated with monoclonal antibody 9E10 anti-*myc* (ATCC, Manassas, VA), diluted 1/500 in 10% phosphate-buffered saline, at room temperature for 1 h. Slices were washed three times in phosphate-buffered saline, incubated with 7.5 μg/ml Texas red-conjugated goat anti-mouse (Molecular Probes, Leiden, The Netherlands) at room temperature for 1 h, washed three times in phosphate-buffered saline, and mounted in Immunofluore (ICN, Madrid, Spain).

RESULTS

Our goal was to identify any new member of the amino acid transporter-related family expressed in the kidney and potentially involved in reabsorption of amino acids. For this purpose, reverse transcription-PCR amplification of total RNA from opossum kidney cells was performed with degenerated primers as described for the identification of y$^+$LAT-1 (3). Electrophoretic analysis of the PCR reaction showed one band of 286 bp, which was subcloned into pGEMT-easy vector and amplified in *Escherichia coli*. The deduced amino acid sequence of one clone (b2c2) showed a significant degree of identity to the amino acid transporter-related proteins: 46, 45, 43, 41, and 43% with human y$^+$LAT-1 and y$^+$LAT-2 and *Xenopus*, rat, and human LAT-1, respectively. This homology is compatible with the assumption that b2c2 is part of a cDNA corresponding to a

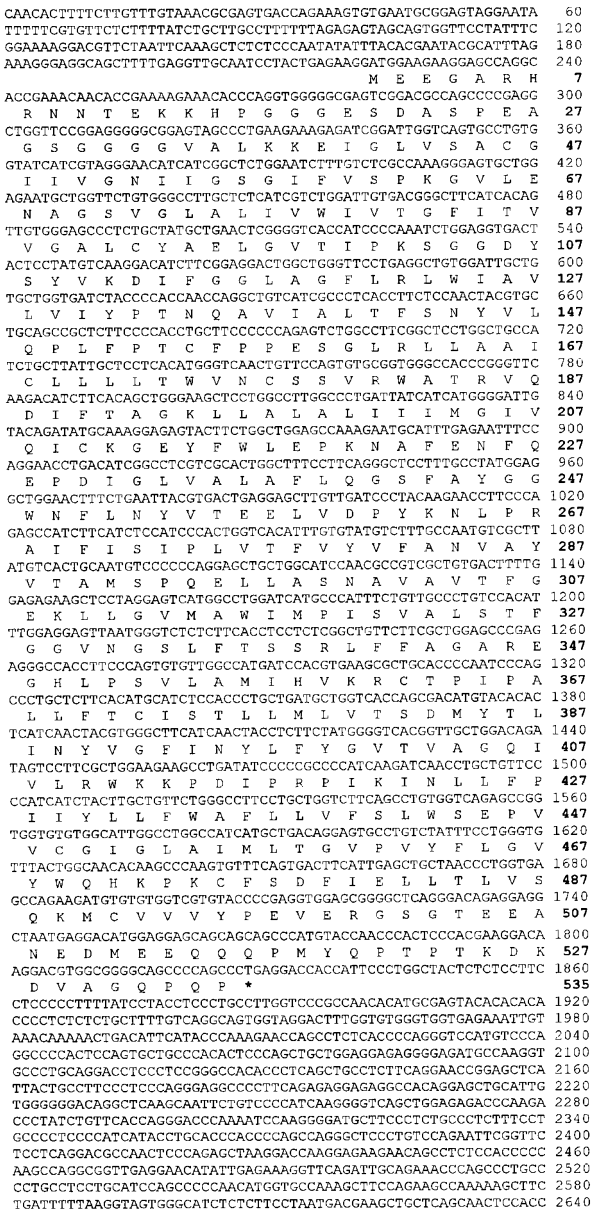

FIG. 1. **Nucleotide and deduced amino acid sequence of LAT-2 cDNA.** The size of the cDNA contig is 3733 bp, containing a 5′-untranslated region of 221 bp followed by an ORF of 535 amino acids and a 3′-untranslated region of 1904 bp that contains a 23-bp long poly(A) tail. The stop codon (TGA) is indicated by a *star*. The possible polyadenylation signal is *underlined*. The sequences of the overlapping segments were identical in all three clones except for nt 1422. At this position, clone 267204 has a G, as in the ESTs N32639 and N31874, and the corresponding amino acid residue is Val^{401}. In contrast, clone 249835 has an A, and the corresponding amino acid is Ile^{401}. This is probably a polymorphism. In agreement with the majority of available EST sequences, the contig LAT-2 shown in this figure and the cRNA injected in this study has G at position 1422 (*i.e.* Val^{401}).

new member of this family. By using the same computer approach (BLAST and EST Cluster Assembly Machine) as we recently used for the identification of y^+LAT-1 (3), a human EST (W39098, IMAGE clone 322502) that shows homology with the b2c2 fragment (92% identity in the amino acid sequence) was identified. Subsequently, EST W39098 was used to identify two other ESTs from the same cluster (N23973 and H84042, from IMAGE clones 267204 and 249835, respectively). Sequences of these overlapping EST clones revealed a 3′-polyadenylated cDNA contig (LAT-2) of 3733 bp (Fig. 1). The first ATG codon lies within a good consensus initiation sequence (5′-GAAGG) (21). The ORF continues to the first stop codon (TGA) at base 1827 and codes for a protein of 535 amino acid residues with a predicted molecular mass of 58,577 Da. The nucleotide sequences of EST clones 322502, 267204, and 249835 and LAT-2 cDNA have been deposited in the GenBank™/EBI data base (accession numbers AF135828, AF135829, AF135830, and AF135831, respectively).

A multiple sequence alignment of the predicted amino acid sequence of human LAT-2, LAT-1, y^+LAT-1, and y^+LAT-2 is shown in Fig. 2. Human LAT-2 shows an amino acid sequence identity of 50, 44, and 45% to human LAT-1, y^+LAT-1 and y^+LAT-2, respectively. Hydrophobicity studies show 12 transmembrane domains with both C- and N-terminal segments intracytoplasmatic, which is the same protein structure suggested for the other members of this family (1–3, 19). Only the consensus for the position of the transmembrane segment III can vary for the proteins presented in Fig. 2. There is only one putative N-glycosylation site (Fig. 2, *boxed*) between the putative transmembrane segments VIII and IX. In our predicted model this segment is cytoplasmic and cannot be glycosylated. This finding is in full agreement with previous expression studies with rat and human LAT-1 (1–2). 4F2hc is associated with its subunits in a disulfide bond-dependent manner (2–4, 22) through cysteine residue 109 of human 4F2hc (18) and cysteine residue 164 of *Xenopus* LAT-1 (23). This cysteine residue is conserved in all human 4F2 light chains including LAT-2 (cysteine residue 154) (Fig. 2).

The human *LAT-2* gene was chromosome-mapped by using a radiation hybrid panel (see "Experimental Procedures") with primers corresponding to the 3′-untranslated region of the LAT-2 cDNA. From this screening we obtained 16 positive and 66 negative results. Chromosome mapping results, obtained from the SGHC server, linked Lat-2, with a logarithem odds score of 12.6, to a distance of 13 cR (286 kb) from the marker SHGC-13507 (D14S1349). The nearest centromeric marker to this one, marker SHGC-6999 (*X*52889), is located at chromosome 14q11.2–13.

cRNA from LAT-2 was injected into oocytes alone or in combination with an equimolar quantity of human 4F2hc cRNA and tested for amino acid transport (Fig. 3). 4F2hc alone induced, as previously reported (16, 18, 24–27), y^+L amino acid transport activity (*i.e.* sodium-independent L-arginine transport and sodium-dependent L-leucine transport). LAT-2 alone induced weakly sodium-independent L-leucine transport. Interestingly, when 4F2hc and LAT-2 were co-injected, the induction of L-arginine transport was lower than that induced by 4F2hc alone, whereas the induction of sodium-independent L-leucine transport increased dramatically (Fig. 3). From four independent experiments the average co-expression of L-leucine transport relative to the induction of 4F2hc alone was 30-fold (ranging from 6- to 100-fold). The co-expression of leucine transport by 4F2hc and LAT-2 is sodium-independent, suggesting induction of a system L-type amino acid transport activity (Fig. 3). Kinetic analysis revealed an apparent K_m of 221 ± 54 μM for the transport of L-leucine induced by 4F2hc/

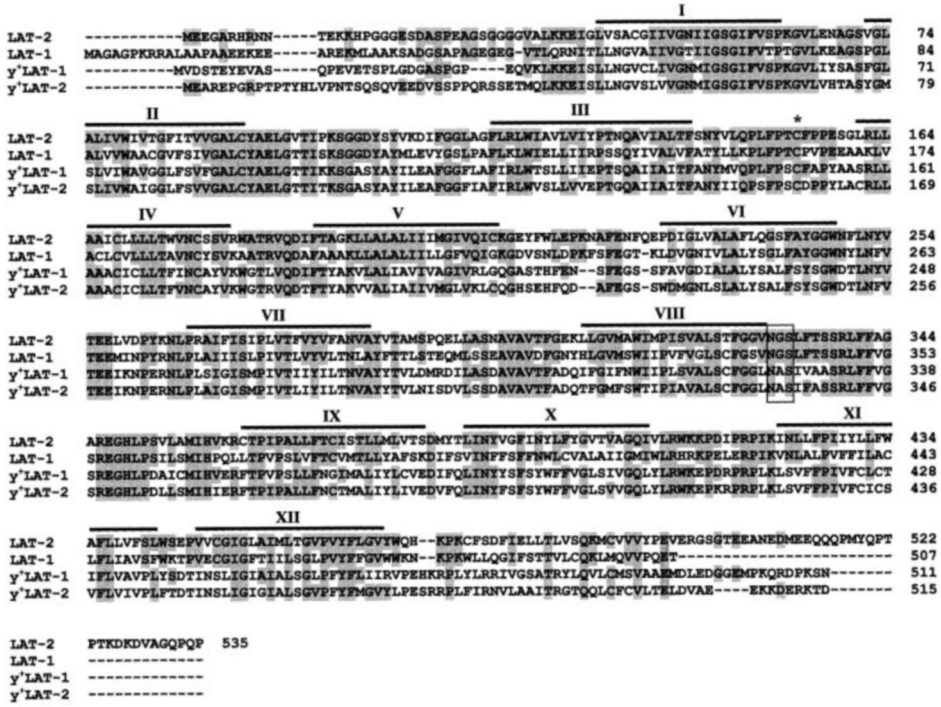

FIG. 2. **Amino acid sequence comparison of the four human members of the family of amino acid transporters that are subunits of 4F2hc.** Multialignment was done using the program CLUSTALW Sequence Alignment from the Baylor College of Medicine. The *thin horizontal lines* indicate the 12 putative transmembrane domains determined by computer analysis (see "Experimental Procedures"). The amino acid residues identical to LAT-2 sequence are indicated in *gray boxes*. The *solid frame box* indicates a potential N-glycosylation site, but according to our membrane topology prediction, this site is intracellular and cannot be glycosylated. The conserved cysteine residue is indicated with a *star*.

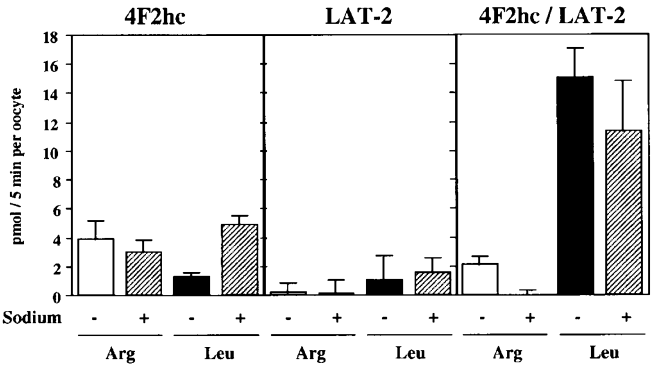

FIG. 3. **Co-expressed amino acid transport activity by 4F2hc and LAT-2.** Oocytes were injected with LAT-2 cRNA alone or in combination with human 4F2hc cRNA. Three days after the injection, the uptake of 50 μM L-[^{3}H]arginine (*Arg*) and 50 μM L-[^{3}H]leucine (*Leu*) in the presence (+, *slashed bars*) or absence (−, *open* or *closed bars*) of 100 mM NaCl was determined for 5 min. Transport of L-[^{3}H]leucine in the absence of sodium is *highlighted* in the *closed bars*. Amino acid uptake rates (pmol/5 min per oocyte) were calculated by subtracting the uptake of the noninjected group from that of the cRNA-injected groups. The amino acid uptake activity of uninjected oocytes was as follows: L-[^{3}H]arginine uptake, 3.0 ± 0.2 (choline medium) and 4.0 ± 0.7 (sodium medium); L-[^{3}H]leucine uptake, 1.8 ± 0.2 (choline medium) and 3.3 ± 0.4 (sodium medium). Data (mean ± S.E.) correspond to a representative experiment with 7–8 oocytes per group.

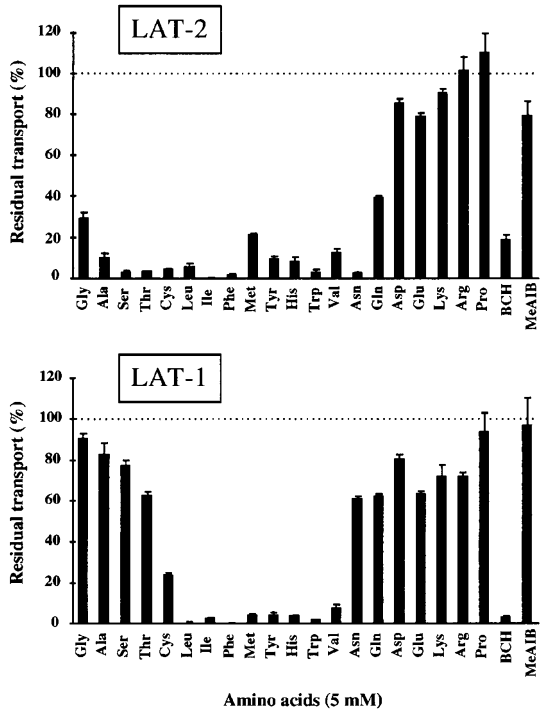

FIG. 4. **Inhibition pattern of the amino acid transport activity co-expressed by 4F2hc and LAT-2.** Two or 3 days after injection of 4F2hc cRNA together with LAT-2 or LAT-1 cRNA, the uptake of 50 μM L-[^{3}H]leucine in the absence of sodium was measured for 5 min in the absence (Control) or in the presence of the indicated amino acids or analogs at 5 mM. The expressed transport (*i.e.* subtracting transport of noninjected oocytes) in control groups was 15.3 ± 1.5 and 26.6 ± 4.0 pmol/5 min per oocyte for 4F2hc/LAT-2- and 4F2hc/LAT-1-injected oocytes, respectively. Data (mean ± S.E.) represent percentages of the amino acid residual transport in the presence of inhibitors. Transport of L-[^{3}H]leucine in noninjected oocytes was 1.6 ± 0.2 pmol/5 min per oocyte. Data correspond to two to four independent experiments, in which 7–8 oocytes were used per group in each experiment.

LAT-2 (data not shown).

To further characterize the uptake activity co-expressed by LAT-2 and 4F2hc, we measured the inhibition of sodium-independent leucine uptake by different amino acids at a 100-fold excess concentration (5 mM). Fig. 4 shows the inhibition pattern for the transport activity induced by LAT-2 and 4F2hc compared with that induced by *X. laevis* LAT-1 and 4F2hc. These results showed clearly that the transport activity induced by 4F2hc/LAT-2 and 4F2hc/LAT-1 is restricted to zwitterionic amino acids. The pattern of inhibition in the case of *X. laevis* LAT-1, restricted to large zwitterionic amino acids and analogs (*i.e.* BCH), is in full agreement with the pattern described for rat LAT-1 (1). In contrast, 4F2hc/LAT-2-induced transport activity was also practically abolished by small zwitterionic amino acids (*i.e.* glycine, alanine, serine, threonine, and cysteine), and it is clearly inhibited by glutamine and asparagine. To demonstrate transport of small zwitterionic amino acids via

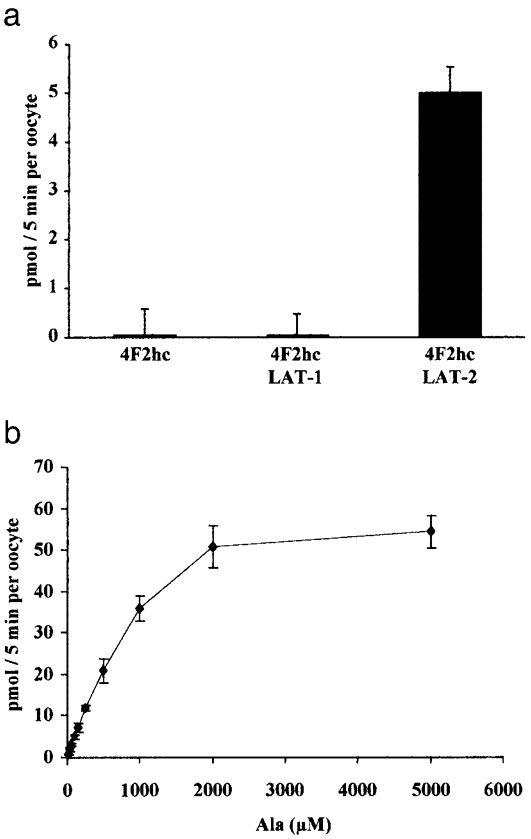

FIG. 5. **4F2hc/LAT-2 co-expresses alanine transport in oocytes.** *a*, oocytes were injected with 4F2hc cRNA alone or in combination with LAT-1 or LAT-2 cRNA. Three days after the injection, the uptake of 50 μM L-[^{3}H]alanine in the absence of sodium was determined for 5 min. Transport of noninjected oocytes was 6.5 ± 1.1 pmol/5 min per oocyte. Data (mean ± S.E.) correspond to a representative experiment with 7–8 oocytes per group. Another two independent experiments showed similar results. *b*, kinetic analysis of L-alanine transport co-expressed by 4F2hc/LAT-2. Oocytes were injected with 4F2hc and LAT-2 cRNA. Two days after the injection, the uptake of L-[^{3}H]alanine in the absence of sodium was determined for 5 min at different substrate concentrations (10, 25, 50, 100, 150, 250, 500, 1000, 2000, and 5000 μM). The transport activity level in noninjected oocytes was subtracted from that of cRNA-injected oocytes. Data (mean ± S.E.) correspond to a representative experiment with 5–6 oocytes per group. Kinetic parameters were: V_{max} = 64 ± 7 pmol/5 min per oocyte, K_m = 978 ± 143 μM, and r (correlation coefficient) = 0.92 ($p \leq 0.001$).

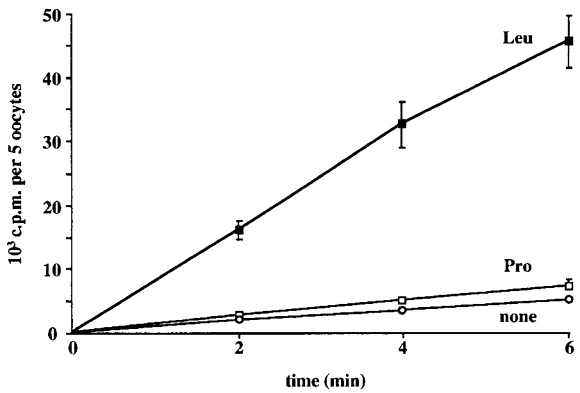

FIG. 6. ***Trans*-stimulation of efflux via 4F2hc/LAT-2 system L.** Oocytes were injected with 4F2hc cRNA alone or in combination with LAT-2 cRNA. Three days after the injection, oocytes were loaded with 50 μM L-[^{3}H]isoleucine for 60 min reaching the following uptake level of radioactivity: 21900 ± 1900, 3700 ± 400, and 2200 ± 400 cpm per oocyte in 4F2hc/LAT-2-injected, 4F2hc-injected, and noninjected oocytes, respectively. The efflux of radioactivity was then measured in the indicated periods of time in media containing 1 mM L-leucine (*Leu*, ■) or proline (*Pro*, □) or no amino acids (*none*, ○). Data (mean ± S.E.) correspond to a representative experiment with three groups of 5 oocytes per data point. The efflux rates in 4F2hc-injected and noninjected oocytes in the presence of 1 mM L-leucine in the medium were 1630 ± 200 and 1350 ± 100 × 10^3 cpm/5 oocytes per min, respectively. These efflux rates are indistinguishable from those of 4F2hc/LAT-2-injected oocytes in medium containing 1 mM proline or no amino acids.

this variant of system L, the uptake of 50 μM L-[^{3}H]alanine was determined in oocytes expressing 4F2hc/LAT-2 or 4F2hc/LAT-1. Interestingly, co-expression of 4F2hc/LAT-2 in oocytes, but not of 4F2hc/LAT-1, resulted in the induction of L-alanine transport above background (*i.e.* noninjected or 4F2hc-injected oocytes) (Fig. 5*a*). Kinetic analysis of this transport revealed an apparent K_m of 978 ± 142 μM (Fig. 5*b*). Similarly to alanine, 4F2hc/LAT-2 induced sodium-independent L-glutamine transport. Two days after injection, the induced uptake of 200 μM L-[^{3}H]glutamine was 0.3 ± 0.4 and 34.6 ± 4.0 pmol/5 min per oocyte for 4F2hc- and 4F2hc/LAT-2-injected oocytes, respectively. All of the above suggests that 4F2hc/LAT-2 represents a broad specificity variant of system L transporter for small and large zwitterionic amino acids.

In agreement with previous reports for the transport activity induced by 4F2hc in oocytes (18, 20) by LAT-1/4F2hc (1–2) and y^+LAT-1/4F2hc (4), LAT-2/4F2hc showed a high level of *trans*-stimulation. Fig. 6 shows that the efflux of L-[^{3}H]isoleucine in oocytes expressing LAT-2/4F2hc is dependent on the presence of a substrate in the medium (*e.g.* leucine), but it is not *trans*-stimulated by amino acids that are not substrates (*e.g.* proline) of LAT-2/4F2hc amino acid transporter. The level of efflux in $trans_0$ conditions (no amino acid substrates in the medium) in oocytes expressing LAT-2/4F2hc is identical to that of noninjected oocytes or oocytes expressing 4F2hc alone (Fig. 6). This result suggests a high level of exchanger coupling via LAT-2/4F2hc.

Recently, it has been shown that y^+LAT-1, LAT-1, and SPRM1 form a disulfide bond heterodimeric complex with 4F2hc (2–4, 22, 23). Moreover, Verrey and co-workers (2) have shown that 4F2hc brings SPRM1 to the oocyte plasma membrane. Fig. 7 shows that 4F2hc also brings LAT-2 to the oocyte plasma membrane. To follow the expression of LAT-2, a tagged LAT-2 cRNA (N-*myc*-LAT-2) was expressed in oocytes. N-*myc*-LAT-2 co-expresses with 4F2hc L-transport activity, but N-*myc*-LAT-2 alone does not induce amino acid transport activity (see legend to Fig. 7). Confocal immunofluorescence detected N-*myc*-LAT-2 at the oocyte plasma membrane when co-expressed with 4F2hc, but its expression was intracellular when expressed alone in oocytes (Fig. 7).

The tissue expression of the mRNA corresponding to LAT-2 was examined by Northern blot analysis at high stringency conditions (Fig. 8). mRNA species of ~5 and ~3.7 kb hybridize with the LAT-2 cDNA; the size of the shorter transcript corresponds to that of the LAT-2 cDNA identified here. Both transcripts are expressed most conspicuously in the kidney. Placenta ≫ brain, liver > skeletal muscle, and heart also express these transcripts. The last two also showed a very faint band of ~7 kb. Long exposures also revealed the 5- and 3.7-kb transcripts in the small intestine and the lung. *In situ* hybridization studies specifically localized the renal expression of LAT-2 mRNA to the epithelial cells of proximal tubules, most probably in the convoluted part (Fig. 9). No other components of the nephron, including distal tubules and glomeruli, were reactive with LAT-2 cRNA probe. A similar pattern of expression has been shown on inmunolocalization of 4F2hc protein on kidney cortex (Ref. 28, and data not shown).

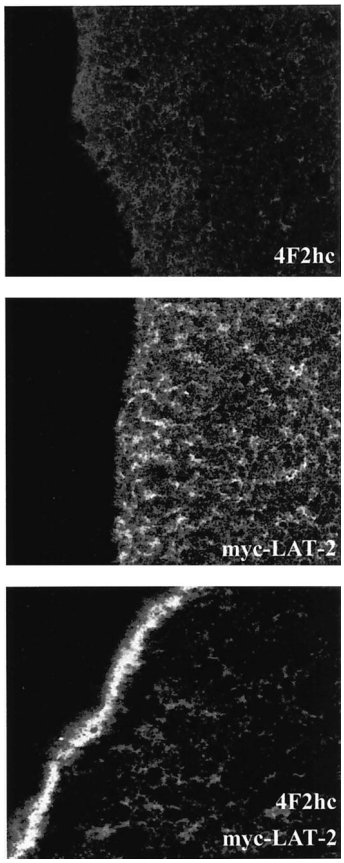

FIG. 7. **Localization of N-*myc*-LAT-2 in oocytes.** Oocytes were injected with *myc*-tagged LAT-2 or 4F2hc cRNA alone or in combination. Three days later oocytes were processed for immunocytochemistry with mAb 9E10 anti-*myc* as primary antibody and Texas red-conjugated goat anti-mouse as secondary antibody (see "Experimental Procedures"). Micrographs show that 4F2hc brings N-*myc*-LAT-2 to the oocyte plasma membrane. The *myc*-immunodetected signal (*white*) is visible inside the oocyte when expressed alone, but it is at the plasma membrane when co-expressed with 4F2hc. The signal is almost absent in 4F2hc-injected oocytes. Two days after injection the induced uptake of 100 μM L-[^{3}H]leucine in pmol/5 min per oocyte was 0.2 ± 1.0 for N-*myc*-LAT-2, 0.9 ± 0.3 for 4F2hc, 37.8 ± 3.9 for 4F2hc/LAT-2, and 19.2 ± 3.3 for 4F2hc/N-*myc*-LAT-2.

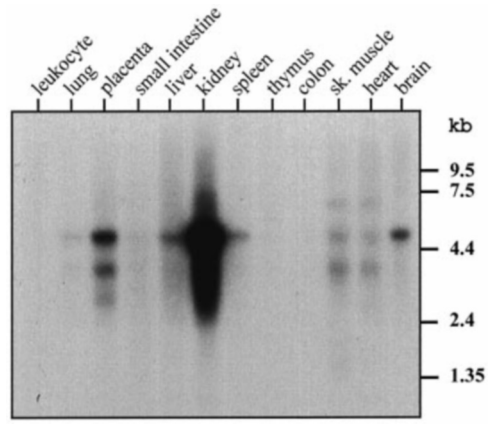

FIG. 8. **Northern blot analysis for LAT-2 mRNA in human tissues.** A poly(A) RNA membrane (2 μg per lane) containing 12 different human adult tissues was purchased from CLONTECH. Blots were probed with ^{32}P-labeled human IMAGE clone 267204 and washed at high stringency conditions (see "Experimental Procedures"). Human LAT-2 cDNA hybridizes to transcripts of ~5 and ~3.7 kb and is expressed in kidney ⋙ placenta ≫ brain, liver > spleen, skeletal muscle, heart, small intestine, and lung. A transcript of ~7 kb is also visible in skeletal muscle and heart. The leukocyte sample is from peripheral blood.

DISCUSSION

In this study we have identified a new member (LAT-2) of the family of amino acid transporters, which are subunits of 4F2hc and in humans are composed also of LAT-1, y^+LAT-1, and y^+LAT-2. We report here on the human LAT-2 cDNA sequence, chromosomal location, and pattern of expression of its mRNA. Moreover, we show that 4F2hc brings LAT-2 to the oocyte plasma membrane, which induces a system L amino acid transport activity with broad specificity. Therefore, LAT-2 is a putative new light subunit of the surface antigen 4F2.

Before the identification of the 4F2hc subunits, functional expression experiments in oocytes revealed that 4F2hc induced both system y^+L (16, 24–27) and system L (29) transport activities. In agreement with this finding, the 4F2hc subunits y^+LAT-1, y^+LAT-2, LAT-1, and LAT-2 are isoforms of systems y^+L and L, respectively. Before our cloning of system y^+L, there were no reports of variants of system y^+L in the literature (30). y^+LAT-1 is defective in lysinuric protein intolerance (5, 6), and y^+LAT-2 might be responsible for system y^+L in the cell types in which this transport activity is not defective in lysinuric protein intolerance (*e.g.* erythrocytes (31)). In contrast, system L variants L1 (substrate affinity in the micromolar range) and L2 (substrate affinity in the millimolar range) have been described previously (10). These subtypes are expressed in hepatoma cell lines and hepatocytes, respectively (11). An L3 subtype was described in fibroblasts with an affinity between that of the L1 and L2 subtypes (12). LAT-1 fits the transport characteristics and the tissue distribution of subtype L1 (Ref. 1 and present study). LAT-2 shows characteristic features of system L (*i.e.* sodium-independent transport of zwitterionic amino acids inhibitable by the analog BCH), but it also shows features that are dissimilar to both system L subtypes. Thus, LAT-2 is expressed in the liver and has a substrate affinity in the micromolar range for L-leucine (K_m ≈220 μM). This is a lower affinity than has been described for rat (18 μM) and *Xenopus* (32 μM) LAT-1 (1, 2). Moreover, in contrast to the hepatic system L, LAT-2 also transports small zwitterionic amino acids (*e.g.* L-alanine with an apparent K_m ≈ 1 mM). A transport system with similar characteristics (sodium-independent, *trans*-stimulated transport for large and small zwitterionic amino acids and a similar apparent K_m for L-alanine) to that of LAT-2 has been described in the basolateral membrane of the intestinal enterocyte (32, 33) and the placental syncytiotrophoblast (34).

What is the physiological role of a system L transporter with broad specificity for zwitterionic amino acids, including the small ones? Christensen (10) hypothesized that system L serves the exchange (efflux and influx) of zwitterionic amino acids through the plasma membrane to fulfill the inter-organ fluxes of these amino acids (10). The transport activity induced by 4F2hc/LAT-2 is highly *trans*-stimulated in oocytes. Indeed, LAT-2 behaves as an exchanger, because the efflux via this transporter in oocytes is totally dependent on the presence of a substrate in the medium. The kidney showed the highest LAT-2 mRNA expression (present study), whereas LAT-1, the other system L isoform transporter, is not expressed in the kidney (1). The involvement of *rBAT* and *y^+LAT-1* in Type I cystinuria and lysinuric protein intolerance, respectively (5, 6, 9), demonstrates the role of these transporters in the apical reabsorption of cystine and dibasic amino acids (rBAT) and in the basolateral efflux of dibasic amino acids (4F2hc/y^+LAT-1). LAT-2 mRNA is restricted to the epithelial cells of the proximal tubule of the human kidney (present study). In these cells 4F2hc has a basolateral location (28). This result suggests that

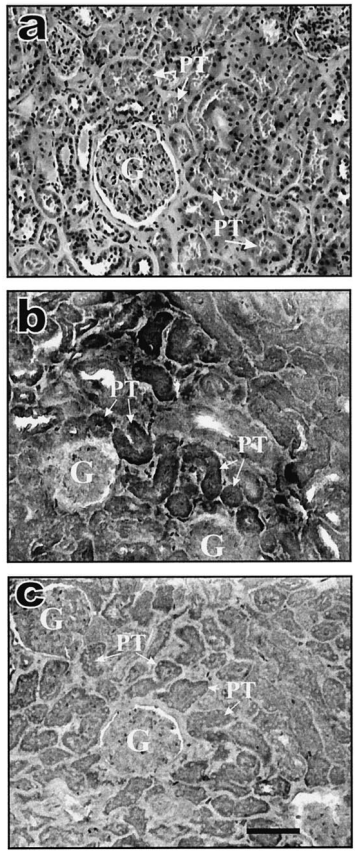

FIG. 9. ***In situ*** **hybridization of LAT-2 mRNA in human adult kidney.** Serial paraffin-embedded sections of human kidney cortex were stained with hematoxylin-eosin (*a*) or incubated with antisense (*b*) or sense (*c*) LAT-2 cRNA probes as described under "Experimental Procedures." Results are representative of two independent experiments. LAT-2 mRNA-specific detection is restricted to proximal tubule (*PT*) epithelial cells (some proximal tubules are indicated by *arrows*). The proximity of these tubules to the glomerulus (*G*) suggests a localization of LAT-2 mRNA signal in the proximal convoluted part. No other specific signal was detected in the renal cortex. *Bar* =100 μm.

4F2hc/LAT-2 might mediate the efflux of zwitterionic amino acids, including those with a short side chain, through the basolateral plasma membrane. Moreover, the fact that cysteine, the main intracellular form of cystine (35, 36), strongly inhibits 4F2hc/LAT-2 transport activity suggests the role of this transporter in the renal reabsorption of cystine. The expression of LAT-2 mRNA in the small intestine suggests a similar role in the intestinal absorption of zwitterionic amino acids. The expression of LAT-2 mRNA in the placenta also implicates it in the transfer of zwitterionic amino acids from the placenta to the fetus. This hypothesis needs confirmation by the localization of LAT-2 at the basolateral plasma membrane in the epithelial cells of the proximal tubule and in the placental trophoblast. The expression of LAT-2 mRNA in the brain, liver, spleen, and skeletal muscle suggests a role in the release of glutamine and short zwitterionic amino acids from these tissues as follows: (i) glial cells release glutamine to neurons as a substrate for glutamate and γ-aminobutyric acid synthesis (37); (ii) hepatocytes in the perivenous zone release glutamine as an ammonium detoxification pathway (38); (iii) the skeletal muscle releases most of the aminic nitrogen as glutamine and alanine (39); and (iv) spleen and small intestinal enterocytes metabolize glutamine and produce alanine and other small zwitterionic amino acids (*e.g.* serine and glycine) (40). Identification of the cells expressing LAT-2 in these tissues will help us to understand the role of this system L amino acid transporter with broad specificity.

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