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## Review

# The SLC3 and SLC7 families of amino acid transporters <sup>☆</sup>

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## ABSTRACT

Amino acids are necessary for all living cells and organisms. Specialized transporters mediate the transfer of amino acids across plasma membranes. Malfunction of these proteins can affect whole-body homeostasis giving rise to diverse human diseases. Here, we review the main features of the SLC3 and SLC7 families of amino acid transporters. The SLC7 family is divided into two subfamilies, the cationic amino acid transporters (CATs), and the L-type amino acid transporters (LATs). The latter are the light or catalytic subunits of the heteromeric amino acid transporters (HATs), which are associated by a disulfide bridge with the heavy subunits 4F2hc or rBAT. These two subunits are glycoproteins and form the SLC3 family. Most CAT subfamily members were functionally characterized and shown to function as facilitated diffusers mediating the entry and efflux of cationic amino acids. In certain cells, CATs play an important role in the delivery of L-arginine for the synthesis of nitric oxide. HATs are mostly exchangers with a broad spectrum of substrates and are crucial in renal and intestinal re-absorption and cell redox balance. Furthermore, the role of the HAT 4F2hc/LAT1 in tumor growth and the application of LAT1 inhibitors and PET tracers for reduction of tumor progression and imaging of tumors are discussed. Finally, we describe the link between specific mutations in HATs and the primary inherited aminoacidurias, cystinuria and lysinuric protein intolerance.

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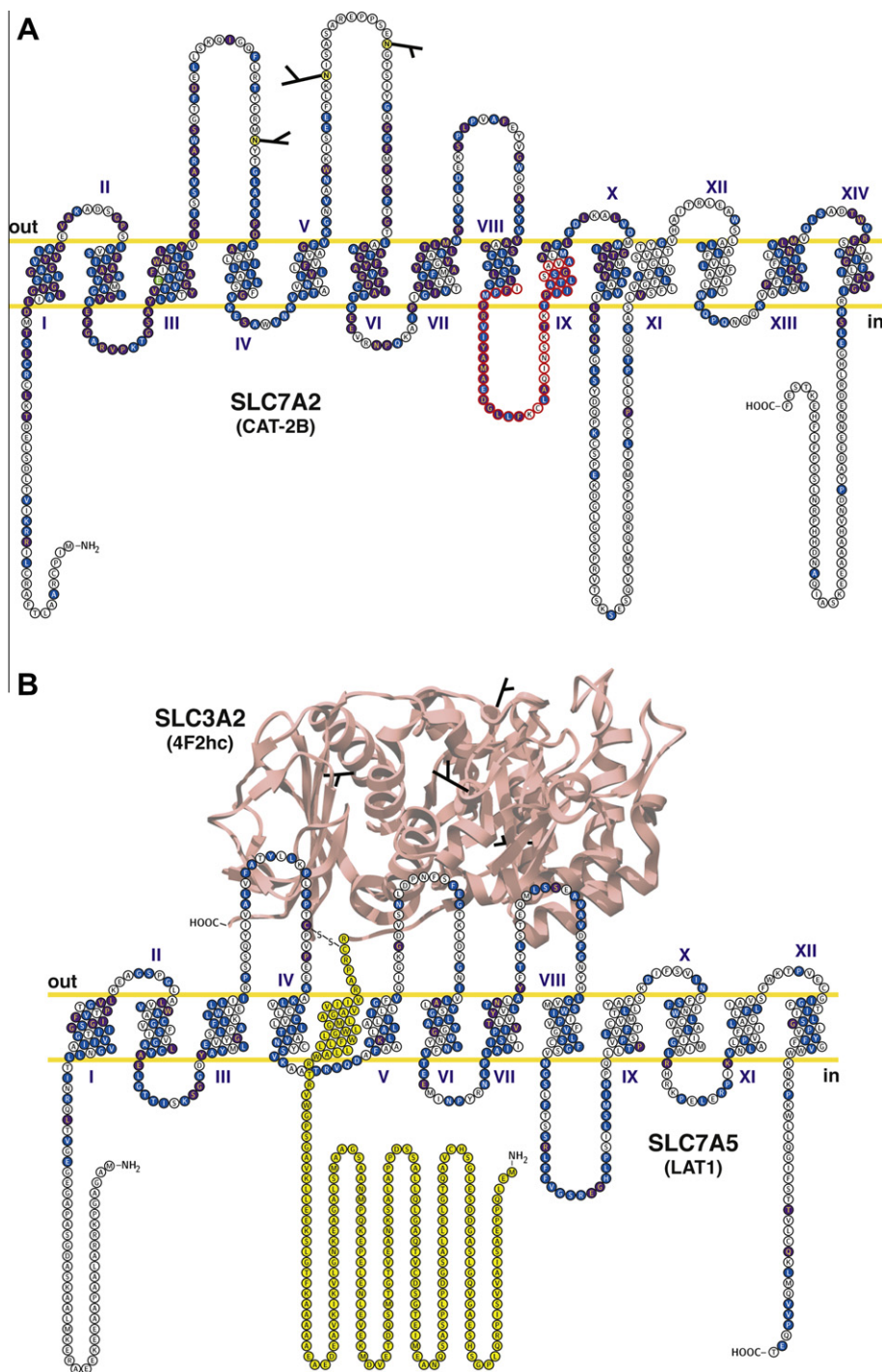
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## 1. Introduction

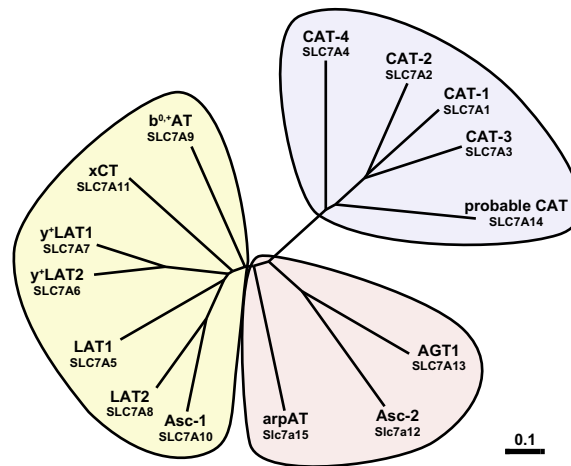
Amino acids are essential as building blocks of proteins, energy sources, precursors of metabolites and signaling molecules in all living cells. Membrane transport proteins mediate the transfer of amino acids between compartments in cells, between different cells and between organs. Absence, overexpression and malfunction of certain amino acid transporters can be associated with diseases. In this review, we address the biochemistry, function, structure, physiology and pathology of the SLC3 and SLC7 families of amino acid transporters: the names SLC3 and SLC7 are according to the SLC (solute carrier) gene nomenclature (Hediger et al., 2004). To allow a link to the Transporter Classification (TC) system (Saier et al., 2006, 2009), TC codes are indicated in the titles of the subchapters describing the SLC3 and SLC7 family members (see below), and where appropriate. The SLC7 family is divided into two subgroups, the cationic amino acid transporters (CATs, SLC7A1–4 and SLC7A14) and the light or catalytic subunits (L-type amino acid transporters (LATs), SLC7A5–13 and SLC7A15) of the heteromeric amino acid transporters (HATs). CATs and LATs belong to the large amino acids, polyamines and organocations (APC; TC 2.A.3) superfamily of transporters. LATs are also called glycoprotein-associated amino acid transporters. The associated heavy subunits (glycoproteins) 4F2hc (SLC3A2, TC 8.A.9.2.1) or rBAT (SLC3A1, TC 8.A.9.1.1) of HATs form the SLC3 family.

CATs are facilitated diffusers and the major entry path in most cells for cationic amino acids and play a key role in nitric oxide synthesis by delivering L-arginine for nitric oxide synthase in certain cells. Among the CATs, CAT-1, CAT-2 and CAT-3 are more closely related to each other (58–59% amino acid sequence (AAS) identity) than to SLC7A4 (tentatively named CAT-4; 38–40% AAS identity) and SLC7A14 (~35% AAS identity). CAT members are N-glycosylated and have 14 putative transmembrane domains (TMDs) with cytosolic N- and C-termini according to transmembrane topology prediction and the available experimental evidence (Closs et al., 2006) (Fig. 1A). In contrast to CATs, LATs are not N-glycosylated and only have 12 TMDs (Gasol et al., 2004) (Fig. 1B), which show significant similarity to the first 12 TMDs of CATs. Sequence analyses indicate that both, CATs and HATs, originate from an ancestral 12 TMD protein and that duplication of the last two TMDs of this ancestral protein is the origin of the 14 TMD CAT structure (Hansen et al., 2011). It is estimated that this duplication happened about 2.6 billion years ago, probably soon after the bifurcation of the eukaryotic and archaean branches (Hansen et al., 2011). Homologous CAT and LAT proteins are found in prokaryotes, but the cysteine residue of the LATs that is involved in the disulfide bridge with the heavy subunit is not conserved (Reig et al., 2007). SLC3 proteins and HATs are only found in metazoans.

HATs are mostly exchangers with a broad spectrum of substrates, ranging from neutral amino acids (systems L and asc) to aromatic amino acids (transporter arpAT), negatively charged amino acids (system x<sub>c</sub><sup>-</sup>) and cationic amino acids plus neutral amino acids (systems y<sup>+</sup>L and b<sup>0+</sup>). Transporters y<sup>+</sup>L are the only sodium-dependent co-transporter HAT members, and only with neutral amino acids as co-substrates. As mentioned above, HATs are disulfide-linked heterodimers of SLC3 members (4F2hc (SLC3A2) or rBAT (SLC3A1)) and eukaryotic LATs from the SLC7 family. The cysteine residue involved in the disulfide bridge with the heavy subunit is located between TMD III and IV of LATs (Fig. 1B). Six LATs form heterodimers with 4F2hc (the heavy chain of the 4F2 antigen), i.e., LAT1-2 (SLC7A5, SLC7A8), y<sup>+</sup>LAT1-2 (SLC7A7, SLC7A6), the cystine/glutamate



**Fig. 1.** Topology models of SLC7 proteins and HATs. (A) Model of human CAT-2B as a representative member of CAT proteins. CATs have 14 predicted TMDs, three putative glycosylation sites in the 2nd and 3rd extracellular loop (corresponding Asp residues in yellow) and intracellular N- and C-termini. The Glu107 (green) residue in TMD III is essential for transport activity (Wang et al., 1994) and conserved in all other known CAT isoforms. Therefore, Glu107 is thought to be part of the substrate translocation pathway. The two splice variants CAT-2A and -B differ in a stretch of 42 amino acid residues (marked in red). In this stretch and in contrast to the high substrate affinity transporters CATs (CAT-1, CAT-2B and CAT-3), the low affinity isoform CAT-2A has an Arg residue at position 369 (instead of a Glu residue) and a missing residue at position 383 (instead of an Asn or His residue) (Habermeier et al., 2003). (B) Model of human 4F2hc/LAT1 as a representative member of HAT proteins. Light subunits of HATs consist of 12 predicted TMDs and are associated with the heavy subunit 4F2hc or rBAT through a conserved disulfide bridge. Heavy subunits are type II membrane N-glycoproteins (1 TMD, in yellow; the 4 glycosylation sites of 4F2hc are indicated) with an intracellular N-terminus (in yellow) and an extracellular C-terminus (ED). In contrast, light subunits have intracellular termini and are not glycosylated. The crystal structure of human 4F2hc-ED was solved and is displayed (Fort et al., 2007). Identical and conserved residues within the corresponding families, i.e. CATs and the light subunits of HATs, are colored in violet and blue, respectively. Transmembrane topologies were predicted using the HMMTOP server (Tusnady and Simon, 2001) and topology data were plotted using T(E)Xtopo (Beitz, 2000).



**Fig. 2.** Phylogenetic tree of SLC7 family members. The SLC7 family is composed of the CATs and the light subunits (glycoprotein-associated amino acid transporters) of HATs. There are two distinct clusters (colored in yellow/pink and blue). One of these has two sub-clusters (colored in yellow and pink). AAS alignments and guide tree were computed using ClustalW2 (Larkin et al., 2007). The phylogenetic tree was drawn using the application PhyloDendron (<http://iubio.bio.indiana.edu/soft/molbio/java/apps/trees/>).

antiporter (xCT, SLC7A11) and the alanine-serine-cysteine transporter 1 (Asc-1, SLC7A10). Only the amino acid transporter  $b^{0,+}$ AT (SLC7A9) forms heterodimers with rBAT. In contrast, arpAT (Slc7a15) forms heterodimers with 4F2hc and rBAT in heterologous expression systems, but the exact heavy subunit of this transporter in the original cells is unknown. 4F2hc- and rBAT-associated LATs share 29–43% AAS identity with arpAT,  $b^{0,+}$ AT having the highest and Asc-2 the lowest AAS identity. There are also two LATs, namely AGT1 (SLC7A13), and Asc-2 (Slc7a12), that associate with not yet identified heavy subunits. They share an AAS identity of ~45% and only 23–32% with the other LATs (Fig. 2). Interestingly, the light subunits Asc-2 (Slc7a12) and arpAT (Slc7a15) are not present or highly inactivated in primate genomes (Fernandez et al., 2005).

## 2. Cationic amino acid transporters

Mouse CAT-1, encoded by the *Slc7a1* gene, was the first CAT cloned and was found to be the receptor for murine ecotropic leukaemia viruses (Kim et al., 1991). CAT function has been described for four members of the CAT subfamily: CAT-1 (encoded by *SLC7A1*), CAT-2A, CAT-2B, and CAT-3 (encoded by *SLC7A3*). CAT-2A and -2B are splice variants encoded by the *SLC7A2* gene that differ only in a stretch of 42 amino acids (see Fig. 1A, stretch between TMDs VIII and IX marked in red); for a more extensive review on CATs, please see (Closs et al., 2006). They mediate sodium-independent transport of cationic L-amino acids. CAT-1, -2B and -3 are the molecular correlates of the classical cationic amino acid transporter system  $y^+$  (White and Christensen, 1982) having *trans*-stimulation (i.e., stimulation by substrate at the *trans*-side of the membrane) and low apparent  $K_m$ s for their substrates, e.g. 38–450  $\mu$ M for L-arginine.  $K_m$  values for L-lysine and L-ornithine are similar to those reported for L-arginine, suggesting that these three substrates are recognized with similar affinity and within the range of the concentration of cationic amino acids in plasma. CAT-1 shows the highest apparent substrate affinity and the strongest *trans*-stimulation and, thus, works almost like an exchanger. In contrast, CAT-2A is a low-affinity carrier for cationic amino acids (apparent  $K_m$  of 2–5 mM) and is relatively insensitive to *trans*-stimulation. At equilibrium all system  $y^+$  isoforms (CAT transporters) maintain an ~8-fold inward gradient of cationic amino acids across the plasma membrane at a membrane potential of –50 mV. Moreover, membrane hyperpolarization increases the  $V_{max}$  for influx of cationic amino acids via CAT-1 and CAT-2A (Kavanaugh, 1993). The principal difference in the substrate specificity among CATs is that L-histidine (at pH 5.5, where it is mostly protonated) is a good substrate for CAT-1, but is not transported by CAT-3 (Vékony et al., 2001). Interaction of CATs with neutral and anionic amino acids, albeit weak, has been detected by means of electrophysiological and competition studies. To our knowledge, the function of the more distantly related isoforms SLC7A4 and SLC7A14 is still unknown (Fig. 2). The features of CATs are summarized in Table 1.

System  $y^+$  transporters are considered the major entry pathway for cationic amino acids in non-epithelial cells, being a relevant determinant of cell metabolism, e.g., protein synthesis, and the formation of nitric oxide, urea, creatine, and agmatine from arginine, and polyamines, proline and glutamine from ornithine (Closs et al., 2006) (Fig. 3A). In contrast, in the renal and small intestinal epithelia the apical system  $b^{0,+}$  (the HAT rBAT/ $b^{0,+}$ AT) and the basolateral system  $y^+L$  (the HAT 4F2hc/ $y^+L$ AT1) mediate the vectorial re-absorption of cationic amino acids from the lumen to the extracellular space (Fig. 3B) (see below).

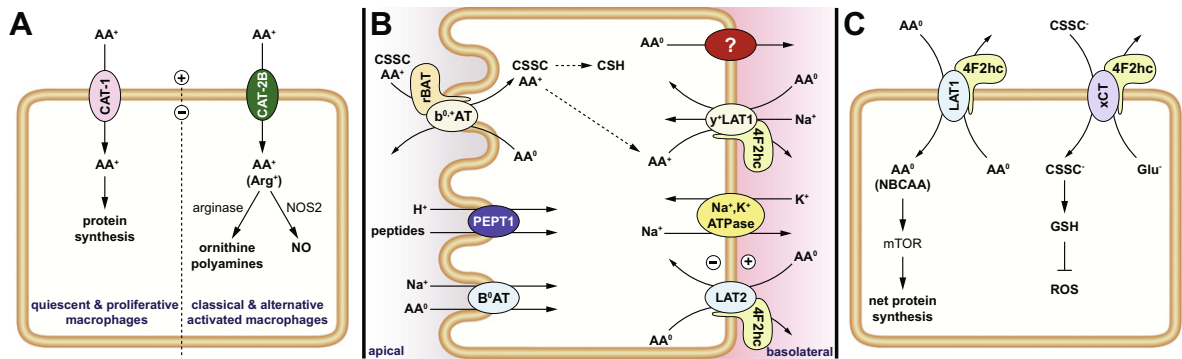
### 2.1. CAT-1 (SLC7A1; TC 2.A.3.3.1)

CAT-1 is expressed almost ubiquitously and constitutively in adult tissues with the exception of adult liver (Closs et al., 2006; Deves and Boyd, 1998) and lacrimal gland (Jäger et al., 2009). CAT-1 is restricted to the basolateral membrane in

**Table 1**SLC7 – cationic amino acid transporters/L-type amino acid transporters. For detailed information about the SLC gene tables, please visit: <http://www.bioparadigms.org>.

Human gene name	Protein name	Aliases [assoc. with]	Predominant substrates	Transport type/coupling ions <sup>a</sup>	Tissue distribution and cellular/subcellular expression	Link to disease	Human gene locus	Sequence accession ID	Splice variants and their features
SLC7A1	CAT-1	ATRC1, system y <sup>+</sup>	Cationic L-amino acids	F <sup>b</sup> (non-obligatory E)	Ubiquitous except for liver and lacrimal gland/basolateral and intracellular membranes in epithelial cells		13q12.3	NM_003045	
SLC7A2	CAT-2 (A or B)	ATRC2, system y <sup>+</sup>	Cationic L-amino acids	F	CAT-2A: liver, skeletal muscle, pancreas; CAT-2B: inducible in many cell types		8p22	NM_003046 NM_001008539	CAT-2A: low affinity; CAT-2B: high affinity
SLC7A3	CAT-3	ATRC3, system y <sup>+</sup>	Cationic L-amino acids	F	Thymus, ovary, testis, brain (neurons)		Xq13.1	NM_032803 NM_001048164	2 splice variants
SLC7A4	CAT-4			O	Brain, testis, placenta. Plasma and intracellular membranes		22q11.21	NM_004173	
SLC7A5	LAT1	[4F2hc], 4F2lc, system L	Large neutral L-amino acids, T <sub>3</sub> , T <sub>4</sub> , L-DOPA, BCH	E (similar intra- and extracellular selectivities, lower intracellular apparent affinity)	Brain, ovary, testis, placenta, spleen, colon, blood–brain barrier, fetal liver, activated lymphocytes, tumor cells	Cancer	16q24.3	NM_003486	
SLC7A6	y <sup>+</sup> LAT2	[4F2hc], system y <sup>+</sup> L	Na <sup>+</sup> indep.: cationic amino acids; Na <sup>+</sup> /large neutral amino acids	E (preferentially intracellular cationic amino acid against extracellular neutral amino acid/Na <sup>+</sup> )	Brain, small intestine, testis, parotids, heart, kidney, lung, thymus/basolateral in epithelial cells		16q22.1	NM_003983 NM_001076785	
SLC7A7	y <sup>+</sup> LAT1	[4F2hc], system y <sup>+</sup> L	Na <sup>+</sup> indep.: cationic amino acids; Na <sup>+</sup> /large neutral L-amino acids	E (preferentially intracellular cationic amino acid against extracellular neutral amino acid/Na <sup>+</sup> )	Small intestine, kidney, spleen, leucocytes, placenta, lung/basolateral in epithelial cells	Lysinuric protein intolerance (LPI)	14q11.2	NM_003982	3 splice variants
SLC7A8	LAT2	[4F2hc], system L	Neutral L-amino acids, T <sub>3</sub> , T <sub>4</sub> , BCH	E (similar intra- and extracellular selectivities, lower intracellular apparent affinity)	Small intestine, kidney, lung, heart, spleen, liver, brain, placenta, prostate, ovary, fetal liver, testis, skeletal muscle/basolateral in epithelial cells		14q11.2	NM_012244 NM_182728	2 splice variants
SLC7A9	b <sup>0,+</sup> AT	[rBAT], system b <sup>0,+</sup>	Cationic amino acids, large neutral amino acids	E (preferentially extracellular cationic amino acid and cystine against intracellular neutral amino acid)	Kidney, small intestine, liver, placenta/apical in epithelial cells	Cystinuria and isolated cystinuria	19q13.1	NM_014270	2 splice variants
SLC7A10	Asc-1	[4F2hc], system asc	Small neutral amino acids	Preferentially E	Brain, CNS, lung, small intestine, heart, placenta, skeletal muscle and kidney		19q13.1	NM_019849	
SLC7A11	xCT	[4F2hc], system x <sub>c</sub> <sup>-</sup>	Cystine (anionic form), L-glutamate	E (preferentially extracellular cystine against intracellular glutamate)	Macrophages, brain, retinal pigment cells, liver, kidney/basolateral in epithelial cells		4q28.3	NM_014331	
SLC7A13	AGT-1	XAT2	L-Aspartate and L-glutamate	E	Proximal straight tubules and distal convoluted tubules (basolateral)		8q21.3	NM_138817	
SLC7A14				O	Highly expressed in CNS/intracellular localization		3q26.2	NM_020949	

<sup>a</sup> C: cotransporter; E: exchanger; F: facilitated transporter; O: orphan transporter.<sup>b</sup> CAT-1 is strongly *trans*-stimulated and works better in the exchange mode, CAT-2B and CAT-3 are moderately *trans*-stimulated, and CAT-2A is completely independent of the presence of *trans*-substrate.



**Fig. 3.** Physiology of CATs and HATs. (A) Roles of CAT-1 and CAT-2B in macrophages. Positively charged amino acids ( $AA^+$ ) are transported into the cells for protein synthesis, and conversion of  $Arg^+$  into ornithine and polyamines (by arginase) and NO (by NO synthase 2). (B) Interplay of HATs and other transporters in epithelial cells of the small intestine and kidney proximal tubule. Cystine (CSSC) and  $AA^+$  are transported into the cell via the apical rBAT/ $b^{0,+}AT$  in exchange with neutral amino acids ( $AA^0$ ), which are accumulated by the function of  $Na^+$ -coupled amino acid transporters (e.g.,  $B^0AT$ ). The apical small intestine transporter PEPT1 also contributes to the absorption of amino acids but in the form of di- and tri-peptides. The basolateral efflux of  $AA^+$  is mediated by 4F2hc/ $y^+LAT1$  in exchange with  $AA^0$  and  $Na^+$ ; this is favored by the  $Na^+$ -gradient generated by the basolateral  $Na^+$ ,  $K^+$  ATPase. The molecular entities of the basolateral transporters mediating efflux of  $AA^0$  has not yet been identified. The involvement of 4F2hc/LAT2 in this process has been proposed. (C) Roles of the HATs 4F2hc/LAT1 and 4F2hc/xCT in non-polarized cells. LAT1 imports neutral branched chain amino acids (NBCAA), which stimulate mTOR in several cell lines. 4F2hc/xCT exchanges anionic glutamate ( $Glu^-$ ) for anionic cystine (CSSC $^-$ ), which is then used to produce reduced glutathione (GSH) and for neutralization of reactive oxygen species (ROS).

transfected epithelial cells and seems to be the major system  $y^+$  transporter in most cells, including nitric oxide-producing cells. The lack of CAT-1 in homozygous *Slc7a1* knockout mice is lethal; the mice die on day 1 after birth (Perkins et al., 1997). They suffer from severe anaemia and are 25% smaller than their wild-type counterparts. In contrast, the heterozygous mice exhibit no phenotypic abnormalities. The expression of CAT-3 during embryogenesis and fetal development in *Slc7a1* $^{-/-}$  mice might explain the relatively normal development of most tissues up to birth (Nicholson et al., 1998).

Amino acid deprivation induces the expression of CAT-1 by up-regulation of transcription, mRNA stability and translation in rat C6 glioma cells (Hatzoglou et al., 2004). Translation of rat *Slc7a1* mRNA during amino acid starvation is initiated from an internal ribosomal entry sequence located in the 5'UTR of the mRNA, which overlaps with an amino acid-responsive element and a micro ORF. In C6 cells, the up-regulation of translation by amino acid deprivation depends on the translation of the micro ORF and binding of the internal ribosomal entry sequence *trans*-acting factors hnRNP L and PTB (Majumder et al., 2009). In contrast, in the human hepatoma cell line Huh7 translation of *SLC7A1* mRNA is suppressed by the liver specific microRNA-122 upon binding to its 3'UTR, and amino acid deprivation or oxidative stress release this suppression. This de-repression is a fast mechanism that causes the release of *SLC7A1* mRNA from cytoplasmic processing bodies and its recruitment to polysomes for protein synthesis (Bhattacharyya et al., 2006a,b). It is not known whether microRNA-122-dependent suppression of *SLC7A1* mRNA is responsible for the lack of expression of CAT-1 in liver. Finally, PKC activation promotes ubiquitination-dependent and clathrin-mediated endocytosis of CAT-1 for recycling, a common mechanism of regulation reported for transporters of other families (e.g., SLC6) (Vina-Vilaseca et al., 2011).

## 2.2. CAT-2 (*SLC7A2*; TC 2.A.3.3.2)

The two CAT-2 splice variants (CAT-2A and CAT-2B) have a more defined expression pattern than CAT-1 (Closs et al., 2006). CAT-2A is most abundant in liver, but is also weakly expressed in cardiomyocytes, cardiac microvascular endothelial cells, the pancreas and both skeletal and vascular smooth muscle. In most cell types, significant expression of CAT-2B is only found after bacterial lipopolysaccharide and pro-inflammatory cytokine treatment, e.g. allergic airway inflammation induces CAT-2 expression in the lung (Niese et al., 2010). Homozygous *Slc7a2* knockout mice appear to have no phenotypic abnormalities (Nicholson et al., 2001). However, spontaneous inflammation in the absence of pathogenic stimuli, reduced nitric oxide (and polyamines and proline) production in activated alveolar macrophages, increased dendritic cell activation and subsequent T cell responses were observed in the lungs of CAT-2-deficient mice (Rothenberg et al., 2006). This suggests that in the lung CAT-2B is required to provide arginine for the synthesis of nitric oxide in macrophages (Fig. 3A) allowing them to suppress dendritic cell activation and inflammation. CAT-2B is induced together with the arginine-metabolizing enzymes nitric oxide synthase 2 and arginase in the classical and alternative activation of macrophages (Yeremian et al., 2006) (Fig. 3A). Activated macrophages present low membrane potential and high consumption of arginine. Under such conditions, influx via CAT-2B is ideal, because of the low dependence on membrane potential and *trans*-stimulation of this transporter (Closs et al., 2006).

It has been proposed that the low-affinity, high-capacity CAT-2A activity in liver serves to remove surplus cationic amino acids from the portal circulation (Closs et al., 2006), but to our knowledge *Slc7a2* KO mice have not been stressed with a high-protein or high-arginine diet to test this hypothesis. A few years ago it was reported that large (8 kb) *Slc7a2* transcripts local-

ize to nuclear paraspeckles in C127I mouse mammary tumor cells, suggesting that gene expression might be regulated by nuclear retention (Prasanth et al., 2005). This hypothesis did not received further support because of a discrepancy between total *Slc7a2* mRNA and protein expression (Closs et al., 2006). Furthermore, no CAT activity was reported.

### 2.3. CAT-3 (*SLC7A3*; TC 2.A.3.3.5)

CAT-3 is thought to play a major role during embryogenesis and fetal development (Nicholson et al., 1998). In mid-streak mouse embryos CAT-3 is expressed in the mesoderm and in several developing tissues, whereas in adult mice and rats CAT-3 is brain-specific (Ito and Groudine, 1997). In humans, weak CAT-3 expression has been observed in the stomach and ovary, moderate expression in the brain, uterus, mammary gland and testis, and strong expression in the thymus (Vékony et al., 2001). Accordingly, no CAT-3 expression was found in healthy conjunctiva, cornea and nasolacrimal ducts (Jäger et al., 2009). No gene knockout studies have been reported so far.

### 2.4. CAT-4 (*SLC7A4*) and *SLC7A14*

The transport function of *SLC7A4* and *SLC7A14* (both members of the TC 2.A.3.3 subfamily) is unknown. Therefore, the name CAT-4 (Cationic Amino acid Transporter-4) might be inappropriate. In heterologous expression systems, *SLC7A4* localizes in the plasma membrane but shows no amino acid transport activity, while *SLC7A14* has an intracellular localization (reviewed in (Closs et al., 2006)). Co-expression with the heavy subunits of HATs (4F2hc or rBAT) neither leads to trafficking of *SLC7A14* to the plasma membrane, nor to cationic amino acid transport activity (Ellen Closs, personal communication). *SLC7A4* mRNA is expressed in testis and placenta (Sperandeo et al., 1998) whereas *SLC7A14* is expressed in CNS with virtually no expression in peripheral tissues (Sreedharan et al., 2011). To our knowledge, no gene knockout studies have been reported for *SLC7A4* and *SLC7A14*.

## 3. HATs and their heavy subunits

The two members of the SLC3 family: rBAT (*SLC3A1*, also named D2 and NBAT) and 4F2hc (*SLC3A2*, also named CD98hc and FRP, for fusion regulatory protein) share about 20% AAS identity. Both proteins are N-glycosylated: ~94 and ~85 kDa for the mature glycosylated forms of rBAT and 4F2hc, respectively. rBAT and 4F2hc are type II membrane N-glycoproteins with an intracellular N-terminus, a single TMD, and a bulky extracellular C-terminus (50–60 kDa) (Fort et al., 2007). The cysteine residue participating in the disulfide bridge with the corresponding light subunit is four to five amino acids away from the TMD (Fig. 1B). The bulky extracellular domain of SLC3 members has sequence and structural homology with insect maltases and bacterial  $\alpha$ -glucosidases (Fort et al., 2007; Gabrisko and Janecek, 2009). The crystal structure of the ectodomain of human 4F2hc has been solved at 2.1 Å resolution. The protein has the characteristic protein fold of these enzymes, i.e., a  $(\beta\alpha)_8$  barrel (domain A) and a C-terminal, anti-parallel  $\beta_8$  sandwich (domain C), but no domain B (Fort et al., 2007). Despite this structural similarity, 4F2hc lacks the key catalytic residues necessary for glucosidase activity (Fort et al., 2007). To our knowledge, the atomic structure of the rBAT ectodomain (rBAT-ED) has not been solved, but sequence homology with glucosidases suggests that in addition to domains A and C, rBAT-ED has also domain B. It is not yet clear whether rBAT has glucosidase-like activity.

The heavy subunit is essential for trafficking of the holotransporter HAT to the plasma membrane, whereas the light subunit catalyzes the transporter function (for reviews see (Palacin and Kanai, 2004; Verrey et al., 2004)). In addition, 4F2hc mediates  $\beta$ -integrin signaling (Feral et al., 2005), cell fusion (Ohgimoto et al., 1995; Takesono et al., 2012) and cell proliferation (Cantor et al., 2009; Fogelstrand et al., 2009). Indeed, 4F2hc-associated transporters are overexpressed in cancers and in activated lymphocytic cells suggesting a role of 4F2hc and these transporters in cell growth (see below). 4F2hc-dependent  $\beta$ -integrin signaling might be at the basis of these roles in the case of 4F2hc, but how 4F2hc mediates integrin signaling is unknown (Feral et al., 2005; Takesono et al., 2012). The dual function of 4F2hc suggests that this protein plays a key role in integrating integrin signaling and amino acid transport, but the impact of integrins modulating amino acid transport via 4F2hc has not been experimentally addressed. No function besides targeting of  $b^{0,+}$ AT to the plasma membrane has been reported for rBAT so far. In agreement with the different roles of both heavy subunits, 4F2hc-associated transporters as a whole are almost ubiquitously expressed, whereas rBAT/ $b^{0,+}$ AT is restricted to the apical domain of the plasma membrane of epithelial cells of the small intestine and of the renal proximal tubule (Fig. 3B). In contrast to rBAT, 4F2hc is expressed in the basolateral plasma membrane in epithelial cells (Broer and Palacin, 2011) (Fig. 3B).

Experimental evidence suggests that in native tissues 4F2hc-associated transporters are single heterodimers whereas rBAT/ $b^{0,+}$ AT is a dimer of heterodimers ( $([rBAT/b^{0,+}AT])_2$ ), where the single rBAT/ $b^{0,+}$ AT heterodimer is the transport functional unit (Fernandez et al., 2006). Chimerical fusion proteins of rBAT with 4F2hc-light subunits also show functional heterotetramers, suggesting that the heavy subunit dictates the oligomerization state (Fernandez et al., 2006).

The biogenesis of rBAT/ $b^{0,+}$ AT has been more widely studied than that of 4F2hc-associated transporters (Bartoccioni et al., 2008; Bauch and Verrey, 2002; Sakamoto et al., 2009). As expected, the co-expression of  $b^{0,+}$ AT and rBAT is required for the functional expression of system  $b^{0,+}$  in the plasma membrane of non-polarized cells and in the apical membrane of polarized cells (Fig. 3B). Alone, i.e. not associated with rBAT,  $b^{0,+}$ AT probably remains in the endoplasmic reticulum (ER), where it has a

**Table 2**

SLC3 – heavy subunits of the heteromeric amino acid transporters. For detailed information about the SLC gene tables, please visit: <http://www.bioparadigms.org>.

Human gene name	Protein name	Aliases	Predominant substrates	Transport type/ coupling ions <sup>a</sup>	Tissue distribution and cellular/subcellular expression	Link to disease	Human gene locus	Sequence accession ID	Splice variants and their features
SLC3A1	rBAT	NBAT, D2	System b <sup>0+</sup> heterodimerizes with light subunit SLC7A9	E (see details in SLC7 table)	Kidney, small intestine, (apical membrane), liver, pancreas	Cystinuria, hypotonia-cystinuria syndrome (HCS) and related Cancer	2p16.3	NM_000341	
SLC3A2	4F2hc	CD98hc, FRP	Systems L, y <sup>+</sup> L, x <sub>c</sub> <sup>-</sup> and asc with light subunits SLC7A5–8 and SLC7A10–11	E (see details in SLC7 table)	Ubiquitous, (basolateral membrane)		11q13	NM_002394 NM_001012661 NM_001012662 NM_001012663 NM_001012664 NM_001013251	6 Splice variants

<sup>a</sup> C: cotransporter; E: exchanger; F: facilitated transporter; O: orphan transporter.

long half-life and from where it can be functionally reconstituted into proteoliposomes (Reig et al., 2002). Thus, folding of b<sup>0+</sup>AT is independent of rBAT. 4F2hc-independent folding of associated light subunits is also expected, but has not been addressed experimentally. In the ER, free rBAT is retained and degraded by the ER-associated protein degradation pathway. Assembly of b<sup>0+</sup>AT and rBAT in the ER abolishes degradation of rBAT. The heterodimer rapidly and efficiently travels along the secretory pathway. Experimental evidence indicates that at least part of the folding of the extracellular domain of rBAT occurs after its assembly with b<sup>0+</sup>AT (Bartoccioni et al., 2008; Rius and Chillaron, 2012).

#### 4. HATs and their light subunits

Substrate selectivity and ion coupling differ among HATs. Thus, these transporters correspond to system L (4F2hc/LAT1 and 4F2hc/LAT2), system y<sup>+</sup>L (4F2hc/y<sup>+</sup>LAT1 and 4F2hc/y<sup>+</sup>LAT2), system x<sub>c</sub><sup>-</sup> (4F2hc/xCT), system asc (4F2hc/Asc-1 and Asc-2 linked to an unknown heavy subunit) and system b<sup>0+</sup> (rBAT/b<sup>0+</sup>AT) (Chillaron et al., 2001; Verrey et al., 2004). In contrast, the AGT1 (SLC7A13) and arpAT (Slc7a15) transport systems were discovered when their cDNAs were cloned (Fernandez et al., 2005; Matsuo et al., 2002). A common feature of HATs is that they are obligatory exchangers with the exception of system asc isoforms that also mediate facilitated diffusion (Pineda et al., 2004). Although there are some small differences, the selectivity of influx and efflux are similar for the L-type and b<sup>0+</sup> transporters, but the apparent substrate affinities are much lower for efflux (inside) (Reig et al., 2002; Verrey et al., 2004). The features of HATs are summarized in Tables 1 and 2.

##### 4.1. System L transporters

###### 4.1.1. LAT1 (SLC7A5; TC 2.A.3.8.1)

L-type amino acid transporter-1 (LAT1) was the first cloned light subunit of HATs (Kanai et al., 1998; Mastroberardino et al., 1998). 4F2hc/LAT1 mediates the sodium-independent obligatory exchange (1:1 stoichiometry) of large neutral amino acids and of the non-metabolizable analog 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) (Mastroberardino et al., 1998; Meier et al., 2002). The apparent affinity for large neutral and aromatic amino acids is in the physiological micromolar range on the extracellular side and up to 100-fold higher on the cytosolic side of the transporter. Transport sidedness also affects substrate selectivity, L-leucine, L-isoleucine and L-methionine being better efflux than influx substrates. The obligatory exchange mechanism allows the relative concentration of large neutral amino acids to equilibrate across the plasma membrane, and represents a tertiary-active mechanism of uptake when coupled with sodium-dependent transporters (i.e., exchange against amino acids accumulated in the cytosol). SLC7A5 mRNA is expressed in the placenta > the brain > the spleen > the testes and the colon (Kanai et al., 1998). Immunohistochemistry with anti-LAT1 antibodies demonstrated that LAT1 is predominantly expressed in microvessels of the central nervous system participating in the transport of L-3,4-dihydroxyphenylalanine (L-DOPA) across the blood–brain barrier (Kageyama et al., 2000). Furthermore, LAT1 is expressed at the inner blood–retinal barrier, where it plays a key role in maintaining large neutral amino acids and neurotransmitters (Tomi et al., 2005). LAT1 is also expressed in placental membranes supplying the developing fetus and the placenta with thyroid hormones and amino acids (Ritchie and Taylor, 2001). In human fibroblasts LAT1 is the major transporter of tyrosine (Vumma et al., 2008). As mentioned above, LAT1 is also a thyroid hormone transporter and might contribute to thyroid hormone transport in several cell types (for a review see (Kinne et al., 2011)). Transport of neutral branched amino acids via LAT1 stimulates mTOR (mammalian target of rapamycin) in several cell lines (Fig. 3C) (Nicklin et al., 2009). Finally, 4F2hc/LAT1 is overexpressed in many tumor cell lines and primary human tumors (Fuchs and Bode, 2005; Kaira et al., 2008; Kobayashi et al., 2008), where it has been shown to play essential roles in growth and survival.



Amino acid transport might contribute to the role of 4F2hc/LAT1 activating cell growth of cancer cells (Imai et al., 2010; Yamauchi et al., 2009) (see below).

#### 4.1.2. LAT2 (SLC7A8; TC 2.A.3.8.6)

4F2hc/LAT2 is a sodium-independent, high-affinity obligatory exchanger. Its transport activity corresponds to a system L with broad substrate selectivity for all neutral amino acids, including small ones (Meier et al., 2002; Pineda et al., 1999; Rossier et al., 1999). Similar to LAT1, LAT2 displays much lower intracellular (in the millimolar range) than extracellular (in the physiological micromolar range) apparent substrate affinities (Meier et al., 2002). Human SLC7A8 mRNA is expressed in many tissues and the expression levels are as follows: kidney  $\gg$  placenta  $\gg$  brain, liver  $>$  spleen, skeletal muscle, heart, small intestine and lung (Pineda et al., 1999). Furthermore, mRNA is also expressed at high levels in the prostate, the testes, the ovaries and in the fetal liver (Park et al., 2005). LAT2 has a basolateral location in renal and intestinal epithelial cells (Rossier et al., 1999) (Fig. 3B). 4F2hc/LAT2 efficiently exports L-cysteine in heterologous expression systems and cell culture models of renal epithelia (Bauch et al., 2003; Fernandez et al., 2003). In contrast, the null knockout of *Slc7a8* in mice did not show renal reabsorption defects of neutral amino acids and cystine in particular (Braun et al., 2011). *Slc7a8* knockout mice present normal development and growth. Only a slightly altered coordination of movements was observed in *Slc7a8*<sup>-/-</sup> mice. Functional compensation by other amino acid transporters might explain the lack of a severe phenotype. LAT2 also transports thyroid hormones (Friesema et al., 2001), but *Slc7a8* KO mice do not show altered circulating thyroid hormones and thyroid hormone-responsive genes, possibly because of functional compensation by other thyroid hormone transporters such as the monocarboxylate transporter 8 (MCT8) or LAT1 (Braun et al., 2011). It has been postulated that 4F2hc/LAT2, like 4F2hc/LAT1 in other cell types, provides neutral-branched amino acids to activate the mTORC1 pathway, particularly in crescentic glomerulonephritis (Kurayama et al., 2011).

### 4.2. System y<sup>+</sup>L transporters

#### 4.2.1. y<sup>+</sup>LAT1 (SLC7A7; TC 2.A.3.8.7)

The HATs 4F2hc/y<sup>+</sup>LAT1 and 4F2hc/y<sup>+</sup>LAT2 (see below) are the molecular correlates of system y<sup>+</sup>L (Deves and Boyd, 1998). These transporters are obligatory exchangers of cationic amino acids (sodium-independent) and neutral amino acids (sodium-dependent) rendering the transporter electroneutral (Kanai et al., 2000; Pfeiffer et al., 1999; Torrents et al., 1998). Under physiological conditions (i.e., 150 mM Na<sup>+</sup> gradient) system y<sup>+</sup>L transporters would maintain a ~5-fold gradient of cationic (lower in the cytosol) and neutral (higher in the cytosol) amino acids. Thus, it is expected that in cells with active influx of cationic amino acids (e.g., cells expressing rBAT/b<sup>0+</sup>AT or CATs) 4F2hc/y<sup>+</sup>LAT1 will mediate export of cationic amino acids (Fig. 3B). y<sup>+</sup>LAT1 is expressed in the small intestine, the kidney, the placenta, the lung, the spleen and in circulating monocytes and macrophages (Barilli et al., 2010; Pfeiffer et al., 1999; Torrents et al., 1998; Yeramian et al., 2006). In the epithelial cells of the small intestine and in the renal proximal tubule, y<sup>+</sup>LAT1 has a basolateral location (Fig. 3B). Similar to LAT2 and b<sup>0+</sup>AT, y<sup>+</sup>LAT1 expression along the proximal kidney tubule follows a decreasing axial gradient (Bauch et al., 2003). In agreement with the cationic amino acid efflux function and its high expression in kidney and small intestine, 4F2hc/y<sup>+</sup>LAT1 has a relevant role in the renal and intestinal re-absorption of cationic amino acids, and mutations in y<sup>+</sup>LAT1 cause the primary inherited aminoaciduria, lysinuric protein intolerance (LPI) (see below).

#### 4.2.2. y<sup>+</sup>LAT2 (SLC7A6)

The transport characteristics of 4F2hc/y<sup>+</sup>LAT2 are very similar to those of 4F2hc/y<sup>+</sup>LAT1 (Kanai et al., 2000; Pfeiffer et al., 1999; Torrents et al., 1998) but 4F2hc/y<sup>+</sup>LAT2 preferentially mediates the efflux of L-arginine in exchange of L-glutamine plus Na<sup>+</sup> (Broer et al., 2000). y<sup>+</sup>LAT2 has a broader tissue distribution than y<sup>+</sup>LAT1 and is expressed in many organs like brain, heart, testis, kidney, small intestine and parotids (Broer et al., 2000). In fibroblasts of LPI patients 4F2hc/y<sup>+</sup>LAT2 is responsible for system y<sup>+</sup>L activity (Dall'Asta et al., 2000). SLC7A6 is a member of the TC 2.A.3.8 subfamily.

### 4.3. System x<sub>c</sub><sup>-</sup>

#### 4.3.1. xCT (SLC7A11; TC 2.A.3.8.5)

4F2hc/xCT is the molecular correlate of system x<sub>c</sub><sup>-</sup> (Sato et al., 1999). It is the Na<sup>+</sup>-independent and electroneutral exchanger of extracellular anionic cystine (for which it has a high-affinity) and intracellular glutamate. The exchange takes place with a stoichiometry of 1:1. Under normal physiological conditions the high intracellular concentration of glutamate and the intracellular reduction of cystine drive the direction of this exchange. (S)-4-carboxy-3-hydroxyphenylglycine is a known and specific system x<sub>c</sub><sup>-</sup> inhibitor, which does not affect the Na<sup>+</sup>-dependent excitatory glutamate transporters EAAT1-5 (SLC1 family) (Patel et al., 2004). xCT is mainly expressed in brain, macrophages and cell culture lines (Fig. 3C) (Bassi et al., 2001; Sato et al., 1999) and has a dual role: it provides cystine for the synthesis of the major endogenous antioxidant (reduced glutathione (GSH)) and supports the non-vesicular release of glutamate (Bridges et al., 2012; Conrad and Sato, 2012). Thus, expression of xCT is up-regulated in cells requiring GSH synthesis as activated macrophages and GSH-depleted culture cells (Bridges et al., 2001; Kim et al., 2001; Sato et al., 1999, 2002; Seib et al., 2011). xCT expression is regulated by competitive binding of positive (Nrf2) and negative (BACH1) factors to an antioxidant/electrophile response element in the xCT promoter (Ishii et al., 2000; Qin et al., 2010; Sasaki et al., 2002).

Defective xCT impairs cell growth and survival. Thus, xCT loss-of-function in cellular and mouse models presents defective protection against oxidative stress in activated macrophages (Nabeyama et al., 2010) and activation of the ROS/autophagy pathway in hepatocellular carcinoma (Guo et al., 2011) (Fig. 3C). In the same sense, up-regulation of xCT increases protection against oxidative stress. Therefore, forced expression of xCT in  $\gamma$ -glutamylcysteine synthetase knock-out cells rescues glutathione deficiency (Mandal et al., 2010). Furthermore, phosphorylation of eIF2 $\alpha$  activates ATF4-mediated transcription of xCT, and constitutively active ATF4 mutations mediate the resistance of neuronal cell lines against oxidative stress by inducing xCT expression (Lewerenz and Maher, 2009; Lewerenz et al., 2012). Besides the claimed role of 4F2hc/xCT in protection against oxidative stress, it is surprising that *Slc7a11* knockout mice present redox imbalance but viable animals (Sato et al., 2005). It is also surprising that ablation of xCT does not exacerbate the phenotype of the superoxide dismutase 1 (*Sod1*) knockout in mice (Iuchi et al., 2008).

In brain, the role of 4F2hc/xCT seems to be more related to the homeostasis of glutamate. Glutamate is released into the extracellular space from synaptic and nonsynaptic sources, and is eliminated via Na<sup>+</sup>-dependent transporters of the SLC1 family, mainly by the major glial transporter GLT1 (Danbolt, 2001). The extracellular nonsynaptic glutamate concentration in several brain areas (hippocampus, nucleus accumbens, striatum) depends on the activity of system x<sub>c</sub><sup>-</sup> as revealed by inhibitor blockade or knockout of the transporter (Baker et al., 2003; De Bundel et al., 2011; Melendez et al., 2005). Therefore, ablation of xCT affects brain excitability and has behavioral consequences. System x<sub>c</sub><sup>-</sup> is required for optimal spatial working memory, and its inactivation decreases susceptibility for limbic epileptic seizures (De Bundel et al., 2011). Glutamate secretion via a prominently expressed 4F2hc/xCT is also at the basis of neurodegeneration and brain edema in the periphery of glioma tumors, and the invasion of glioma cells (Lyons et al., 2007; Savaskan et al., 2008). Moreover, the role of 4F2hc/xCT in the homeostasis of glutamate in brain is involved in the relapse of cocaine addiction, where system x<sub>c</sub><sup>-</sup> is down-regulated after chronic cocaine consumption. This results in reduced extracellular levels of the amino acid and diminished stimulation of extrasynaptic group II metabotropic glutamate receptors in the nucleus accumbens and prefrontal cortex (Baker et al., 2003; Kau et al., 2008; Xi et al., 2002a,b). Indeed, activation of the transporter with *N*-acetylcysteine restores glutamate concentration and attenuates the reinstatement of cocaine-seeking behavior (Moussawi et al., 2009).

In addition to its transport function, xCT is Kaposi's sarcoma-associated herpesvirus (KSHV; human herpesvirus 8) fusion-entry receptor (Kaleeba and Berger, 2006). KSHV binds to the target cell surface interacting with heparan sulfate molecules via its envelope glycoproteins gB and gpK8.1A, with integrins ( $\alpha$ V $\beta$ 5,  $\alpha$ V $\beta$ 3 and  $\alpha$ 3 $\beta$ 1) via gB, with the transporter 4F2hc/xCT complex, and possibly with other molecules (Veetil et al., 2008). Interestingly, KSHV induces the expression of xCT by microRNA miR-K12-11-dependent suppression of the negative regulator factor BACH1 of antioxidant response elements. In this way the virus facilitates *de novo* infection and the survival of KSHV-infected and reactive nitrogen species (RNS)-secreting cells in the tumor microenvironment (Qin et al., 2010).

#### 4.4. System asc transporters

##### 4.4.1. Asc-1 (SLC7A10; TC 2.A.3.8.13)

4F2hc/Asc-1 and Asc-2, which is linked to a yet unknown heavy subunit, are the two isoforms of system asc (alanine-serine-cysteine). 4F2hc/Asc-1 mediates Na<sup>+</sup>-independent transport of glycine, L-alanine, L-serine, L-threonine, L-cysteine,  $\alpha$ -aminoisobutyric acid and  $\beta$ -alanine (Fukasawa et al., 2000; Nakauchi et al., 2000). 4F2hc/Asc-1 has two peculiarities among HATs: first, it also transports D-isomers of the small neutral amino acids, in particular D-serine, with high apparent affinity (*K<sub>m</sub>* values of ~50  $\mu$ M). Second, 4F2hc/Asc-1 also works as a facilitate diffuser, although it preferentially works as an exchanger. These characteristics correspond to an asc isoform with low stereospecificity.

*SLC7A10* mRNA is expressed in the brain, lung, skeletal muscle, heart, kidney, small intestine and placenta (Fukasawa et al., 2000; Nakauchi et al., 2000). It is a neuronal transporter distributed all over the CNS (Helboe et al., 2003). Most probably the main physiological role of 4F2hc/Asc-1 in the CNS is the control of the synaptic concentration of the glutamate co-agonists that activate the N-methyl-D-aspartate (NMDA) receptors, i.e. D-serine and glycine (Hashimoto and Oka, 1997). Consequently, ablation of *Slc7a10* in mice results in tremors, ataxia, seizures and early postnatal death (Xie et al., 2005). These data suggest that the hyper-excitability of *Slc7a10* knockout mice is due to over-activation of NMDA receptors, presumably resulting from elevated extracellular D-serine.

##### 4.4.2. Asc-2 (Slc7a12; TC 2.A.3.8.14)

Asc-2 (asc-type amino acid transporter-2) has a relatively low, but significant, AAS identity and similarity to other HATs (see Fig. 2). Mouse Asc-2 has the cysteine residue in the 2nd extracellular loop (between TMDs III and IV) that is conserved in all HATs. This cysteine is involved in the disulfide bridge connecting Asc-2 with its heavy subunit. Western blot analysis of mouse erythrocytes and kidney detected Asc-2 as multiple bands under non-reducing conditions and as a single band with a lower molecular mass under reducing conditions. This suggested that Asc-2 is associated with other protein(s) via a disulfide bond (Chairoungdua et al., 2001). Besides this, co-expression of Asc-2 and 4F2hc or rBAT does not induce any transport activity at the cell plasma membrane. Mouse Asc-2 was characterized functionally using fusion proteins of Asc-2 and 4F2hc or rBAT that were sorted to the plasma membrane. These fusion proteins exhibited a transport function corresponding to the Na<sup>+</sup>-independent transport system asc (Chairoungdua et al., 2001). Unlike the other system asc isoform (4F2hc/Asc-1), Asc-2 is stereoselective and does not accept some of the high-affinity substrates of Asc-1, such as  $\alpha$ -aminoisobutyric acid and  $\beta$ -alanine. Mouse Asc-2 is expressed in kidney collecting duct cells, placenta, spleen, lung and skeletal muscle (Chairoungdua

et al., 2001). A human ortholog of *Slc7a12* is not present in the human genome. To our knowledge, the phenotype of the *Slc7a12* knockout mouse has not been reported, and the physiological roles of this transporter are unknown.

#### 4.5. System $b^{0,+}$

##### 4.5.1. rBAT/ $b^{0,+}$ AT (SLC3A1/SLC7A9)

The rBAT/ $b^{0,+}$ AT heterodimer mediates the electrogenic exchange of extracellular cationic amino acids and cystine for intracellular neutral amino acids, except imino acids (Bertran et al., 1992; Busch et al., 1994; Chillaron et al., 1996). On the extracellular side, rBAT/ $b^{0,+}$ AT has a high affinity for cationic amino acids and cystine ( $K_m \sim 100 \mu\text{M}$  within the physiological range in plasma), while on the intracellular side the affinity for neutral amino acids is in the millimolar range (e.g.,  $\sim 2.5 \text{ mM}$  for L-leucine) (Reig et al., 2002). In native tissues, disulfide-linked rBAT/ $b^{0,+}$ AT heterodimers were found to form noncovalently-linked heterotetramers (Fernandez et al., 2006). This complex represents system  $b^{0,+}$ , which has a broad specificity for neutral (0) and cationic (+) amino acids. In humans *SLC7A9* mRNA is expressed in kidney, liver, small intestine and placenta (Calonge et al., 1994; Feliubadalo et al., 1999). In small intestine and kidney, the  $b^{0,+}$  system is located in brush-border membranes, where it removes dibasic amino acids and cystine from the intestinal and renal tubular lumen in exchange for intracellular neutral amino acids (Munck, 1980, 1985). The driving forces determining the direction of substrate exchange via system  $b^{0,+}$  are the high intracellular concentration of neutral amino acids, the electric potential across the plasma membrane (negative inside) and the intracellular reduction of cystine to cysteine (Fig. 3B). Mutations in either subunit of system  $b^{0,+}$  cause cystinuria, aminoaciduria of cystine and dibasic amino acids (see below). *SLC7A9* is a member of the TC 2.A.3.8 subfamily.

##### 4.6. AGT1 (SLC7A13; TC 2.A.3.8.8)

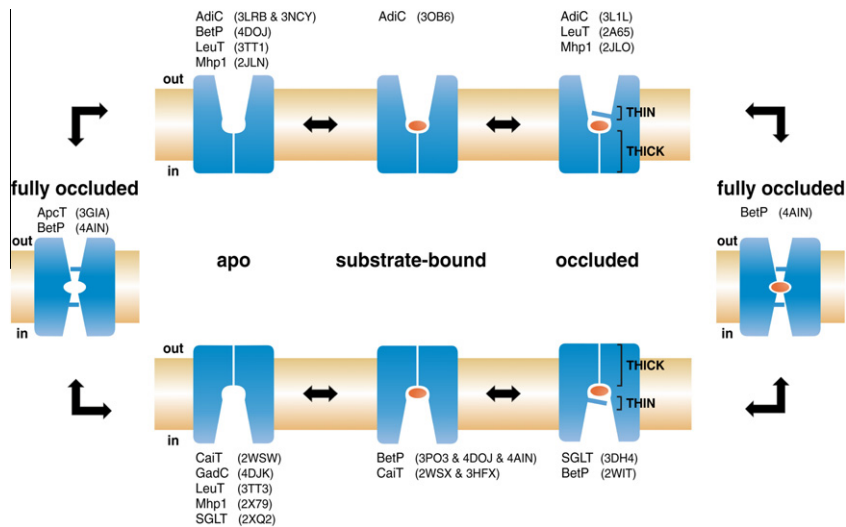
The aspartate/glutamate transporter-1 (AGT1) was identified as a SLC7 family member in 2002 by Endou and coworkers (Matsuo et al., 2002). Similar to the HAT Asc-2, AGT1 is associated with unknown protein(s), i.e. putative heavy chain(s), via a disulfide bridge. AGT1 was successfully expressed in the plasma membrane of *Xenopus* oocytes as fusion proteins of the transporter with 4F2hc or rBAT (Matsuo et al., 2002). The AGT1 fusion proteins transported anionic amino acids in a sodium-independent manner. This transport activity is distinct from that of xCT, since AGT1 shows high affinities for aspartate and glutamate but does not accept cystine, homocysteate or  $\text{l-}\alpha$ -amino adipate. AGT1 is only expressed in kidney at the basolateral membrane of the proximal straight tubules and distal convoluted tubules. To our knowledge, the phenotype of the mouse *Slc7a13* knockout has not been reported and the physiological roles of this transporter are unknown.

##### 4.7. ArpAT (Slc7a15; TC 2.A.3.8.11)

The *Slc7a15* cDNA encoding arpAT (aromatic-preferring amino acid transporter) was identified by a similarity search through vertebrate genomes using known human and mouse light subunit sequences (Fernandez et al., 2005). Co-expression of mouse arpAT with the human heavy subunit rBAT in HeLa cells resulted in the formation of disulfide-linked heterodimers and the induction of sodium-independent transport of several amino acids including L-alanine, L-tyrosine, L-DOPA, L-glutamine, L-serine, L-cystine and L-arginine (Fernandez et al., 2005). Kinetic and cis-inhibition studies showed a  $K_m$  of 1–2 mM for L-alanine and an IC50 in the millimolar range for most amino acids, except L-proline, glycine, anionic and D-amino acids, which were not inhibitory. L-DOPA and L-tyrosine were the most effective competitive inhibitors of L-alanine transport, with IC50 values in the physiological micromolar range. Co-expression of mouse arpAT and human 4F2hc also induced amino acid transport (Fernandez et al., 2005). The associated heavy subunit (rBAT and/or 4F2hc) in the original tissue is unknown. *Slc7a15* mRNA is expressed in some areas of the brain and mainly in the small intestine, where it shows a decreasing expression gradient in the enterocytes from the crypts to the tip of the villi. *SLC7A15* is inactivated in the primate lineage and the gene denoted as *SLC7A15P* (Casals et al., 2008).

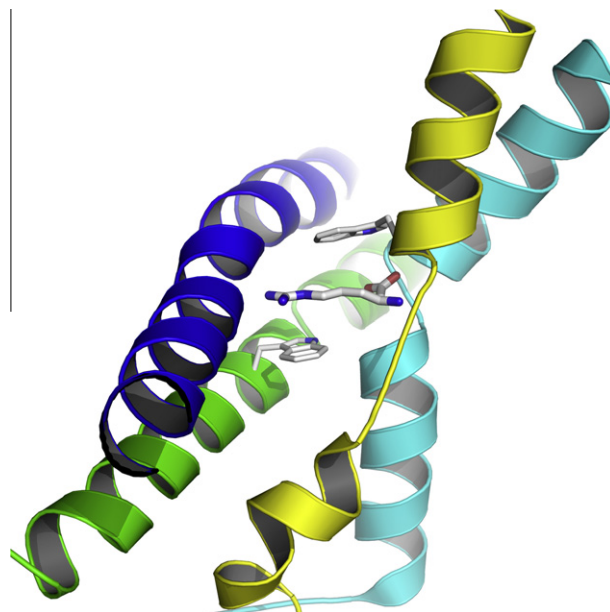
## 5. The structure of the light subunits of HATs

The atomic structure of the bacterial APC transporters AdiC (arginine/agmatine antiporter) (Fang et al., 2009; Gao et al., 2009, 2010; Kowalczyk et al., 2011), ApcT ( $\text{H}^+$ -coupled amino acid transporter) (Shaffer et al., 2009) and GadC (glutamate/GABA antiporter) (Ma et al., 2012) are the present structural models of the light subunits of HATs. These transporters present the same protein fold as the bacterial transporter LeuT (Yamashita et al., 2005), a fold also shared by five transporter families with no apparent homology between them (AAS identity < 10%) (Jeschke, 2012) (Fig. 4). The LeuT fold is characterized by a pseudosymmetry that relates two structurally similar repeats of 5 consecutive TMDs by a pseudo twofold axis of symmetry located in the plane of the membrane, i.e., the first 5 TMDs (1st repeat) and following 5 TMDs (2nd repeat) have an inverted topology in the membrane. Thus, this fold is also named 5 + 5 inverted repeat fold. Sequence analysis indicates that the 5 + 5 inverted TMDs correspond to the first 10 TMDs in the light subunits of HATs and CATs (Bartoccioni et al., 2010). To translocate the substrate, LeuT fold transporters transit through different outward-facing and inward-facing conformations of apo, substrate-bound open and substrate-bound occluded states (Fig. 4). When occluded, the substrate is blocked by a thin



**Fig. 4.** Conformational states forming part of the alternative access mechanism of prokaryotic transporters with the LeuT fold. When substrate (red ellipse) binds to the open-to-out or open-to-in apo states, the substrate-bound state changes to an occluded state, where a thick and thin gate prevent the diffusion of the substrate to either side of the membrane (Krishnamurthy et al., 2009). Transition to the inward-facing (or outward-facing) states requires a transient, fully occluded, pseudosymmetrical intermediate. The transporters indicated here belong to 5 different families: LeuT, (leucine transporter from the neurotransmitter:sodium symporter family), SGLT (sodium-coupled glucose transporter from the solute:sodium symporter family), Mhp (sodium-hydantoin transporter from the nucleobase:cation symporter-1 family), BetP and CaIT (betaine and carnitine transporters, respectively, from the betaine/carnitine/choline transporter family) and the transporters AdiC, ApcT and GadC from the APC superfamily. Figure adapted from (Kowalczyk et al., 2011).

(usually the side chain of a single residue) and a thick (usually several TMDs) gate (see Figs. 4 and 5) preventing the diffusion of the substrate to either side of the membrane (Krishnamurthy et al., 2009). Three atomic structures of LeuT-fold transporters with a bound amino acid substrate have been reported: LeuT, with L-leucine bound in the outward-facing state of the transporter (Yamashita et al., 2005), AdiC with L-arginine bound in the outward-facing open state of the transporter (Kowalczyk et al., 2011) and AdiC with L-arginine bound in the outward-facing occluded state of the transporter (Gao et al., 2010).



**Fig. 5.** Binding pocket and substrate recognition in the bacterial L-arginine/arginine antiporter AdiC, a member of the APC superfamily. Color codes for TMDs: cyan – TMD I, blue – TMD III, yellow – TMD VI and green – TMD VIII. The  $\alpha$ -amino carboxyl moiety of L-arginine interacts with the unwound regions of TMD I (cyan). Trp293 in TMD VIII (green) is the main attractor of the guanidinium group of L-arginine. The movement of Trp202 in TMD VI (yellow) results in the occlusion of L-arginine from the periplasm (thin gate). The thick gate is composed of several TMDs, including those depicted in the figure.

Interestingly, a common feature is apparent in these structures: binding of the  $\alpha$ -amino carboxyl moiety of the substrate to the unwound section of the first TMDs of each repeat (i.e. 5 + 5 inverted repeat) and interaction of the side-chain of the substrate to residues in the third TMD of each repeat. In AdiC, the  $\alpha$ -amino carboxyl moiety of L-arginine interacts with the unwound regions of TMD I (cyan, Fig. 5) and TMD VI (yellow) and the main attractor of the guanidinium group of L-arginine is residue Trp293 in TMD VIII (green). The movement of Trp202 in TMD VI (yellow, Fig. 5) results in the occlusion of L-arginine from the periplasm. In spite of the low AAS identity with LeuT (<10%) and AdiC (<20%), the design of the substrate binding site seems to be conserved in LATs and CATs. In accordance, thiol modification of Cys327 in TMD VIII of the light subunit xCT suggests close proximity to the substrate binding site/permeation pathway (Jimenez-Vidal et al., 2004). Further, mutations of residue Lys295 in TMD VIII (homologous to Trp293 in AdiC) broadened the substrate specificity of SteT, a bacterial LAT that exchanges L-serine and L-threonine (Bartoccioni et al., 2010). Finally, the biochemical phenotype associated to some cystinuria and LPI mutations (e.g., T123M in b<sup>0+</sup>AT and G54V in y<sup>+</sup>LAT1, see below) also support a common substrate binding design.

For CATs a protein fold and design of the binding pocket similar to LATs is expected based on available functional data. Glu107 (marked in green in Fig. 1A) is known to be essential for the transport activity of mouse CAT-1 (Wang et al., 1994). Located in TMD III and conserved in all other known CAT isoforms, Glu107 is likely to be part of the substrate translocation pathway (for a review see: (Closs, 2002)). The third extracellular loop (TMD V-TMD VI) of mouse CAT-1 is the main binding site for ecotropic murine leukemia viruses (reviewed in (Closs et al., 2006)), and is interestingly the most protruding periplasmic loop in AdiC. The currently available atomic structures of APC transporters do not indicate the role played by the 42 amino acid residue stretch that differs between the splice variants CAT-2A and -2B (Fig. 1A). At present, there are no atomic structures of prokaryotic or eukaryotic LAT and CAT proteins. The low AAS identities of LATs and CATs with APC transporters of known structure preclude the generation of robust human transporter models. Finally, APC transporter structures give no clues about the supramolecular organization of HATs, i.e., the architecture and interactions between the heavy and the light subunits.

## 6. HATs and inherited aminoacidurias

Mutations in systems b<sup>0+</sup> (rBAT/b<sup>0+</sup>AT) and y<sup>+</sup>L (4F2hc/y<sup>+</sup>LAT1) can cause cystinuria and LPI, respectively. Cystinuria is the most common primary inherited aminoaciduria (Online Mendelian Inheritance in Man (OMIM) database, entry 220100). Hyperexcretion of cystine and dibasic amino acids into the urine is the hallmark of the disease. The poor solubility of cystine results in the formation of renal calculi, which can cause obstruction, infection and ultimately chronic kidney disease. Cystinuria causes 1–2% of all cases of renal stone formation and even 6–8% in paediatric patients (for a review see (Chillaron et al., 2010)). Cystinuria is due to defective transport of cystine and dibasic amino acids across the apical membrane of epithelial cells of the renal proximal tubule and the small intestine, which is mediated by system b<sup>0+</sup> (rBAT/b<sup>0+</sup>AT heterodimer; Fig. 3B). Specific mutations in the genes coding both subunits cause the disease (Calonge et al., 1994; Feliubadalo et al., 1999). Cystinuria is usually considered to be an autosomal-recessive disorder, requiring two mutated alleles for the disease to occur. Two biochemical phenotypes of cystinuria have been described. In type I, individuals with one mutated allele have normal urinary excretion of amino acids, whereas in non-type-I heterozygotes, urinary hyperexcretion of dibasic amino acids and cystine is observed (Chillaron et al., 2010). Moreover, some individuals carrying a single non-type-I allele also produce cystine calculi (Font-Llitjos et al., 2005). Therefore, non type-I cystinuria should be considered an autosomal dominant disease, where a single mutated allele suffices to cause disease. Worldwide, 133 mutations have been reported in *SLC3A1* (in a total of 579 alleles) and 95 mutations in *SLC7A9* (in a total of 436 mutated alleles) (Chillaron et al., 2010). Type I cystinuria is usually caused by mutations in *SLC3A1* encoding rBAT, with <15% of the mutant alleles involving *SLC7A9* (Font-Llitjos et al., 2005). Some of these type-I-associated *SLC7A9* alleles carry hypomorphic mutations (Chillaron et al., 2010). Similarly, mice harboring the missense mutation D140G in *SLC7A9* present type I cystinuria (Peters et al., 2003). In contrast, non-type I cystinuria is usually caused by mutations in *SLC7A9* encoding b<sup>0+</sup>AT, with <4% having mutations in *SLC3A1* (Chillaron et al., 2010). Accordingly, *SLC7A9*-deficient mice present non-type-I cystinuria (Feliubadalo et al., 2003).

Cystinuria-specific *SLC3A1* mutations show loss of function due to strong trafficking defects, supporting the proposed role of rBAT as a helper subunit for trafficking of the holotransporter to the plasma membrane (Bartoccioni et al., 2008). Two different mechanisms underlie the trafficking defects. The mutation L89P, located in the single TMD, impedes efficient assembly of rBAT with b<sup>0+</sup>AT. However, the small amount of rBAT/b<sup>0+</sup>AT that reaches the plasma membrane has mature glycosylation and forms heterotetramers. In contrast, mutations in the ectodomain of rBAT allow efficient assembly with b<sup>0+</sup>AT to form disulfide-linked heterodimers, but HATs remain core-glycosylated, fail to oligomerize and are degraded, most probably via the proteasome. All cystinuria-specific mutations in b<sup>0+</sup>AT studied so far cause loss-of-function. Similar to other transporters, the molecular causes include lack of protein expression (mutation G105R), defective trafficking to the plasma membrane (mutation A182T) and defective transport function (mutations A354T and P482L) (Font et al., 2001; Reig et al., 2002; Shigeta et al., 2006). The crystal structures of prokaryotic APC transporters (see above) provide further insights into the molecular defects of some b<sup>0+</sup>AT mutations. For instance, Gly105 is located just after the conserved intracellular loop 1 between TMD II and TMD III (Fig. 1B) suggesting that the G105R mutation causes protein misfolding and degradation. The position of residue Ala182 in TMD V suggests an interaction with the TMD of rBAT (Fig. 1B), but experimental evidence

is missing (Chillaron et al., 2010). Finally, mutation T123M of  $b^{0,+}AT$  causes a very rare type of cystinuria characterized by urinary hyperexcretion of cystine alone (i.e. isolated cystinuria) (Eggermann et al., 2007; Font et al., 2001). Thr123 is located in TMD III within the putative substrate cavity of  $b^{0,+}AT$  suggesting a role of this residue in cystine but not in dibasic amino acid recognition. There are no clues why mutations A354T (TMD IX) and P482L (C-terminus) are transport defective.

In addition to cystinuria and isolated cystinuria, three similar syndromes associated with type I cystinuria have been described: 2p21 deletion syndrome, hypotonia–cystinuria syndrome (HCS) and atypical HCS. These syndromes are associated with recessive deletions of *SLC3A1* and genes contiguous to *SLC3A1* (see (Chillaron et al., 2010) for a review).

LPI (OMIM 222700) is a very rare (~200 patients reported) primary inherited aminoaciduria of cationic amino acids (mainly lysine) with a recessive mode of inheritance (Palacin et al., 2001; Simell, 2001). Patients with LPI are usually asymptomatic while being breast-fed. LPI symptoms appear after weaning and may include diarrhoea, vomiting, failure to thrive, hepatosplenomegaly, lung involvement, osteoporosis, bone-marrow abnormalities, mental retardation, episodes of coma, altered immune response and chronic renal disease. Mutations in the *SLC7A7* gene encoding the HAT light-chain  $y^+LAT1$  cause LPI (Borsani et al., 1999; Torrents et al., 1999). Under normal physiological conditions, the high extracellular  $Na^+$  concentration drives the electroneutral efflux of cationic amino acids in exchange for neutral amino acids via  $y^+LAT1$ . This mode of exchange explains why mutations in  $y^+LAT1$  only cause urine hyperexcretion and intestinal malabsorption of cationic amino acids. Impairment of intestinal absorption and renal re-absorption of dibasic amino acids causes a metabolic derangement characterized by increased urinary excretion and low plasma concentration of dibasic amino acids, and dysfunction of the urea cycle leading to hyperammonaemia, orotic aciduria and protein aversion. In contrast with disorders of apical amino acid transporters (e.g., cystinuria, Hartnup disorder), the basolateral location of the LPI transporter suggests that it cannot be bypassed by the apical intestinal absorption of dibasic amino-acid-containing peptides via the intestinal proton-dependent transporter PEPT1 (peptide transporter 1, also called SLC15A1; Fig. 3B) (Nassl et al., 2011). Thus, patients fail to thrive normally. Mice nullizygous for *Slc7a7* that survive the massive neonatal lethality display identical metabolic derangement to LPI patients (Sperandeo et al., 2007).

In *SLC7A7*, 49 LPI-specific mutations in a total of 141 patients from 107 independent families have been described (>95% of the studied alleles) (Sperandeo et al., 2008). No LPI mutations have been identified in the heavy subunit *SLC3A2* (4F2hc). Indeed, 4F2hc serves to traffic six amino acid transporter subunits (Palacin and Kanai, 2004) and is necessary for proper  $\beta 1$  integrin function (Cantor et al., 2009; Feral et al., 2005). Further, its knockout in mice is lethal (Tsumura et al., 2003). All patients with identified *SLC7A7* mutations present aminoaciduria, whereas other symptoms vary widely, even when the *SLC7A7* mutations are the same (e.g. the Finnish founder splice-site mutation 1181-2A  $\rightarrow$  T). This precludes the establishment of genotype–phenotype correlations. The ten LPI point mutations functionally studied showed loss of function. Four mutations (E36del, G54V, F152L and L334R) reach the plasma membrane and show defective system  $y^+L$  transport activity in heterologous expression systems (Mykkanen et al., 2000; Sperandeo et al., 2005a,b). Gly54 corresponds to the third residue in the highly conserved unwound segment GS/AG in TMD I of  $y^+LAT1$  (i.e., the first TMD of the first inverted repeat), which is believed to interact with the  $\alpha$ -carboxy group of the substrate (Fig. 5). Thus, defective substrate binding appears probable in the G54V mutant. Residue Leu334 in TMD VIII is expected to face the substrate binding site and mutation L334R most probably affects the binding of the substrates. There are no clues to help explaining the defective transport function associated to mutants E36del and F152L.

### 6.1. 4F2hc/LAT1 and cancer

A wide range of tumor cell lines, primary human cancers and metastases (Fuchs and Bode, 2005; Ichinoe et al., 2011; Kaira et al., 2009a,b,c, 2008, 2011; Yoon et al., 2005) show very high expression of LAT1, i.e., 4F2hc/LAT1. Expression of 4F2hc/LAT1 correlates with cell proliferation and angiogenesis, and 4F2hc/LAT1 was a significant prognostic factor for predicting poor outcome in non-small cell lung cancer, triple negative breast cancer and prostate cancer (Furuya et al., 2012; Imai et al., 2010; Kaira et al., 2009b,c; Sakata et al., 2009). LAT1 is considered to be oncogenic because inhibition of system L transport activity by 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) decreases the viability of cancer cell lines and potentiates cell death induced by anti-cancer drugs gefitinib, bestatin and cisplatin (Fan et al., 2010; Imai et al., 2010; Yamauchi et al., 2009). Similarly, knockdown of LAT1 in several human cancer cells lines resulted in marked *in vitro* cell growth inhibition and anti-human LAT1 mAb inhibited *in vivo* tumor growth in athymic mice (Kim et al., 2006; Ohkawa et al., 2011). Thus, LAT1 is considered to be both a prognosis marker and a therapeutic target in cancer. This fostered the idea to develop specific LAT1 inhibitors and specific LAT1 PET tracers. Compound KYT-0353 inhibits leucine uptake via LAT1 and cell growth in human colon cancer-derived HT-29 cells with IC50s of 60 nM and 4  $\mu$ M, respectively (Oda et al., 2010). Moreover, intravenous administration of KYT-0353 reduced the growth of HT-29 tumors transplanted into nude mice (Oda et al., 2010). LAT1 PET tracers have been developed as derivatives from tyrosine ( $L$ -[3- $^{18}F$ ]- $\alpha$ -methyl tyrosine) (Kaira et al., 2007, 2009c, 2010), phenylalanine ( $L$ -p-(2-[ $^{18}F$ ]fluoroethyl)-phenylalanine) (Wang et al., 2011), tryptophan (5-(2- $^{18}F$ -fluoroethoxy)- $L$ -tryptophan) (Kramer et al., 2012) and methionine (S-(3-[ $^{18}F$ ]fluoropropyl)- $D$ -homocysteine) (Denoyer et al., 2012). Among them,  $L$ -[3- $^{18}F$ ]- $\alpha$ -methyl tyrosine is the most specific for LAT1 due to its  $\alpha$ -methyl moiety (Wiriyasermkul et al., 2012). Similarly, the LAT1 selective  $\alpha$ -methyl amino acid probe  $L$ -3-[ $^{123}I$ ]- $\alpha$ -methyl tyrosine has been used for tumor imaging by SPECT (Wiriyasermkul et al., 2012). Nowadays, these tracers are being used to image, evaluate and stage tumors *in vivo*.

Often the overexpression of LAT1 correlates with the up-regulation of ASCT2 (Alanine-Serine-Cysteine Transporter 2; SLC1A5) (Fuchs and Bode, 2005). The roles of 4F2hc/LAT1 and ASCT2 in tumor cells are beginning to be described. Human 4F2hc/LAT1 has a high affinity ( $K_m \sim 50 \mu\text{M}$ ) for large neutral amino acids and a lower affinity ( $K_m$  1–2 mM) for glutamine and asparagine exchange (Yanagida et al., 2001). Human ASCT2 is electroneutral and mediates the exchange of neutral amino acids and  $\text{Na}^+$  ions (Broer et al., 2000). Mouse ASCT2 has a high affinity ( $K_m \sim 20 \mu\text{M}$ ) for small neutral amino acids, glutamine and asparagine, and a lower affinity for certain large neutral amino acids. These include substrates of 4F2hc/LAT1: leucine, isoleucine, phenylalanine, methionine, histidine, tryptophan and valine (Broer et al., 1999). In contrast to ASCT2, the substrate affinities of LAT1 are lower for intracellular than extracellular substrates. This suggests that the 4F2hc/LAT1 exchange velocity is modulated by the intracellular amino acid concentration (Meier et al., 2002). ASCT2 is essential for cell growth and survival as it mediates the net uptake of glutamine (Fuchs et al., 2007). Interestingly, the coordinated action of ASCT2 and 4F2hc/LAT1 (system L uses system ASC substrates in the cytosol to drive the influx of essential amino acids) regulates mTOR signaling (Nicklin et al., 2009). Therefore, deprivation of LAT1 essential amino acids or loss of ASCT2 or 4F2hc/LAT1 at the cell surface inhibit mTOR signaling (Fuchs and Bode, 2005). LAT1, ASCT2 and the lactate transporters MCT1 (SLC16A1) and MCT4 (SLC16A3) assemble in a metabolic activation-related CD147/4F2hc complex (Xu and Hemler, 2005). This complex includes the epithelial cell adhesion molecule (EpcAM), which is a regulator of cell proliferation. The monotopic ancillary protein CD147 is required for functional expression of MCT1 and MCT4 at the plasma membrane (Halestrap and Meredith, 2004). At the cell surface, CD147 induces the expression of matrix metalloproteinases and hyaluronan. Furthermore, it is used as a prognostic marker for a wide range of tumors (Weidle et al., 2010). As for 4F2hc (see above), knockdown experiments indicated a strong positive association between CD147/4F2hc complex and tumor malignancy (Chen et al., 2006; Schneiderhan et al., 2009). Moreover, the CD147/4F2hc complex has a positive association with cell proliferation, and a negative association with the activity of the metabolic-sensing AMPK (Xu and Hemler, 2005), which is one of the signal pathways integrated by mTOR (Gleason et al., 2007; Gwinn et al., 2008; Xu and Hemler, 2005). Therefore, the CD147/4F2hc complex might co-ordinate the transport of nutrients and survival signals of tumor cells. Another mechanism that gives an advantage to tumor cells is the exchange of tryptophan (influx)/kynurenine (efflux) via 4F2hc/LAT1. This tumor immune-escape mechanism protects tumor cells from accumulation of the apoptosis inducing catabolite kynurenine (Kaper et al., 2007).

Integrins are essential receptors involved in cellular behavior. The heavy subunit 4F2hc has been reported to mediate  $\beta\alpha 1$ - and  $\beta\alpha 3$ -integrin signaling (Feral et al., 2005). In B-lymphocytes, 4F2hc induces the integrin-dependent rapid cell proliferation during clonal growth (Cantor et al., 2009). Furthermore, migration and proliferation of vascular smooth muscle cells after arterial injury is dependent of 4F2hc (Fogelstrand et al., 2009). The molecular mechanism of 4F2hc-mediated  $\beta\alpha$ -integrin signaling is not fully elucidated yet. Integrin signaling seems to be independent of the transport activity of the 4F2hc-associated transporters (Fenczik et al., 2001). The transmembrane domain of 4F2hc is necessary and sufficient for integrin association (Henderson et al., 2004). Cross-linking of 4F2hc in the plasma membrane by specific antibodies has been shown to promote clustering of  $\beta$ -integrin to form high-density complexes. This resulted in integrin activation and signaling, and anchorage-independent growth (Kolesnikova et al., 2001; Rintoul et al., 2002). Considering the high expression levels of LAT1 in proliferating tumor cells, it is reasonable to assume that the HAT 4F2hc/LAT1 participates in  $\beta$ -integrin signaling in tumor cells.

## 7. Conclusions and future perspectives

As illustrated, SLC3 and SLC7 family members play central roles in human physiology and are involved in diseases such as inherited aminoacidurias, viral infection and cancer. For the last, a first inhibitor (KYT-0353) has been published setting the stage for improvement and future therapeutic applications. Although promising, we are still at the beginning and several transporters need to be characterized and mouse models established in order to get a full image of the roles of CATs and HATs in human physiology and health. Finally, atomic structures of CATs and HATs have not been solved. When available, these structures will facilitate the comprehension of their molecular mechanisms of transport, the generation of CAT- and HAT-specific ligands by structure-based drug design and the understanding of the mechanisms involved in the functional interaction between integrins and 4F2hc-associated transporters.

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