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Reabsorption of Neutral Amino Acids Mediated by Amino Acid Transporter LAT2 and TAT1 in The Basolateral Membrane of Proximal Tubule

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In order to understand the renal reabsorption mechanism of neutral amino acids *via* amino acid transporters, we have isolated human L-type amino acid transporter 2 (hLAT2) and human T-type amino acid transporter 1 (hTAT1) in human, then, we have examined and compared the gene structures, the functional characterizations and the localization in human kidney. Northern blot analysis showed that hLAT2 mRNA was expressed at high levels in the heart, brain, placenta, kidney, spleen, prostate, testis, ovary, lymph node and the fetal liver. The hTAT1 mRNA was detected at high levels in the heart, placenta, liver, skeletal muscle, kidney, pancreas, spleen, thymus and prostate. Immunohistochemical analysis on the human kidney revealed that the hLAT2 and hTAT1 proteins coexist in the basolateral membrane of the renal proximal tubules. The hLAT2 transports all neutral amino acids and hTAT1 transports aromatic amino acids. The basolateral location of the hLAT2 and hTAT1 proteins in the renal proximal tubule as well as the amino acid transport activity of hLAT2 and hTAT1 suggests that these transporters contribute to the renal reabsorption of neutral and aromatic amino acids in the basolateral domain of epithelial proximal tubule cells, respectively. Therefore, LAT2 and TAT1 play essential roles in the reabsorption of neutral amino acids from the epithelial cells to the blood stream in the kidney. Because LAT2 and TAT1 are essential to the efficient absorption of neutral amino acids from the kidney, their defects might be involved in the pathogenesis of disorders caused by a disruption in amino acid absorption such as blue diaper syndrome.

Key words: L-Type amino acid transporter 2 (LAT2), T-Type amino acid transporter 1 (TAT1), Amino acids, Reabsorption mechanism, Kidney

INTRODUCTION

Amino acid transport across the plasma membrane is needed in order for the cells to be supplied with amino acids for cellular nutrition (Christensen, 1990). This process is mediated *via* the amino acid transporters that are responsible for the translocation of amino acids through the membrane (Kanai and Endou, 2001). In the epithelia of the kidney and small intestine, distinct amino acid transporters develop in the apical and basolateral membranes of the epithelial cells, which ensure the vectorial transport of the amino acids through the epithelia (Stevens *et al.*, 1984).

Previously, a large number of amino acid transport systems in mammals were distinguished based on the differences in the substrate selectivity and ion dependence (Christensen, 1990). Over the 10 years, molecular cloning techniques have been successfully used to reveal the nature of the amino acid transport systems (Palacin *et al.*, 1998). Currently, most transport systems have been identified at the molecular level. These transporters are

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classified into several transporter families based on the differences in the substrate selectivity and ion dependence. The transporter families include Na^+-dependent and Na^+-independent neutral amino acid transporters, Na^+-dependent and Na^+-independent basic amino acid transporters and Na^+-dependent and Na^+-independent acidic amino acid transporters (Kanai and Endou, 2001). Although it is known that the amino acid transporters have differences in the apical and basolateral membranes of the epithelial cells, which ensures the vectorial transport of amino acids through the epithelia, the molecular characterization of the amino acid transporters in addition to the role of amino acid transporters in kidney are unclear.

Among the amino acid transport systems, the system L amino acid transporter transports neutral amino acids, which comprise most of the amino acids with the exception of basic amino acids such as lysine and arginine and acidic amino acids such as glutamate and aspartate (Oxender and Christensen, 1963; Christensen, 1990). The system L amino acid transporter is a Na^+-independent neutral amino acid transport system, which is the major route for providing living cells including tumor cells with neutral amino acids including several essential amino acids (Oxender and Christensen, 1963; Christensen, 1990). Because of its broad substrate selectivity, the system L amino acid transporter is regarded as a drug transporter, which transports not only naturally occurring amino acids but also the following amino acid-related drugs: L-dopa, a therapeutic drug for Parkinsonism; melphalan, an anticancer phenylalanine mustard; triiodothyronine and thyroxine, two thyroid hormones; gabapentin, an anticonvulsant; and *S*-(1,2-dichlorovinyl)-L-cysteine, a neurotoxic cysteine conjugate (Goldenberg *et al.*, 1979; Christensen, 1990; Lakshmanan *et al.*, 1990; Blondeau *et al.*, 1993; Su *et al.*, 1995; Gomes and Soares-da-Silva, 1999; Kanai and Endou, 2001).

By means of expression cloning, we isolated a rat L-type amino acid transporter 1 (rLAT1), the first isoform of the system L amino acid transporter, from C6 rat glioma cells (Kanai *et al.*, 1998). In addition, we isolated and characterized a human LAT1 (hLAT1) from PA-1 human teratocarcinoma cells (Yanagida *et al.*, 2001). The LAT1 is a predicted 12-membrane-spanning protein (Kanai *et al.*, 1998; Yanagida *et al.*, 2001), which mediates Na^+-independent amino acid exchange and prefers large neutral amino acids with bulky or branched side chains as its substrates (Kanai *et al.*, 1998; Yanagida *et al.*, 2001). The LAT1 requires an additional single-membrane-spanning protein the heavy chain of the 4F2 antigen (4F2hc) for its functional expression in the plasma membrane (Kanai *et al.*, 1998; Mannion *et al.*, 1998; Yanagida *et al.*, 2001). LAT1 and 4F2hc form a heterodimeric complex *via* a disulfide bond (Kanai *et al.*, 1998; Mannion *et al.*, 1998; Yanagida *et al.*, 2001). The LAT1 mRNA is only expressed in restricted organs such as the brain, spleen, placenta and testis (Kanai *et al.*, 1998; Nakamura *et al.*, 1999; Prasad *et al.*, *1999*; Yanagida *et al.*, 2001). However, the 4F2hc mRNA is ubiquitously expressed in all embryonic and adult tissues (Kanai *et al.*, 1998; Nakamura *et al.*, 1999; Yanagida *et al.*, 2001). In addition, the LAT1 is highly expressed in malignant tumors presumably to support their continuous growth and proliferation (Sang *et al.*, 1995; Wolf *et al.*, 1996; Kanai *et al.*, 1998; Yanagida *et al.*, 2001).

Subsequently, we cloned a rat L-type amino acid transporter 2 (rLAT2), the second isoform of system L amino acid transporter, from the rat small intestine (Segawa *et al.*, 1999). In addition, the human L-type amino acid transporter 2 (hLAT2) was isolated (Pineda *et al.*, 1999). The LAT2 is also a Na^+-independent amino acid transporter, which requires the 4F2hc for its functional expression (Pineda *et al.*, 1999; Segawa *et al.*, 1999). Although the hLAT2 was cloned, the tissue distributions in human, the localization in human tissues and the transport mechanisms are unclear.

The system T amino acid transporter is a plasma membrane amino acid transport system that mediates the Na^+-independent transport of aromatic amino acids across the plasma membrane (Rosenberg *et al.*, 1980; Christensen, 1990; van Winkle *et al.*, 1990). Recently, we have identified the transporters subserving system T in rat and human (Kim *et al.*, 2001, 2002b). The transporters, which were designated the rat T-type amino acid transporter 1 (rTAT1) and human T-type amino acid transporter 1 (hTAT1), are predicted to be 12-membrane-spanning proteins (Kim *et al.*, 2001, 2002b). When expressed in *Xenopus laevis* oocytes, TAT1 exhibits the Na^+-independent facilitative transport of aromatic amino acids such as tryptophan, phenylalanine and tyrosine, which is consistent with the properties of system T amino acid transporter (Kim *et al.*, 2001, 2002b).

It is known that the system L amino acid transporter and the system T amino acid transporter play an important role in the absorption of neutral amino acids in the small intestine and kidney (Silbernagl, 1979; Christensen, 1990; Kanai and Endou, 2001). However, the absorption mechanisms of the neutral amino acids in the small intestine and kidney are unknown.

Therefore, this study investigated the absorption mechanisms of neutral amino acids *via* the system L amino acid transporter and the system T amino acid transporter in the kidney. To accomplish this, the cDNAs of the system L amino acid transporter and the system T amino acid transporter in human were cloned and the gene structures, the functional characterizations and the localization in the human kidney were examined and compared.

MATERIALS AND METHODS

Materials

The radiolabeled amino acids were purchased from Perkin-Elmer Life Sciences Inc. (Boston, MA, USA). The affinity-purified anti-LAT2 and anti-TAT1 polyclonal antibodies were kindly provided by Kumamoto Immunochemical Laboratory, Transgenic Inc. (Kumamoto, Japan). Other chemicals were purchased from Sigma (St. Louis, MO, USA).

Identifications and sequencings of human LAT2 (hLAT2) and human TAT1 (hTAT1)

The cDNA for LAT2 for a human expressed sequence tag (EST) (GenBankTM/EBI/DDBJ accessions no. N32639), which we found in the course of EST database searches to show nucleotide sequence similarity to rat LAT1, was obtained from the Integrated and Molecular Analysis of Genomes and their Expressions (IMAGE). The 0.38 kb cDNA fragment was amplified by polymerase chain reaction (PCR) using the synthetic oligonucleotide primers 5'-CTCTTCACATGCATCTCCAC-3' and 5'-GGTACACGACCACACACATC-3' (corresponding to nucleotides 35-54 and 397-416 of the nucleotide sequence submitted to GenBankTM/EBI/DDBJ under accession no. N32639). The cDNA fragment was labeled with [^{32}P]dCTP (^{T7}QuickPrime, Amersham Pharmacia Biotech, Tokyo, Japan) in order to use it as a probe to screen the nondirectional cDNA library. The cDNA library was prepared from the human kidney poly(A)$^+$RNA (Clontech, Palo Alto, CA, USA) using a superscript Choice System (Life Technologies, Grand Island, NY, USA). The synthesized cDNA was ligated to the lZipLox EcoRI arms (Life Technologies, Grand Island, NY, USA). Screening cDNA library and isolation of positive plques were performed as described elsewhere (Utsunomiya-Tate *et al.*, 1996, 1997). Hybridization was performed in 50% formamide at 37°C in 0.1 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0)/0.1% sodium dodecyl sulfate (SDS). The cDNAs in the positive lZipLox phages were rescued into plasmid pZL1 by *in vivo* excision according to the manufacturer's instruction (Life Technologies, Grand Island, NY, USA). The cDNA was sequenced in both directions by the dye terminator cycle sequencing method (Perkin-Elmer, Norwalk, CT, USA and Applied Biosystems, Foster City, CA, USA). The transmembrane regions of proteins were predicted based on the amino acid sequence using the SOSUI algorithm (Hirokawa *et al.*, 1998).

The cDNA for TAT1 for a human expressed sequence tag (EST) (GenBankTM/EBI/DDBJ accession no. AW058100), which we found in the course of EST database searches to show nucleotide sequence similarity to rat TAT1, was obtained from the Integrated and Molecular Analysis of Genomes and their Expression (IMAGE) (IMAGE clone number 2536142). The cDNA was sequenced in both directions by the dye terminator cycle sequencing method (Perkin-Elmer, Norwalk, CT, USA and Applied Biosystems, Foster City, CA, USA).

Northern bolt analysis

The northern blot analyses were performed as described elsewhere (Lee *et al.*, 2000, 2004) with minor modifications. Human Multiple Tissue Northern (MTM™) blots containing approximately 2 mg of mRNA for each lane isolated from different human tissues were obtained from Clontech (Palo Alto, CA, USA). The *HincII* fragment of hLAT2 cDNA corresponding to the 1126-1591 base pairs for hLAT2 and the *ApaI*fragment of hTAT1 cDNA corresponding to 165-1328 base pairs for hTAT1 were labeled with ^{32}P using a ^{T7}QuickPrime kit (Amersham Pharmacia Biotech, Tokyo, Japan), and used as the probes for the northern bolt analyses. Hybridization and the following washing of the filter were performed according to the manufacturer's instruction (Clontech, Palo Alto, CA, USA).

Anti-peptide antibody

The oligopeptides (EEANEDMEEQQQC) corresponding to the amino acid residues 506-517 of hLAT2 and the oligopeptides (SQEEPDSARGTSEC) corresponding to the amino acid residues 4-16 of hTAT1 were synthesized. The C-terminal cysteine residues were introduced for conjugation with the keyhole limpet hemocyanine. The anti-peptide antibodies were produced as described elsewhere (Altman *et al.*, 1984). For immunohistochemical analysis, the antisera were affinity-purified as described previously (Hisano *et al.*, 1996).

Immunohistochemical analysis

The five-micrometer paraffin sections of the nephrectomized human kidney were processed for light microscopic immunohistochemical analysis as described elsewhere (Hisano *et al.*, 1996; Mizoguchi *et al.*, 2001) with minor modifications. The renal tissues obtained from a tumor patient and approved by the Kyorin University Institutional Research Board (IRB) to be used for medical study. For immunostaining, serial sections (5 mm) were dewaxed, rehydrated and treated with H_2O_2 for 10 min in order to eliminate the endogenous peroxidase activity. Then, the sections were incubated with the affinity-purified anti-hLAT2 (1:250) or anti-hTAT1 (1:100) antibodies overnight at 4°C. Thereafter, they were treated with Envision (+) rabbit peroxidase (Dako, Carpinteria, CA, U.S.A.) for 30 min. To detect immunoreactivity, the sections were treated with 0.8 mM diaminobenzidine. The sections counterstained with hematoxylin. To verify the specificity of immunoreactions by absorption experiments, the tissue sections

were treated with the primary antibodies in the presence of antigen peptides (200 mg/mL).

Xenopus laevis oocyte expression

The cRNAs were obtained by *in vitro* transcription using SP6 RNA polymerase for hLAT2 in the plasmid pSPORT1 linearized with *RsrII* and T7 RNA polymerase for human 4F2hc (h4F2hc) in the plasmid pBluescriptII linearlized *BamHI* as described elsewhere (Kanai and Hediger, 1992).

For expression in *Xenopus laevis* oocytes, the hTAT1 cDNA was subcloned into pcDNA3.1(-) (Invitrogen, Carlsbad, CA, U.S.A.). The hTAT1 cRNA was obtained by *in vitro* transcription using T7 RNA polymerase in the plasmid pcDNA3.1(-) linearized with *EcoRI*.

The *Xenopus* oocyte expression studies and uptake measurements were performed as described elsewhere (Utsunomiya-Tate *et al.*, 1996). Briefly, the oocytes were treated with collagenase A for 30-50 min at room temperature in a Ca^{2+}-free collagenase treatment buffer (OR II buffer containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 5 mM HEPES, pH 7.5) to remove follicular layer and then maintained in a modified Barth's solution (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$ and 5 mM HEPES, pH 7.5). The oocytes were injected with cRNA using a manual injector.

The functional properties of hLAT2 were examined by co-expression with h4F2hc in the *Xenopus* oocytes because LAT2 required 4F2hc for its functional expression in *Xenopus* oocytes as described previously (Pineda *et al.*, 1999; Rossier *et al.*, 1999; Segawa *et al.*, 1999). For the co-expression experiments of hLAT2 and h4F2hc, 17 ng of hLAT2 cRNA and 8 ng h4F2hc cRNA giving the molar ratio of 1:1 were mixed and injected into each oocyte. In the case of hTAT1, the oocytes were injected with 25 ng of hTAT1 cRNA. The uptake experiments of the radiolabeled amino acids were measured 2-3 days after injection of cRNA.

Amino acid uptake measurements in *Xenopus laevis* oocytes

The amino acid uptake measurements in the *Xenopus laevis* oocytes were performed as described elsewhere (Kanai *et al.*, 1998; Segawa *et al.*, 1999; Kim *et al.*, 2001). Before the uptake experiments, groups of 6-8 oocytes were washed in a Na^+-free uptake buffer (100 mM choline-Cl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM Tris, pH 7.4). The functional properties of hLAT2 and hTAT1 were examined in the Na^+-free uptake buffer because it was reported that the amino acid uptakes *via* LAT2 and TAT1 were not dependent on Na^+ in the buffer (Segawa *et al.*, 1999; Kim *et al.*, 2001). The oocytes were then incubated in 500 mL of the uptake buffer containing the indicated concentration of the amino acids and 0.5-3.0 mCi of the corresponding radiolabeled amino acids. Preliminary experiments to determine the time-course of the [^{14}C]L-leucine uptake into the oocyte expressing hLAT2 and h4F2hc, and time-course of [^{14}C]L-tryptophan uptake into the oocyte expressing hTAT1 showed that the uptakes were linearly dependent on the incubation time up to 30 min (data not shown), so for all the following experiments, uptakes were measured for 30 min. The oocytes were then washed 5 - 6 times with ice-colded uptake buffer and distributed to scintillation vials separately. After lysis of oocytes in the 10 % SDS solution, the radioactivity was counted by liquid scintillation, and the values were expressed as pmol/oocyte/min.

The substrate selectivity of hLAT2 was investigated by the inhibition experiments in which the uptake of 50 μM [^{14}C]L-leucine was measured in the presence of 5 mM of the non-labeled amino acids. In the case of hTAT1, the substrate selectivity of hTAT1 was investigated by the inhibition experiments in which 10 μM [^{14}C]L-tryptophan uptake was measured in the presence of 10 mM of the non-labeled amino acids.

The K_m and V_{max} of the amino acid substrates were determined using the Eadie-Hofstee equation based on the hLAT2-mediated amino acid uptakes measured at 0.003, 0.01, 0.03, 0.1, 0.3, 1, and 3 mM. The K_m and V_{max} of the amino acid substrates were determined using the Eadie-Hofstee equation based on the hTAT1-mediated amino acid uptakes measured at 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 mM. The hLAT2 and hTAT1 mediated amino acid uptakes were calculated as the differences between the means of the uptakes into the oocytes, which had been injected with the cRNA for either hLAT2 or hTAT1 and those of the control oocytes injected with water.

For the measurements of the uptake of radiolabeled amino acids in the present study, six to eight oocytes were used for each data point. Each datum in the figures represents the min ± S.E.M. of the uptake values (n = 6 – 8). In order to confirm the reproducibility of the results, three separate experiments were performed for each measurement using different batches of oocytes and *in vitro* transcribed cRNAs. The results from the representative experiments are shown in the figures.

Chromosomal localization by fluorescence *in situ* hybridization mapping for hLAT2

Fluorescence *in situ* hybridization (FISH) detection was performed as described elsewhere (Heng *et al.*, 1992; Heng and Tsui, 1993). The full cDNA insert of hLAT2 was used as a probe. The probe was labeled with biotinylated dUTP (the BRL BioNick labeling system) (Heng *et al.*, 1992). Chromosomes were stained before anlysis with

propidium iodide as a counterstain and 4,6-diamino-2-phenylindole (DAPI) for chromosome identification. The FISH signals and the DAPI banding pattern were separately recorded by taking photographs. The assignment of the FISH mapping data with the chromosomal bands was achieved by superimposing the FISH signals with the DAPI banded chromosomes.

RESULTS

Structural feature of hLAT2

The hLAT2 cDNA (4215 bp) contained an open reading frame from the nucleotides 731 to 2335 encoding a 536-amino acid protein as described elsewhere (Pineda *et al.*, 1999). The hLAT2 and rat LAT2 (rLAT2) were highly conserved with an amino acid sequence identity of 92% (data not Shown). The 12-transmembrane regions were predicted based on the hLAT2 amino acid sequence. There was a conserved cysteine residue (hLAT2 amino acid residue 155) in the putative extracellular loop between the predicted transmembrane domains 3 and 4, which is supposed to link to 4F2hc by a disulfide bond (Pfeiffer *et al.*, 1998). A tyrosine kinase-dependent phosphorylation site was predicted at the amino acid residue 110 in the putative intracellular loop between the predicted transmembrane domain 2 and 3, which is conserved the LAT1 but not for the system y^+L transporters, y^+LAT1 and KIAA0245/y^+LAT2 (Segawa *et al.*, 1999).

The structural features of hTAT1 were described in our previous paper (Kim *et al.*, 2002b).

Tissue distribution of hLAT2 and hTAT1

The northern blot analyses were examined using commercially available multiple human tissue blots (Clontech, Palo Alto, CA, USA). In most of the tissues examined, the positive signal for the hLAT2 transcript was ubiquitously detected (Fig. 1A). The 4.4 kb message was expressed at high levels in the heart, brain, placenta, kidney, spleen, prostate, testis, ovary, lymph node, thymus and the fetal liver (Fig.1A).

In the case of hTAT1, the northern blot analysis indicated that 2.6 kb hTAT1 message was expressed at high levels in the heart, placenta, liver, skeletal muscle, kidney, pancreas, spleen, thymus, testis and fetal liver (Fig. 1B). On longer exposure, faint message were also detected in the lung, prostate, ovary, small intestine and lymph node (Fig. 1B). In heart, placenta, liver, skeletal muscle, kidney, pancreas, spleen, thymus and fetal liver, an additional 4.4 and 9.5 kb messages were also detected (Fig. 1B).

Immunolocalizations of hLAT2 and hTAT1

Immunohistochemical analysis of human kidney tissue revealed a low magnification view showing that the hLAT2 (Fig. 2A) and hTAT1 (Fig. 2B) immunoreactivities were located in the renal cortex. The higher magnifications showed that the hLAT2 (Fig. 2C) and hTAT1 (Fig. 2D) immunoreactivities were located in the proximal tubule of the human kidney. The high magnification view showed that the hLAT2 (Fig. 2E) and hTAT1 (Fig. 2F) immunoreactivities were observed in the basolateral membrane but not in the apical membrane. In the absorption experiments in which the tissue sections were treated with the primary antibodies in the presence of antigen peptides, the immunostaining was not detected, confirming the specificity of the immunoreactions (data not shown).

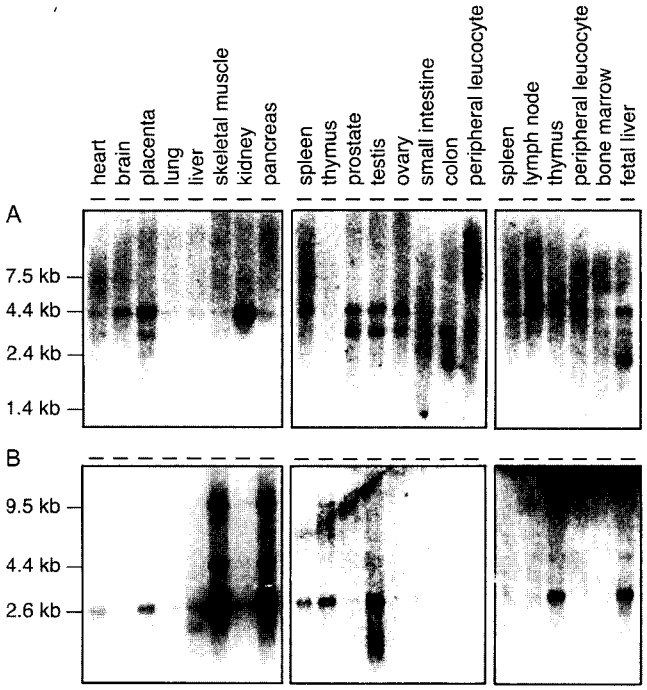

Fig. 1. Tissue distribution of hLAT2 and hTAT1 mRNA. Northern blot analysis of poly(A)$^+$RNA (2 μg) from the human tissues for hLAT2 and hTAT1. **(A)** High stringency northern hybridization analysis using a hLAT2 probe was performed against the poly(A)$^+$RNA membrane (2 μg per lane) containing the human tissues (Clontech, Palo Alto, CA, USA). The 4.4 kb message was expressed at high levels in the heart, brain, placenta, kidney, spleen, prostate, testis, ovary, lymph node, thymus and the fetal liver. **(B)** High stringency northern hybridization analysis using a hTAT1 probe was performed against the poly(A)$^+$RNA membrane (2 μg per lane) containing the human tissues (Clontech, Palo Alto, CA, USA). The hybridization signals (2.6, 4.4 and 9.5 kb) were detected in the heart, placenta, liver, skeletal muscle, kidney, pancreas, spleen, thymus, testis and fetal liver. Upon longer exposure, the faint message was also detected in the lung, prostate, ovary, small intestine and lymph node.

Functional expressions of hLAT2 and hTAT1 in *Xenopus laevis* oocytes

The substrate selectivities of hLAT2 and hTAT1 were

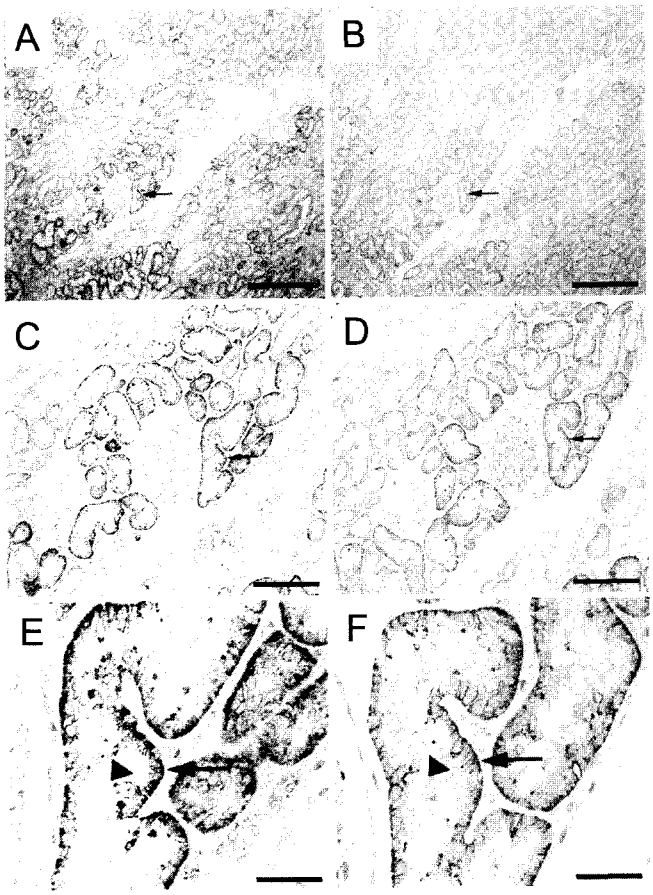

Fig. 2. Localization of hLAT2 and hTAT1 protein in the proximal tubule of the human kidney. The results of immunohistochemical analysis of serial paraffin embedded sections of the human kidney (5 μm) showing the localization of the hLAT2 **(A, C, E)** and hTAT1 **(B, D, F)** proteins. Low magnifications showing that the hLAT2 **(A)** and hTAT1 **(B)** immunoreactivities were located in the renal cortex (*arrows*) (Scale bars = 250 μm). The higher magnifications showing that the hLAT2 **(C)** and hTAT1 **(D)** immunoreactivities were located in the proximal tubule (*arrows*) of the human kidney (Scale bars = 100 μm). High magnifications view showing that the hLAT2 **(E)** and hTAT1 **(F)** immunoreactivities were located in the basolateral membrane (*arrows*) but not in the apical membrane (*arrow heads*) (Scale bars = 25 μm).

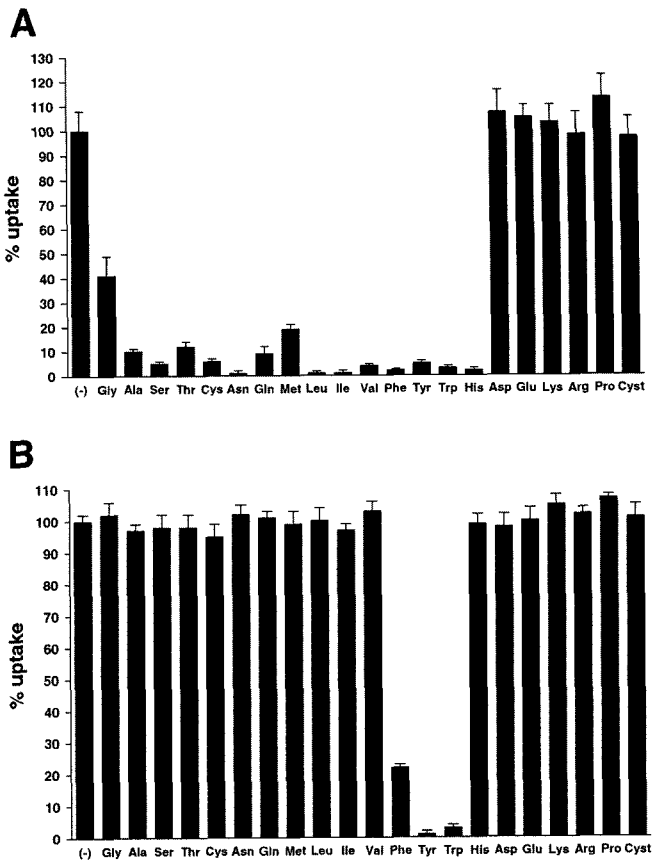

Fig. 3. Inhibition of hLAT2-mediated [^{14}C]L-leucine uptake and hTAT1-mediated [^{14}C]L-tryptophan uptake by the amino acids. **(A)** The hLAT2-mediated [^{14}C]L-leucine uptake (50 μM) was measured in the presence of 5 mM non-radiolabeled L-amino acids. The uptake was measured in a Na^+-free uptake solution and the values are expressed as the percentage of the control L-leucine uptake in the absent of the inhibitors (labeled as (-)). The L-leucine uptake was highly inhibited by neutral L-amino acids. **(B)** The hTAT1-mediated [^{14}C]L-tryptophan uptake (10 μM) was measured in the presence of 10 mM non-radiolabeled L-amino acids. The uptake was measured in a Na^+-free uptake solution and the values are expressed as the percentage of the control L-tryptophan uptake in the absence of inhibitors (labeled as (-)). The L-tryptophan uptake was strongly inhibited by the aromatic L-amino acids. Cyst: L-cystine.

investigated by inhibition experiments in which 50 mM [^{14}C]L-leucine uptake was measured in the presence of 5 mM amino acids for hLAT2 and 10 μM [^{14}C]L-tryptophan uptake was measured in the presence of 10 mM amino acids. The [^{14}C]L-leucine uptake by hLAT2 was markedly inhibited by the glycine and L-isomers of the neutral amino acids alanine, serine, threonine, cysteine, asparagine, glutamine methionine, leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan and histidine (Fig. 3A). The acidic amino acids, L-aspartate and L-glutamate, and the basic amino acids, L-lysine and L-arginine, L-proline and L-cystine did not inhibit hLAT2-mediated L-leucine uptake at 5 mM (Fig. 3A). The [^{14}C]L-tryptophan uptake by hTAT1 was strongly inhibited by the L-isomers of the aromatic amino acids phenylalanine, tyrosine and tryptophan, whereas the other L-amino acids did not inhibit hTAT1-mediated [^{14}C]L-leucine uptake at 10 mM (Fig. 3B).

In order to examine the hLAT2-mediated transport of the 100 μM radiolabeled amino acids, the hLAT2 cRNA and h4F2hc cRNA were injected into the *Xenopus* oocytes. Consistent with the results from the inhibition experiments (Fig. 3A), the oocytes expressing hLAT2 and h4F2hc transported the glycine, L-isomers of the neutral amino acids including small neutral amino acids such as alanine, serine, threonine and cysteine, branched or aromatic chained amino acids such as asparagines, glutamine, methionine, leucine, isoleucine, valine, phenylalanine,

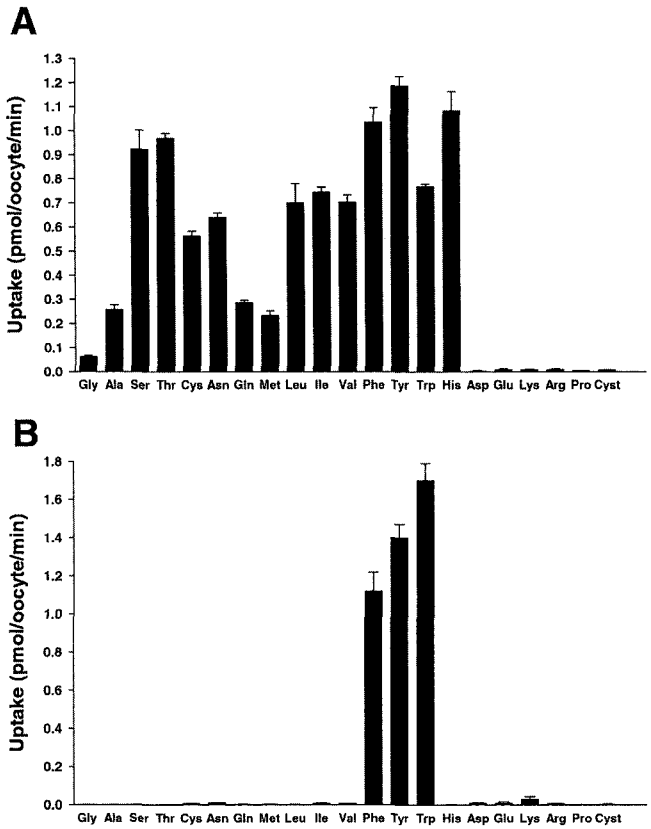

Fig. 4. The hLAT2-mediated and hTAT1-mediated amino acid uptakes. **(A)** The uptake of 100 μM radiolabeled L-amino acids was measured in Na^+-free uptake solution on the oocytes co-expressed with hLAT2 and h4F2hc cRNAs. Neutral L-amino acids and L-histidine were transported by hLAT2. Acidic amino acids, basic amino acids, L-proline and L-cystine were not transported by hLAT2. **(B)** The uptake of the 1 mM radiolabeled L-amino acids was measured in a Na^+-free uptake solution on the oocytes expressed with hTAT1 cRNAs. Aromatic L-amino acids were transported by hTAT1. The other amino acids tested were not transported by hTAT1. Cyst: L-cystine.

tyrosine, tryptophan and histidine (Fig. 4A). However, the acidic amino acids as L-aspartate and L-glutamate, and the basic amino acids as L-lysine and L-arginine, L-proline and L-cystine were not transported by hLAT2 (Fig. 4A).

The hTAT1 cRNA was injected into *Xenopus* oocytes in order to examine the hTAT1-mediated transport of the 1 mM radiolabeled amino acids. Consistent with the results from the inhibition experiments (Fig. 3B), the hTAT1 only transported aromatic amino acids such as L-phenylalanine, L-tyrosine and L-tryptophan (Fig. 4B).

The kinetic parameters of the aromatic amino acids among the amino acid substrates are listed in Table I.

Chromosomal localization of *hLAT2*

The *hLAT2* was mapped on the human chromosomes using the FISH. Since the DAPI banding was used to identify the specific chromosome, the assignment between signals from the probe and the long arm of chromosome 14 was obtained (Fig. 5 left panel and Fig. 5 middle panel). The detailed position was determined based on a summary of 10 photographs (Fig. 5). As shown in Fig. 5, *hLAT2* was mapped to chromosome 14, region q11.2.

The chromosome localization of *hTAT1* was described in our previous paper (Kim *et al.*, 2002b).

Table I. Kinetic parameters of amino acid substrates

Amino acids	hLAT2		hTAT1	
	K_m[a]	V_{max}[b]	K_m[a]	V_{max}[b]
	μM		μM	
L-Tryptophan	57.3 ± 8.9	(1.00)	452.2 ± 27.8	1.08
L-Tyrosine	48.8 ± 5.6	0.95	636.3 ± 41.8	1.03
L-Phenylalanine	30.7 ± 4.1	0.83	740.5 ± 36.7	1.24
L-Leucine	93.9 ± 8.9	0.98	-	-

The [a]K_m and [b]V_{max} values were determined as described in 'MATERIALS AND METHODS'. The table was constructed based on the three separate experiments using different batches of oocytes. In each experiment, L-tryptophan uptake measurements for hLAT2 were performed to compare V_{max} values among different experiments. The V_{max} value for each amino acid was normalized to that of hLAT2 for L-tryptophan in the same experiment. The K_m values of hLAT2 and hTAT1 for amino acid substrates are the mean of three measurements (mean±SEM). For comparison, the previous data of the K_m and V_{max} for hTAT1 is described (Kim *et al.*, Genomics, 79, 95-103 (2002b)).

Gene structure of *hLAT2*

A search of the NCBI human genome nucleotide database revealed a contig, RP11-244E17 (Accession No. AL117258.4), on chromosome 14 sharing an identity with the hLAT2 cDNA sequence. Aligning the nucleotide sequence of the hLAT2 cDNA with the genomic sequence allowed the exon-intron organization of the *hLAT2* (*SLC7A8*) to be deduced. Approximately 99% of the hLAT2 cDNA (nucleotides 1-4188) was found to be present on this contig using sequence comparison. The region corresponding to the hLAT2 cDNA spanned ~584 kb, with 11 exons and 10 introns (Table II). Each size of the exon/intron and each nucleotide sequence of the splice junction are shown in Table II. The translation initiation site was present on exon 1. The translation termination site TAA was present on exon 11. The exon sizes varied from 97 to 2,042 bp and the intron sizes varied from 675 to 22,080 bp (Table II). The nucleotide sequences at the exon/intron boundaries of *hLAT2 (SLC7A8)* conformed to the GT/AG rule for the intron donor and acceptor splice sites (Table II).

The gene structure of *hTAT1* (*SLC16A10*) was described in our previous paper (Kim *et al.*, 2002b).

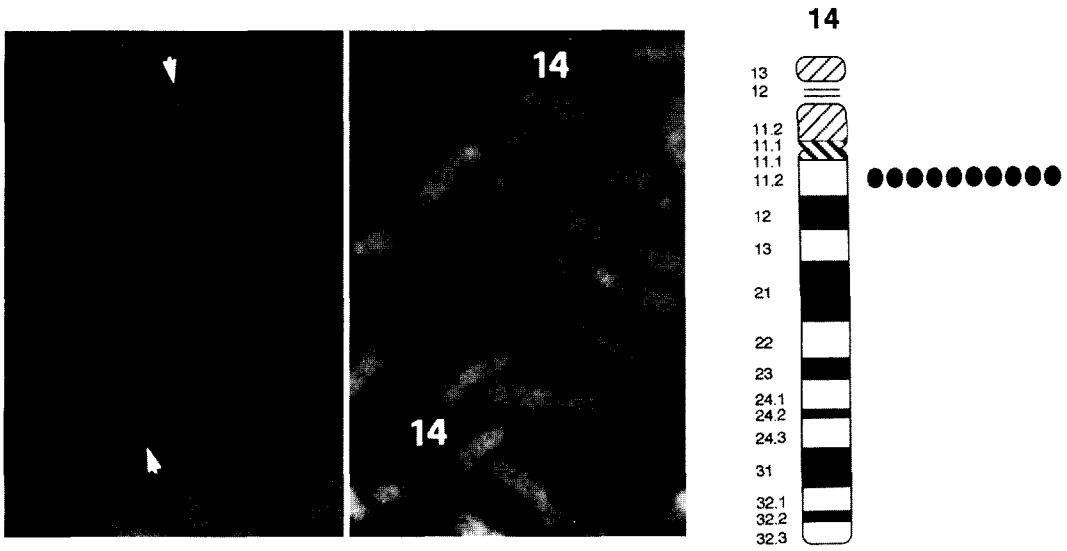

Fig. 5. Chromosomal localization of the *hLAT2* gene (human *SLC7A8* gene). Example of fluorescence *in situ* hybridization (FISH) mapping and diagram summarizing the mapping results of the probe hLAT2. **(Left panel)** The FISH signals on the human chromosome. **(Middle panel)** The same mitotic figure stained with 4,6-diamino-2 phenylindole (DAPI) to identify the human chromosome 14. **(Right panel)** Each dot represents double FISH signals detected on human chromosome 14.

Table II. Exon-intron organization of *hLAT2* gene (human *SLC7A8* gene)

Exon				Intron			Exon	
No.	Size (bp)	3'Junction	5' Junction	Size[a] (bp)	No.	3' Junction	5' Junction	No.
1	856	...TCATCGTAG	gtgagttcc...	16223	1	...ctcatgcag	GGAACATCA...	2
2	205	...ACTGGCTGG	gtaagagga...	899	2	...ctccttcag	GTTCCTGAG...	3
3	152	...TCTGCTTAT	gtaagtgtc...	22080	3	...tcatttcag	TGCTCCTCA...	4
4	126	...TATGCAAAG	gtgagtatc...	2454	4	...tgtccacag	GAGAGTACT...	5
5	154	...TCCCTACAA	gtgagttga...	923	5	...cctctacag	GAACCTTCC...	6
6	124	...GTCGCTGTG	gtaagaaat...	1399	6	...ctctcccag	ACTTTTGGA...	7
7	104	...CTCCTCTCG	gtgagtgct...	6363	7	...ccctgacag	GCTGTTCTT...	8
8	97	...CTCTTCACA	gtaagggcc...	1661	8	...ctttcccag	TGCATCTCC...	9
9	150	...CCCATCAAG	gtgagagaa...	1453	9	...gctccacag	ATCAACCTG...	10
10	178	...ACTTCATTG	gtgagttgt...	675	10	...ttcctacag	AGCTGCTAA...	11
11	2042	...TTAAAATGG						

[a]Intron sizes were determined from sequence analysis of contig RP11-244E17 (AL117258.4).

DISCUSSION

One of the most important roles of the kidney is the reabsorption of endogenous and exogenous materials. Amino acids are reabsorbed *via* the amino acid transporters present in the apical and basolateral membrane of the renal proximal tubule epithelial cells (Silbernagl, 1979; Kanai and Endou, 2001). The amino acid transporters play pivotal roles in the transepithelial transport of amino acids. Acidic amino acids are absorbed from the luminal fluid *via* the Na^+-dependent system $X^-_{A,G}$ glutamate transporter excitatory amino acid carrier 1 (EAAC1), which is located on the apical membrane of the epithelial cells (Shayakul *et al.*, 1997). The defect of EAAC1 results in the acidic amino aciduria in which glutamate and aspartate are excreted in the urine (Peghini *et al.*, 1997). It is believed that the absorbed glutamate is converted into neutral amino acids in the epithelial cells. The converted glutamate then leaves the cells to the blood stream *via* the neutral amino acid transport systems, which include the system L amino acid transporter LAT2. It has been suggested that in the renal proximal tubules and small intestine, cystine and basic amino acids are absorbed from the luminal fluid *via* the system $b^{0,+}$ transporter located on the apical membrane of the epithelial cells (Chillaron *et al.*, 1996; Palacin *et al.*, 1998). The basic

amino acids then pass through the basolateral membrane into the extracellular fluid and blood stream via the system y^+L transporter (Chillaron *et al.*, 1996; Palacin *et al.*, 1998). Neutral amino acids are absorbed from the luminal fluid *via* the Na^+-dependent system B^0 that is located on the apical membrane of the epithelial cells (Broer *et al.*, 2004). The exit path for neutral amino acids to the blood stream is believed to be the system L. This transporter is well suited for the exit path at the basolateral membrane because the facilitated transport mode is more predominant in LAT2 (Rossier *et al.*, 1999). In the present study, therefore, in order to examine the absorption mechanisms of neutral amino acids in the kidney, we have cloned the cDNAs of the system L amino acid transporter and the system T amino acid transporter in human. The gene structures, the functional characterizations and the localization in the human kidney were then examined and compared.

Northern blot analysis revealed that the hLAT2 transcript was ubiquitously expressed (Fig. 1A). The message was expressed at high levels in the heart, brain, placenta, kidney, spleen, prostate, testis, ovary, lymph node, thymus and the fetal liver (Fig.1A). In contrast to hLAT2, the hLAT1, which is another isoform of the system L amino acid transporter, is only expressed in restricted organs such as the brain, spleen, placenta and testis (Yanagida *et al.*, 2001). In addition, LAT1 is strongly expressed in malignant tumors presumably to support their continuous growth and proliferation (Sang *et al.*, 1995; Wolf *et al.*, 1996; Kanai *et al.*, 1998; Yanagida *et al.*, 2001). The previous studies reported that T24 human bladder carcinoma cells and KB human oral epidermoid carcinoma cells express hLAT1, but not hLAT2 (Kim *et al.*, 2002a; Yoon *et al.*, 2004). The results in this study, in conjunction with other reports (Sang *et al.*, 1995; Wolf *et al.*, 1996; Kanai *et al.*, 1998; Yanagida *et al.*, 2001; Kim *et al.*, 2002a; Yoon *et al.*, 2004) show that LAT1 is strongly expressed in malignant tumors presumably to support their continuous growth and proliferation and T24 human bladder carcinoma cells and KB human oral epidermoid carcinoma cells express hLAT1, but not hLAT2, suggests that the LAT1 and LAT2 play important roles in transport of neutral amino acid in cancer cells and normal cells, respectively. In the case of hTAT1, the hTAT1 message is expressed at high levels in the heart, placenta, liver, skeletal muscle, kidney, pancreas, spleen, thymus, testis and fetal liver (Fig. 1B). It should be noted that the hTAT1 message is expressed at high levels in the kidney in humans whereas it is not detected in the rat kidney (Kim *et al.*, 2001). This variation might be due to the differences in the promoter sequences between hTAT1 and rTAT1, which results in the differences in the cell specific promoter activity. As the results of northern blot analysis in this study, it is suggested that the hLAT2 and hTAT1 play important roles in the transport of neutral amino acids in the human kidney.

In order to understand the role of hLAT2 and hTAT1 in the human kidney, immunohistochemical analyses were performed on human kidney. The results showed that the hLAT2 and hTAT1 proteins co-exist in the basolateral membrane of the renal proximal tubule (Fig. 2). To our knowledge, this is the first study to report the expression of the hLAT2 and hTAT1 proteins in the basolateral membrane of the epithelial cells from human tissues. Because these results correspond to our previous study showing that the rTAT1 protein is present in the basolateral membrane of the epithelial cells in the rat small intestine (Kim *et al.*, 2001), it is concluded that the hLAT2 and hTAT1 play important roles in the *trans*-epithelial transport of neutral amino acids in the human kidney.

In this study, the hLAT2 transported the glycine, and the L-isomers of neutral amino acids including small neutral amino acids such as alanine, serine, threonine and cysteine, branched or aromatic chained amino acids such as asparagines, glutamine, methionine, leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan and histidine (Fig. 3A and Fig. 4A). In a previous study, hLAT1 preferred larger neutral amino acids with bulky or branched side chains for its substrates (Yanagida *et al.*, 2001). The hLAT2 is expressed mainly in normal cells. However, the hLAT1 is expressed primarily in cancer cells (Yanagida *et al.*, 2001). These results suggest that the LAT2 plays an important role in all neutral amino acid transport in normal cells, and the LAT1 plays an important role in the transport of large neutral amino acid in cancer cells. Although the LAT1 activity in cancer cells is completely blocked, the growth and proliferation of normal cells is possible because the LAT2 is present in normal cells. Therefore, the specific inhibition of LAT1 would be a new rationale for the suppression of cancer cell growth. However, these events need to be further delineated.

The hTAT1 transports aromatic amino acids such as L-tryptophan, L-tyrosine and L-phenylalanine (Fig. 3B and Fig. 4B). The hLAT2 also transports aromatic amino acids. The affinity of hLAT2 to aromatic amino acids is higher than that of hTAT1 when expressed in the *Xenopus laevis* oocytes: The K_m for aromatic amino acids is 30.7-57.3 μM for hLAT2 v.s. 452.2-740.5 μM for hTAT1 (Table I).

Using the FISH mapping, the *hLAT2 (SLC7A8)* was found in human chromosome 14, region q11.2 (Fig. 5 and Table II). This was further confirmed by the identification of *hLAT2* (*SLC7A8*) on the contig RP11-244E17 on the human chromosome 14 (Table II). A previous study reported that the *hTAT1 (SLC16A10)* is mapped in human chromosome 6, region q21-q22 (Kim *et al.*, 2002b). The *hLAT1 (SLC7A5)* is also mapped in separate chromosomes (human chromosome 16, region q24.3 (Nii *et al.*, 2001). Therefore, the genes for these related proteins are not

clustered in the human chromosomes. This study showed that the *hLAT2 (SLC7A8)* is constructed with 11 exons (Table II). However the *hLAT1 (SLC7A5)* is assembled with 10 exons (Nii *et al.*, 2001). The locations of the exon-intron boundaries are not conserved between the *hLAT2 (SLC7A8)* and *hLAT1 (SLC7A5)*. The genomic structure of *hLAT2 (SLC7A8)* also differs considerably from that of the *hLAT1 (SLC7A5)*.

In the rat small intestine, it was previously shown that the rTAT1 protein is located in the basolateral membrane of the epithelial cells (Kim *et al.*, 2001). This study showed that the hLAT2 and hTAT1 proteins co-exist in the basolateral membrane of the epithelial cells of the renal proximal tubule. In addition, it is known that the LAT2 and TAT1 are the facilitated amino acid transporters (Pineda *et al.*, 1998; Kim *et al.*, 2001). These results suggest that the hLAT2 and hTAT1 function as an exit path for amino acids from the epithelial cells to the blood stream in the *trans*-epithelial transport of amino acids. Although the affinity of hLAT2 to the substrate amino acids is higher than that of hTAT1, it would be beneficial to have additional selective transporters for the efficient *trans*-epithelial transports because the hLAT2 exhibits a broad substrate selectivity, which covers all neutral amino acids. Although hTAT1 functions as one of the redundant transport pathways for the transport of aromatic amino acids through the basolateral membrane, the aromatic amino acid-selective hTAT1 is essential for maintaining efficient *trans*-epithelial transport of aromatic amino acids so they are not out competed by the other neutral amino acids.

Overall, these results suggest that the hLAT2 plays important roles in the reabsorption of all neutral amino acids including aromatic amino acids, and the hTAT1 plays essential roles in the reabsorption of aromatic amino acids in the human kidney. In conclusion, the neutral amino acids are absorbed from the luminal fluid of the renal proximal tubule *via* the system B^0 amino acid transporter located on the apical membrane of the epithelial cells, and are reabsorped from the epithelial cells to the blood stream *via* the system L amino acid transporter and system T amino acid transporter. Although the specific diseases are, at the moment, not mapped to a particular region of the human chromosome where the *hLAT2 (SLC7A8)* and *hTAT1 (SLC16A10)* are located, the defect of these amino acid transporters might be involved in the pathogenesis of disorders caused by a disruption of amino acid absorption such as blue diaper syndrome (Rosenberg *et al.*, 1980).

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REFERENCES

Altman, A., Cardenas, J. M., Houghten, R. A., Dixon, F. J., and Theofilopoulos, A. N., Antibodies of predetermined specificity against chemically synthesized peptides of human interleukin 2. *Proc. Natl. Acad. Sci. U.S.A.*, 81, 2176-2180 (1984).

Blondeau, J. P., Beslin, A., Chantoux, F., and Francon, J., Triiodothyronine is a high-affinity inhibitor of amino acid transport system L1 in cultured astrocytes. *J. Neurochem.*, 60, 1407-1413 (1993).

Broer, A., Klingel, K., Kowalczuk, S., Rasko, J. E., Cavanaugh, J., and Broer, S., Molecular cloning of mouse amino acid transport system B^0, a neutral amino acid transporter related to Hartnup disorder. *J. Biol. Chem.*, 279, 24467-24476 (2004).

Chillaron, J., Estevez, R., Mora, C., Wagner, C. A., Suessbrich, H., Lang, F., Gelpi, J. L., Testar, X., Busch, A. E., Zorzano, A., and Palacin, M., Obligatory amino acid exchange *via* systems $b^{o,+}$-like and y^+L-like. A tertiary active transport mechanism for renal reabsorption of cystine and dibasic amino acids. *J. Biol. Chem.*, 271, 17761-17770 (1996).

Christensen, H. N., Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol. Rev.*, 70, 43-77 (1990).

Goldenberg, G. J., Lam, H. Y., Begleiter, A., Active carrier-mediated transport of melphalan by two separate amino acid transport systems in LPC-1 plasmacytoma cells *in vitro*. *J. Biol. Chem.*, 254, 1057-1064 (1979).

Gomes, P. and Soares-da-Silva, P., L-DOPA transport properties in an immortalized cell line of rat capillary cerebral endothelial cells, RBE 4. *Brain Res.*, 829, 143-150 (1999).

Heng, H. H. Q., Squire, J., and Tsui, L. -C., High resolution mapping of mammalian genes by *in situ* hybridization to free chromosome. *Proc. Natl. Acad. Sci. U.S.A.*, 89, 9509–9513 (1992).

Heng, H. H. Q. and Tsui, L. -C., Modes of DAPI banding and simultaneously *in situ* hybridization. *Chromosoma*, 102, 325–332 (1993).

Hirokawa, T., Boon-Chieng, S., and Mitaku, S., SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics*, 14, 378-379 (1998).

Hisano, S., Haga, H., Miyamoto, K., Takeda, E., and Fukui, Y., The basic amino acid transporter (rBAT)-like immunoreactivity in paraventricular and supraoptic magnocellular neurons of the rat hypothalamus. *Brain Res.*, 710, 299-302 (1996).

Kanai, Y. and Hediger, M. A., Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature*, 360, 467-471 (1992).

Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E., and Endou, H., Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J. Biol. Chem.,* 273, 23629-23632 (1998).

Kanai, Y. and Endou, H., Heterodimeric amino acid transporters: molecular biology and pathological and pharmacological relevance. *Curr. Drug Metab.,* 2, 339-354 (2001).

Kim, D. K., Kanai, Y., Chairoungdua, A., Matsuo, H., Cha, S. H., and Endou, H., Expression cloning of a Na^+-independent aromatic amino acid transporter with structural similarity to H^+/monocarboxylate transporters. *J. Biol. Chem.,* 276, 17221-17228 (2001).

Kim, D. K., Kanai, Y., Choi, H. W., Tangtrongsup, S., Chairoungdua, A., Babu, E., Tachampa, K., Anzai, N., Iribe, Y., and Endou, H., Characterization of the system L amino acid transporter in T24 human bladder carcinoma cells. *Biochim. Biophys. Acta,* 1565, 112-121 (2002a).

Kim, D. K., Kanai, Y., Matsuo, H., Kim, J. Y., Chairoungdua, A., Kobayashi, Y., Enomoto, A., Cha, S. H., Goya, T., and Endou, H., The human T-type amino acid transporter-1: characterization, gene organization, and chromosomal location. *Genomics,* 79, 95-103 (2002b).

Lakshmanan, M., Goncalves, E., Lessly, G., Foti, D., and Robbins, J., The transport of thyroxine into mouse neuroblastoma cells, NB41A3: the effect of L-system amino acids. *Endocrinology,* 126, 3245-3250 (1990).

Lee, S. H., Chae, K. S., Nan, J. X., and Sohn, D. H., The increment of purine specific sodium nucleoside cotransporter mRNA in experimental fibrotic liver induced by bile duct ligation and scission. *Arch. Pharm. Res.,* 23, 613-619 (2000).

Lee, S. H., Chae, K. S., and Sohn, D. H., Identification of expressed sequence tags of genes expressed highly in the activated hepatic stellate cell. *Arch. Pharm. Res.,* 27, 422-428 (2004).

Mannion, B. A., Kolesnikova, T. V., Lin, S. -H., Thompson, N. L., and Hemler, M. E., The light chain of CD98 is identified as E16/TA1 protein. *J. Biol. Chem.,* 273, 33127-33129 (1998).

Mizoguchi, K., Cha, S. H., Chairoungdua, A., Kim, D. K., Shigeta, Y., Matsuo, H., Fukushima, J., Awa, Y., Akakura, K., Goya, T., Ito, H., Endou, H., and Kanai, Y., Human cystinuria-related transporter: localization and functional characterization. *Kidney Int.,* 59, 1821-1833 (2001).

Nakamura, E., Sato, M., Yang, H., Miyagawa, F., Harasaki, M., Tomita, K., Matsuoka, S., Noma, A., Iwai, K., and Minato, N., 4F2 (CD98) heavy chain is associated covalently with an amino acid transporter and controls intracellular trafficking and membrane topology of 4F2 heterodimer. *J. Biol. Chem.,* 274, 3009-3016 (1999).

Nii, T., Segawa, H., Taketani, Y., Tani, Y., Ohkido, M., Kishida, S., Ito, M., Endou, H., Kanai, Y., Takeda, E., and Miyamoto, K., Molecular events involved in up-regulating human Na^+-independent neutral amino acid transporter LAT1 during T-cell activation. *Biochem. J.,* 358, 693-704 (2001).

Oxender, D. L. and Christensen, H. N., Evidence for two types of mediation of neutral amino acid transport in Ehrlich cells. *Nature,* 197, 765-767 (1963).

Palacin, M., Estevez, R., Bertran, J., and Zorzano, A., Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol. Rev.,* 78, 969-1054 (1998).

Peghini, P., Janzen, J., and Stoffel, W., Glutamate transporter EAAC-1-deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. *EMBO J.,* 16, 3822-3832 (1997).

Pfeiffer, R., Spindler, B., Loffing, J., Skelly, P. J., Shoemaker, C. B., and Verrey, F., Functional heterodimeric amino acid transporters lacking cysteine residues involved in disulfide bond. *FEBS Lett.,* 439, 157-162 (1998).

Pineda, M., Fernandez, E., Torrents, D., Estevez, R., Lopez, C., Camps, M., Lloberas, J., Zorzano, A., and Palacin, M., 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. *J. Biol. Chem.,* 274, 19738-19744 (1999).

Prasad, P. D., Wang, H., Huang, H., Kekuda, R., Rajan, D. P., Leibach, F. H., and Ganapathy, V., Human LAT1, a subunit of system L amino acid transporter: molecular cloning and transport function. *Biochem. Biophys. Res. Commun.,* 255, 283-288 (1999).

Rosenberg, R., Young, J. D., and Ellory, J. C., L-tryptophan transport in humanred blood cells. *Biochim. Biophys. Acta,* 598, 375-384 (1980).

Rossier, G., Meier, C., Bauch, C., Summa, V., Sordat, B., Verrey, F., and Kuhn, L. C., LAT2, a new basolateral 4F2hc/CD98-associated amino acid transporter of kidney and intestine. *J. Biol. Chem.,* 274, 34948-34954 (1999).

Sang, J., Lim, Y. -P., Panzia, M., Finch, P., and Thompson, N. L., TA1, a highly conserved oncofetal complementary DNA from rat hepatoma, encodes an integral membrane protein associated with liver development, carcinogenesis, and cell activation. *Cancer Res.,* 55, 1152-1159 (1995).

Segawa, H., Fukasawa, Y., Miyamoto, K., Takeda, E., Endou, H., and Kanai, Y., Identification and functional characterization of a Na^+-independent neutral amino acid transporter with broad substrate selectivity. *J. Biol. Chem.,* 274, 19745-19751 (1999).

Shayakul, C., Kanai, Y., Lee, W. S., Brown, D., Rothstein, J. D., and Hediger, M. A., Localization of the high-affinity glutamate transporter EAAC1 in rat kidney. *Am. J. Physiol.,* 273, F1023-F1029 (1997).

Silbernagl, S., Renal transport of amino acids. *Klin. Wochenschr.,* 57, 1009-1019, (1979).

Stevens, B. R., Kaunitz, J. D., and Wright, E. M., Intestinal transport of amino acids and sugars: advances using membrane vesicles. *Annu. Rev. Physiol.,* 46, 417-433 (1984).

Su, T. Z., Lunney, E., Campbell, G., and Oxender, D. L.,

Transport of gabapentin, a gamma-amino acid drug, by system I alpha-amino acid transporters: a comparative study in astrocytes, synaptosomes and CHO cells. *J. Neurochem.,* 64, 2125-2131 (1995).

Utsunomiya-Tate, N., Endou, H., and Kanai, Y., Cloning and functional characterization of a system ASC-like Na^+-dependent neutral amino acid transporter. *J. Biol. Chem.,* 271, 14883-14890 (1996).

Utsunomiya-Tate, N., Endou, H., and Kanai, Y., Tissue specific variants of glutamate transporter GLT-1. *FEBS Lett.,* 416, 312-316 (1997).

van Winkle, L. J., Mann, D. F., Campione, A. L., and Farrington, B. H., Transport of benzenoid amino acids by system T and four broad scope systems in preimplantation mouse conceptuses. *Biochim. Biophys. Acta,* 1027, 268–277 (1990).

Wolf, D. A., Wang, S., Panzia, M. A., Bassily, N. H., and Thompson, N. L., Expression of a highly conserved oncofetal gene, TA1/E16, in human colon carcinoma and other primary cancers: homology to *Schistosoma mansoni* amino acid permease and *Caenorhabditis elegans* gene products. *Cancer Res.,* 56, 5012-5022 (1996).

Yanagida, O., Kanai, Y., Chairoungdua, A., Kim, D. K., Segawa, H., Nii, T., Cha, S. H., Matsuo, H., Fukushima, J., Fukasawa, Y., Tani, Y., Taketani, Y., Uchino, H., Kim, J. Y., Inatomi, J., Okayasu, I., Miyamoto, K., Takeda, E., Goya, T., and Endou, H., Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. *Biochim. Biophys. Acta,* 1514, 291-302 (2001).

Yoon, J. H., Kim, Y. B., Kim, M. S., Park, J. C., Kook, J. K., Jung, H. M., Kim, S. G., Yoo, H., Ko, Y. M., Lee, S. H., Kim, B. Y., Chun, H. S., Kanai, Y., Endou, H., and Kim, D. K., Expression and functional characterization of the system L amino acid transporter in KB human oral epidermoid carcinoma cells. *Cancer Lett.,* 205, 215-226 (2004).