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REVIEW ARTICLE

The role of amino acid transporters in inherited and acquired diseases

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Amino acids are essential building blocks of all mammalian cells. In addition to their role in protein synthesis, amino acids play an important role as energy fuels, precursors for a variety of metabolites and as signalling molecules. Disorders associated with the malfunction of amino acid transporters reflect the variety of roles that they fulfil in human physiology. Mutations of brain amino acid transporters affect neuronal excitability. Mutations of renal and intestinal amino acid transporters affect whole-body homoeostasis, resulting in malabsorption and renal problems. Amino acid transporters that are integral parts of metabolic pathways reduce the function of these pathways. Finally, amino acid uptake is essential for cell growth, thereby explaining their role in tumour progression. The present review summarizes the involvement of amino acid transporters in these roles as illustrated by diseases resulting from transporter malfunction.

Key words: aminoaciduria, cancer metabolism, epithelial transport, mitochondrion, neurotransmitter, urea cycle disorder.

INTRODUCTION

Amino acid transporters are essential for the absorption of amino acids from nutrition, mediating the interorgan and intercellular transfer of amino acids and the transport of amino acids between cellular compartments [1]. Amino acid transporter-associated diseases are related to metabolic disorders for transporters that are tightly connected to metabolism, particularly when it involves different organs, cell types or cell compartments. In mammalian genomes, transporters are grouped according to sequence similarity into SLC (solute carrier) families (Table 1). To date, almost 50 different SLC families have been identified of which 11 are known to comprise amino acid transporters. One notable exception is cystinosin, a lysosomal cystine transporter, which has not received an SLC number as it belongs to a family of proteins that appear to be involved in protein glycosylation [2]. In addition, researchers in the field use a nomenclature based on functional criteria, such as substrate preference and Na^+-dependence, which categorizes amino acid transporters into systems (Table 1). Although the majority of amino acid transporters have been identified and characterized, a significant number of orphan transporters remain, for instance in the SLC16 and SLC38 families. The physiological function of amino acid transporters is prominently highlighted by inherited and acquired (developing postnatally) diseases that result from transporter malfunction. The present review tries to summarize cases where a firm association has been made between an amino acid transporter and a disease status. We have excluded loose associations, such as up-regulation or down-regulation of a gene in a certain disease state. The diseases are grouped by the organ that is mainly affected by the gene dysfunction. The description of the diseases is preceded by a brief description of the biochemistry of the transporters involved.

BIOCHEMISTRY OF AMINO ACID TRANSPORTERS INVOLVED IN DISEASES

Biochemistry of SLC1 amino acid transporters

Glutamate uptake in mammalian cells is mediated by a family of closely related glutamate/aspartate transporters named EAAT (excitatory amino acid transporter) 1–5 (also called SLC1A1–A3, SLC1A6 and SLC1A7) [3]. In addition, the family comprises two ASC-type neutral amino acid transporters (SLC1A4 and SLC1A5, ASC indicating a preference for alanine, serine and cysteine). Members EAAT1–5 transport glutamate and aspartate with affinities of 10–100 μM. A hallmark of these transporters is the preference of D-aspartate over L-aspartate, whereas the inverse is true for glutamate stereoisomers [4]. The substrate is taken up in co-transport with $3Na^+$ and $1H^+$. Return of the carrier is facilitated by K^+ antiport [5]. The mechanism of these transporters has been characterized in extensive detail due to their role in neurotransmitter removal [6].

The high-resolution structure of a bacterial homologue of the glutamate transporter family has revealed detailed insight into the structure and mechanism of the transporter [7]. The transporter from the thermophilic bacterium *Pyrococcus horikoshii* (PDB code 1XFH) forms a trimer of functionally independent subunits (Figure 1A). The trimer adopts a shape similar to a deep bowl, which reaches almost halfway through the membrane.

Abbreviations used: ACE2, angiotensin-converting enzyme 2; AdiC, arginine/agmatine antiporter; AGC, aspartate/glutamate carrier; AMPK, AMP-dependent kinase; Apc, amino acid, polyamine and organocation; ASC, preference for alanine, serine and cysteine; ASCT, neutral amino acid transporter; ASS argininosuccinate synthetase; B^0AT, broad neutral (0) amino acid transporter; CTNL2, type 2 citrullinaemia; EA, episodic ataxia1; EAAT, excitatory amino acid transporter; EEG, electroencephalogram; 4F2hc, 4F2 cell-surface-antigen heavy chain; GABA, γ-aminobutyric acid; GC1, mitochondrial glutamate carrier 1; HAT, heteromeric amino acid transporter; HHH, hyperammonaemia–hyperornithinaemia–homocitrullinuria; IL1, intracellular loop 1; LeuT, leucine transporter; $LeuT_{Aa}$, LeuT from *Aquifex aeolicus*; LPI, lysinuric protein intolerance; MCT, monocarboxylate transporter; MeAIB, *N*-methylaminoisobutyric acid; mTOR, mammalian target of rapamycin; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; OCD, obsessive–compulsive disorder; OMIM, Online Mendelian Inheritance in Man; ORC, ornithine/citrulline exchanger; PAT, proton–amino acid transporter; rBAT, related to $b^{0,+}$ amino acid transport; SLC solute carrier; SNP, single nucleotide polymorphism; TM, transmembrane domain; VGLUT, vesicular glutamate transporter.

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Table 1 Amino acid transporters, their properties and involvement in diseases

Substrates are given in one-letter code. Cit, citrulline; Cn, cystine; O, ornithine. The 'Function' column includes references to amino acid transport systems. These systems have acronyms indicating the substrate specificity of the transporter. Upper-case letters indicate Na^+-dependent transporters (with the exception of system L, system T and the proton amino acid transporters); lower case is used for Na^+-independent transporters (for example asc, y^+ and x^-_c). X^- or x^- indicates transporters for anionic amino acids (as in X^-_{AG} and x^-_c). The subscript AG indicates that the transporter accepts aspartate and glutamate, and the subscript c indicates that the transporter also accepts cystine. Y^+ or y^+ refer to transporters for cationic amino acids (an Na^+-dependent cationic amino acid transporter has not been unambiguously defined and as a result Y^+ is not used), B or b refers to amino acid transporters of broad specificity with superscript ^{0} indicating a transporter accepting neutral amino acids and superscript $^+$ indicating a transporter for cationic amino acids. T stands for a transporter for aromatic amino acids, and system N indicates selectivity for amino acids with nitrogen atoms in the side chain. In the remaining cases, the preferred substrate is indicated by the one-letter code for amino acids. For example, system L refers to a leucine-preferring transporter and system ASC to a transporter preferring alanine, serine and cysteine. Proline and hydroxyproline are referred to as imino acids. Owing to historic idiosyncrasies, the nomenclature for plasma-membrane amino acid transport systems is not completely consistent, but is widely used in the field. AAT, amino acid transporter.

SLC	Acronym	Substrate(s)	Function	Disease/phenotype
SLC1A1	EAAT3	D,E,Cn	System X^-_{AG}	Dicarboxylic aminoaciduria, OCD
SLC1A2	EAAT2	D,E	System X^-_{AG}	
SLC1A3	EAAT1	D,E	System X^-_{AG}	Episodic ataxia?
SLC1A4	ASCT1	A,S,C	System ASC	
SLC1A5	ASCT2	A,S,C,T,Q	System ASC	Tumour growth
SLC1A6	EAAT4	D,E	System X^-_{AG}	
SLC1A7	EAAT5	D,E	System X^-_{AG}	
SLC3A1	rBAT	Trafficking subunits	Heavy chains of heteromeric AAT	Cystinuria
SLC3A2	4F2hc	Trafficking subunits	Heavy chains of heteromeric AAT	Tumour growth
SLC6A5	GlyT2	G	System Gly	Hyperekplexia
SLC6A7	PROT	P	Proline transporter	
SLC6A9	GlyT1	G	System Gly	
SLC6A14	$ATB^{0,+}$	All neutral and cationic amino acids	System $B^{0,+}$	Obesity?
SLC6A15	B^0AT2	P,L,V,I,M	System B^0	
SLC6A17	$NTT4/B^0AT3$	L,M,P,C,A,Q,S,H,G	System B^0	
SLC6A18	$XT2/B^0AT3$	G, A	System Gly	Hyperglycinuria? Hypertension?
SLC6A19	B^0AT1	All neutral amino acids	System B^0	Hartnup disorder, hypertension?
SLC6A20	IMINO	P	System IMINO	Iminoglycinuria
SLC7A1	CAT-1	K,R,O	System y^+	
SLC7A2	CAT-2	K,R,O	System y^+	
SLC7A3	CAT-3	K,R,O	System y^+	
SLC7A5	LAT1/4F2hc	H,M,L,I,V,F,Y,W	System L	Tumour growth
SLC7A6	$y^+LAT2/4F2hc$	K,R,Q,H,M,L	System y^+L	
SLC7A7	$y^+LAT1/4F2hc$	K,R,Q,H,M,L,A,C	System y^+L	Lysinuric protein intolerance
SLC7A8	LAT2/4F2hc	All neutral amino acids, except P	System L	
SLC7A9	$b^{0,+}AT/rBAT$	R,K,O,Cn	System $b^{0,+}$	Cystinuria
SLC7A10	Asc-1/4F2hc	G,A,S,C,T	System asc	
SLC7A11	xCT/4F2hc	D,E,Cn	Sytem x^-_c	
SLC7A12	Asc-2	G,A,S,C,T	System asc	
SLC7A13	AGT1	D,E	Asp, Glu transporter	
SLC16A10	TAT1	W,Y,F	System T	Blue diaper syndrome?
SLC17A6	VGLUT2	E	Vesicular Glu transporter	
SLC17A7	VGLUT1	E	Vesicular Glu transporter	
SLC17A8	VGLUT3	E	Vesicular Glu transporter	Non-syndromic deafness
SLC25A2	ORC2	K,R,H,O,Cit	Orn/Cit carrier	
SLC25A12	AGC1	D,E	Asp/Glu carrier	Global cerebral hypomyelination
SLC25A13	AGC2	D,E	Asp/Glu carrier	Type II citrullinaemia, neonatal intrahepatic cholestasis
SLC25A15	ORC1	K,R,H,O,Cit	Orn/Cit carrier	HHH syndrome
SLC25A18	GC2	E	Glu carrier	
SLC25A22	GC1	E	Glu carrier	Neonatal myoclonic epilepsy
SLC32A1	VIAAT	G,GABA	Vesicular Gly/GABA transporter	
SLC36A1	PAT1	G,P,A	Proton AAT	Hair colour (horses)
SLC36A2	PAT2	G,P,A	Proton AAT	Iminoglycinuria
SLC36A4	PAT4	P,W	Amino acid sensor	
SLC38A1	SNAT1	G,A,N,C,Q, H,M	System A	
SLC38A2	SNAT2	G,P,A,S,C,Q,N,H,M	System A	
SLC38A3	SNAT3	Q,N,H	System N	
SLC38A4	SNAT4	G,A,S,C,Q,N,M	System A	
SLC38A5	SNAT5	Q,N,H,A	System N	
SLC43A1	LAT3	L,I,M,F,V	System L	
SLC43A2	LAT4	L,I,M,F,V	System L	
Not assigned	Cystinosin	Cn	Lysosomal Cys transporter	Cystinosis

Although the subunits are functionally independent, trimerization is important for assembly of the transporter and trafficking to the plasma membrane. The transporter occludes the substrates between two hairpin loops (Figure 1B), which belong to a domain that is thought to translocate like an 'elevator' perpendicular to the plasma membrane together with the substrates [8].

Biochemistry of the SLC3 and SLC7 families

The HATs (heteromeric amino acid transporters) are composed of a heavy subunit (SLC3 family) and a light subunit (SLC7 family), which are linked by a conserved disulfide bridge [9,10]. The heavy subunit is essential for trafficking of the holotransporter

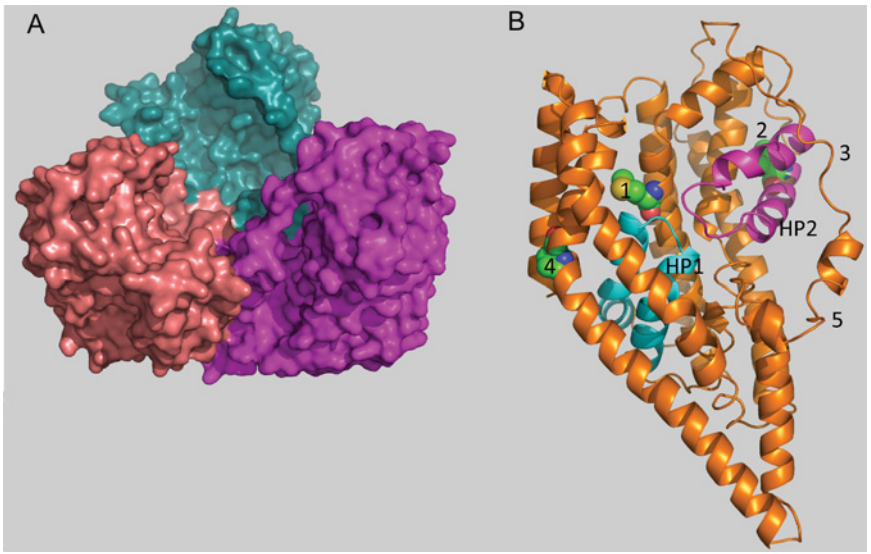

Figure 1 Overview of the trimeric and monomeric structure of the glutamate transporter Glt_{Ph} from *P. horikoshii*

The structure (PDB code 1XFH) was visualized using PyMol (DeLano Scientific; http://www.pymol.org). (**A**) Holotransporter viewed tilted from the side. The monomers are indicated by different colours. The bowl formed by the three subunits is viewed from the extracellular side. (**B**) View of a glutamate transporter monomer. The two hairpin loops (HP1 and HP2) are indicated in blue and magenta. Residues in Glt_{Ph} that are equivalent to human mutations are depicted: 1, Met^{395} (equivalent to Arg^{445} in SLC1A1); 2, Ile^{339} (equivalent to Ile^{395} in SLC1A1); 3, analogous position to Thr^{164} in SLC1A1; 4, Pro^{206} (equivalent to Pro^{290} in SLC1A3); and 5, approximate location of C186S in SLC1A3. An interactive three-dimensional structure for Figure 1 is available at http://www.BiochemJ.org/bj/4360193/bj4360193add.htm.

to the membrane [11,12], whereas the light subunit catalyses the transporter function [13]. Two heavy subunits, rBAT (related to $b^{0,+}$ amino acid transport, also called SLC3A1) and 4F2hc [4F2 cell-surface-antigen heavy chain; also named CD98 (SLC3A2)] form the human SLC3 family. The SLC7 family comprises eight members in humans, which act as the light subunits of HAT. Six SLC7 members (named LAT1, LAT2, y^+LAT1, y^+LAT2, asc1 and xCT) heterodimerize with 4F2hc, but only one (named $b^{0,+}$AT) with rBAT. The heavy subunit associating with AGT1 (aspartate/glutamate transporter 1, also called AGC1) and asc2 are presently unknown. SLC3 members are type II membrane N-glycoproteins with a single TM (transmembrane domain) segment, an intracellular N-terminus and a large extracellular C-terminus. The extracellular domain of these proteins has sequence [14] and structural [15] homology with bacterial α-amylases. Despite this homology, 4F2hc lacks key catalytic residues and has no glucosidase activity [15]. Similarly, it is not clear whether rBAT has any glucosidase activity. The SLC7 family proteins have a 12-TM topology [16] and their structure is expected to be homologous with that of the sequence-related (<20% amino acid identity) AdiC (arginine/agmatine antiporter) from *Escherichia coli* [17–20] (PDB codes 3NCY and 3L1L) and to the amino acid transporter ApcT (where Apc is amino acid, polyamine and organocation) [21] (PDB code 3GIA). These structures are characterized by the so-called '5+5 inverted repeat' fold. This structural element is characterized by five transmembrane helices in the N-terminal half of the transporter, which are repeated as a pseudo-two-fold symmetry in the C-terminal half of the transporter (Figure 2). This protein fold was first described in $LeuT_{Aa}$ [LeuT (leucine transporter) from *Aquifex aeolicus*] [22] (PDB code 2A65) and is shared by at least four other apparently non-sequence-related families of transporters, including SLC7.

Functionally, HATs are amino acid antiporters (exchangers) with a 1:1 stoichiometry [23,24]. The rBAT/$b^{0,+}$AT heterodimer mediates the exchange of cationic amino acids, cystine and other

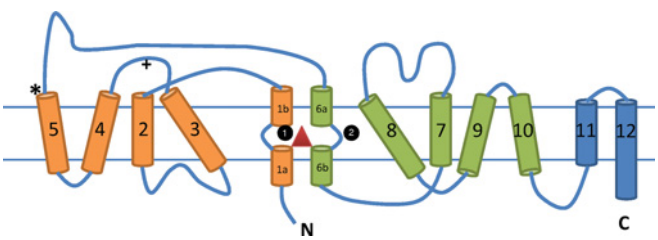

Figure 2 Topology plot of the structure adopted by SLC6, SLC7, SLC36 and SLC38 family members

Twelve TMs are found in the SLC6 and SLC7 families, whereas SLC36 and SLC38 members share the first 11 TMs. A hallmark of this protein fold is the 5+5 inverted repeat, which is indicated by orange and green colours. The two repeats are related by a pseudo-two-fold symmetry. In the SLC6 family, two Na^+-binding sites are found, which are indicated by black circles, and the substrate is indicated by a red triangle. The substrate-binding site is enclosed by helices 1 and 6, which are unwound in the centre. The Na^+-binding site 1 is co-ordinated by residues from helices 1 and 6 and the substrate, whereas Na^+-binding site 2 is co-ordinated by residues from helices 1 and 8. Transporters of the SLC7, SLC36 and SLC38 families do not bind Na^+ ions. * indicates the position of Arg^{240} in SLC6A19, which is thought to interact with trafficking subunits; + indicates the position of the disulfide bridge between SLC7 transporters and the SLC3 trafficking subunits. Intracellular loop 1 between helices 2 and 3 is highly conserved in the SLC7 family and is mutated in both cystinuria and LPI. Reprinted by permission from Macmillan Publishers Ltd: Nature [22], © 2005.

neutral amino acids, except imino acids [24,25]. Thus the exchange of dibasic and neutral amino acids is electrogenic [23]. The transporter has a high affinity for external cationic amino acids and cystine (K_m ~100 μM) and a slightly lower affinity for neutral amino acids. The apparent affinity is lower at the intracellular binding site [13]. Two disulfide-linked rBAT/$b^{0,+}$AT heterodimers form a non-covalent heterotetramer in native tissues [26]. This complex is the molecular correlate of the renal and intestinal cationic amino acid transport system $b^{0,+}$ [indicating a transporter of broad specificity for neutral (0) and cationic (+) amino acids] previously

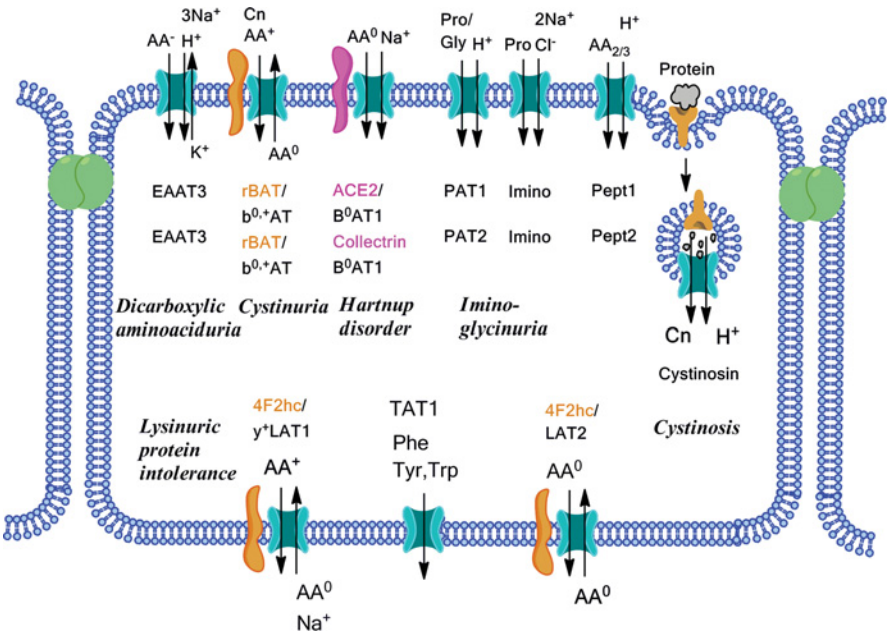

Figure 3 Transporters involved in renal and epithelial amino acid transport

Amino acid and peptide transporters in epithelial cells are depicted. Transporter names are given in two rows: the upper row indicates apical transporters in the intestine, whereas the lower row indicates the corresponding transporters in the kidney. Low-molecular-mass protein absorption is a renal function. As a result, cystinosin dysfunction predominantly affects renal tubules. Diseases associated with the different transporters are shown in italics. Trafficking subunits associated with epithelial transporters are shown in magenta (SLC6-associated) and orange (SLC7-associated). AA^+, cationic amino acids; AA^0, neutral amino acids; AA^-, anionic amino acids; $AA_{2/3}$, di- or tri-peptides; Cn, cystine; Pept1, peptide transporter 1; Pept2, peptide transporter 2. SLC6A18 (B^0AT3) has been omitted because its role in humans is unclear. The basolateral transporters TAT1 and 4F2hc/LAT2 are indicated for completeness.

detected in brush-border membranes from the small intestine and kidney [27,28] (Figure 3). Physiologically, dibasic amino acids and cystine are removed from the intestinal and renal tubular lumen in exchange for intracellular neutral amino acids. The high intracellular concentration of neutral amino acids, the electric potential across the plasma membrane and the intracellular reduction of cystine to cysteine are the driving forces that determine the direction of the exchange of substrates via system $b^{0,+}$. The 4F2hc-linked light chains carry out a variety of transport functions: 4F2hc/LAT1 and 4F2hc/LAT2 form two variants of transport system L (a transporter for large neutral amino acids, exemplified by leucine). Both isoforms carry out exchange of neutral amino acids, the former preferring large neutral amino acids, whereas the latter transports a wide variety of neutral amino acids. The HAT members 4F2hc/y^+LAT1 and 4F2hc/y^+LAT2 (SLC3A2/SLC7A6 and SLC3A2/SLC7A7) correspond to amino acid transport system y^+L, mediating electroneutral exchange of cationic (y^+) and large neutral amino acids (L) plus Na^+ with a stoichiometry of 1:1:1. Physiologically, the transporter mediates the efflux of a dibasic amino acid in exchange for an extracellular neutral amino acid plus Na^+, with an apparent K_m of ~20 μM [12,29–31]. It is speculated that the Na^+ ion replaces the positive charge of the side chain of dibasic amino acids when neutral amino acids are transported. In support of this notion, the affinity of neutral, but not cationic, amino acids increases by approximately two orders of magnitude in the presence of Na^+. Similar to system $b^{0,+}$, the apparent affinity for substrates is also lower at the intracellular binding site for 4F2hc/y^+LAT1 [31].

Biochemistry of the SLC6 family

The SLC6 family comprises 20 members in humans that can be grouped into four subfamilies, namely the monoamine transporter branch, the GABA (γ-aminobutyric acid) transporter branch, and the amino acid transporter branches I and II [32]. The structure of SLC6 family transporters is homologous with $LeuT_{Aa}$ [22] (PDB code 2A65), which is characterized by the 5+5 inverted repeat structure (Figure 2). Helix 1 and helix 6 are unwound in the centre to provide contact points for Na^+ and substrate binding. The bacterial transporter has two Na^+-binding sites, one of which (Na^+-binding site 1) involves the carboxy group of the substrate amino acid. The transporter crystallizes as a dimer, which is in agreement with functional studies from the mammalian SLC6 family. Careful comparison of the structure of LeuT with mammalian homology models revealed a cavity in the latter close to Na^+-binding site 1, which is occupied by a glutamate residue in LeuT and was shown to provide a Cl^--binding site in the mammalian transporters [33–35]. *SLC6A5* (GlyT2) and *SLC6A9* (GlyT1) encode glycine transporters, which are involved in the removal of this inhibitory neurotransmitter in the brain. GlyT1 transports glycine together with $2Na^+$ and $1Cl^-$, whereas GlyT2 transports glycine together with $3Na^+$ and $1Cl^-$ and is therefore less likely to run in reverse [36]. *SLC6A14* ($ATB^{0,+}$) encodes a transporter for neutral and cationic amino acids (system $B^{0,+}$) [37]. With the exception of glutamate, aspartate and proline, $ATB^{0,+}$ accepts all amino acids and in addition transports carnitine [38]; K_m values range from 6 to 600 μM, with a preference for large neutral amino acids. Neutral amino acids are co-transported together with $2Na^+$ and $1Cl^-$. SLC6A19 [B^0AT1, broad neutral (0) amino acid transporter 1] transports all 16 neutral amino acids in co-transport with $1Na^+$ [39]. Functional analysis suggests that Na^+-binding site 1 is used in B^0AT1 and that the transporter is Cl^--independent. B^0AT1 shows similar V_{max} values for its substrates, but the K_m values differ. Large aliphatic amino acids have the highest affinity ($K_m = 1$ mM), glycine has a low affinity ($K_m = 12$ mM) and other amino acids have intermediate values. Histidine and proline are

transported very slowly [40]. SLC6A18 (B^0AT3) is closely related to B^0AT1. It prefers alanine and glycine as substrates, but does transport neutral amino acids to some extent [41]. In contrast with B^0AT1, it is Cl^- dependent. Transport of amino acids generates inward currents, suggesting that it mediates substrate uptake in co-transport with $2Na^+$ and $1Cl^-$. SLC6A20 [IMINO or SIT (system imino transporter)] is specific for proline and hydroxyproline and also mediates substrate uptake in co-transport with $2Na^+$ and $1Cl^-$ [35].

Both B^0AT1 and B^0AT3 transporters require either collectrin [also called TMEM27 (transmembrane protein 27)] or ACE2 (angiotensin-converting enzyme 2) as a trafficking protein for surface expression in the kidney (B^0AT1 and B^0AT3) and intestine (B^0AT1) respectively [42,43] (Figure 3). Collectrin and ACE2 are type-I membrane proteins, with an extracellular N-terminus and a single transmembrane helix. ACE2 is a carboxypeptidase, which plays an important role in the inactivation of angiotensin II [44]. However, it is also a general carboxypeptidase, which aids in the digestion of nutrient-derived peptides in the intestine [43]. ACE2 preferentially releases large neutral amino acids, which are also the preferred substrates of the transporter B^0AT1. Collectrin lacks the catalytic domain of ACE2, but shares sequence homology in the transmembrane and cytosolic regions [44]. Collectrin is thought to interact with the SNARE (soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor) complex by binding to snapin [45]. As a result, collectrin could fuse B^0AT1-containing vesicles with the membrane, allowing surface expression. In the intestine, this function would be carried out by ACE2, with the added benefit of bringing a peptidase to the surface that provides substrates for B^0AT1.

Biochemistry of the SLC17 family

The SLC17 family comprises a total of eight members encoding vesicular and epithelial anion transporters [46]. In two seminal studies, SLC17A6 was identified as VGLUT (vesicular glutamate transporter) 1, which is essential for glutamatergic neurotransmission [47,48]. Subsequently, two more vesicular glutamate transporters were identified in this family [49]. VGLUTs are stereospecific and, unlike the plasma-membrane transporters (SLC1 family), are Na^+-independent and do not accept aspartate [50]. The K_m for glutamate is in the low millimolar range. The rate of uptake and accumulation is mainly driven by the inside-positive membrane potential and not by ΔpH. As a result, it is thought that L-glutamate is taken up as an anion and the final concentration inside vesicles can reach up to 100 mM. In the case of VGLUT1 it has been suggested that the transporter has an endogenous Cl^- conductance, which allows replacement of Cl^- ions by glutamate ions inside the vesicles [51]. SLC17A8 (VGLUT3) is an unusual member of this family because it is expressed in neurons that are not considered to be glutamatergic, such as serotonergic and cholinergic neurons and also in astrocytes. In addition, its subcellular localization is not restricted to synaptic boutons, but also includes the cell soma and dendrites [49].

Biochemistry of the SLC25 family

The SLC25 family comprises a total of ~30 members, including three ADP/ATP carrier isoforms five uncoupling protein isoforms and six amino acid transporters [52]. Two of them are ORC (ornithine/citrulline exchanger) isoforms of the inner mitochondrial membrane, ORC1 (SLC25A15) and ORC2 (SLC25A2) [53–55]. Both exchange the L-stereoisomers of ornithine, lysine, arginine and citrulline with a 1:1 stoichiometry and, with less activity, exchange dibasic amino acids for H^+. ORC2 has a broader substrate specificity than ORC1, accepting L- and D-histidine, L-homoarginine and D-stereoisomers of ornithine, lysine and arginine as efficient substrates. Ornithine/citrulline exchange links the enzyme activities of the urea cycle in the liver cytosol to those in the mitochondria (Figure 4). It is believed that ORC1 is the main mediator of this exchange because it has higher expression levels in liver and also in other tissues (e.g. lung, pancreas and testis) [56].

Two SLC25 family members are transporters of aspartate and glutamate, namely SLC25A12 [AGC (aspartate/glutamate carrier) 1 or aralar1] and SLC25A13 (AGC2 or citrin) [52]. AGC1 is highly expressed in the inner mitochondrial membrane in brain, heart and skeletal muscle, whereas AGC2 is found in several tissues, but most abundantly in liver, where AGC1 is absent [57,58]. Both transporters catalyse the electrogenic exchange of L-aspartate (anion)/ L-glutamate (acid) with a stoichiometry of 1:1, with similar K_m values for aspartate (~50 μM) and glutamate (~200 μM) from the cytosolic side [59]. Thus, in energized mitochondria (i.e. positive membrane potential outside), the exit of aspartate and entry of glutamate via AGC transporters are essentially irreversible. Both proteins contain two domains, the C-terminal domain with all the prototypical features of the SLC25 transporter family, and a long N-terminal domain with four EF-hand Ca^{2+}-binding motifs, which protrude into the periplasmic space. The C-terminal domain is the catalytic part, whereas the N-terminal domain binds cytosolic Ca^{2+} and activates these transporters [59]. SLC25 transporters have six transmembrane helices divided into three similar domains each with two transmembrane helices [60] (PDB code 1OKC). In the crystal structure, the three 2-TMs are related by a pseudo-3-fold axis of symmetry and have a disposition similar to a photo-camera diaphragm, delineating a barrel with a large cavity towards the mitochondrial intermembrane space.

Biochemistry of the SLC36 family

The SLC36 family comprises a total of four members, three of which have been functionally characterized. Both SLC36A1 (PAT1) and SLC36A2 (PAT2) are proton–amino acid transporters, which transport small neutral amino acids, particularly glycine and proline, together with $1H^+$ [61]. PAT1 appears to be a lysosomal transporter in many cell types, but is also found in the apical membrane of intestinal epithelial cells [62,63] (Figure 3). Both transporters accept D- and L-amino acids with similar affinity; however, PAT1 (K_m glycine = 7 mM; K_m proline = 2.8 mM) has much lower affinities for its substrates than does PAT2 (K_m glycine = 0.59 mM; K_m proline = 0.12 mM) [61]. In addition to the imino acids and glycine, PAT1 accepts alanine, β-alanine, betaine, sarcosine (*N*-methylglycine), MeAIB (*N*-methylaminoisobutyric acid) and GABA. PAT2 has similar substrate specificity, but a much lower affinity for GABA and β-alanine. PAT1 is significantly more active at acidic pH, due to the H^+-co-transport mechanism. PAT2 is less pH-dependent because the proton-binding site is already saturated at neutral pH [64]. Human PAT4 is a high-affinity (K_m in the low micromolar range) equilibrative transporter for proline and tryptophan, which is not coupled to proton co-transport [65]. The structure of these transporters is expected to adopt the same 5+5 inverted repeat fold as the SLC6 and SLC7 families [66].

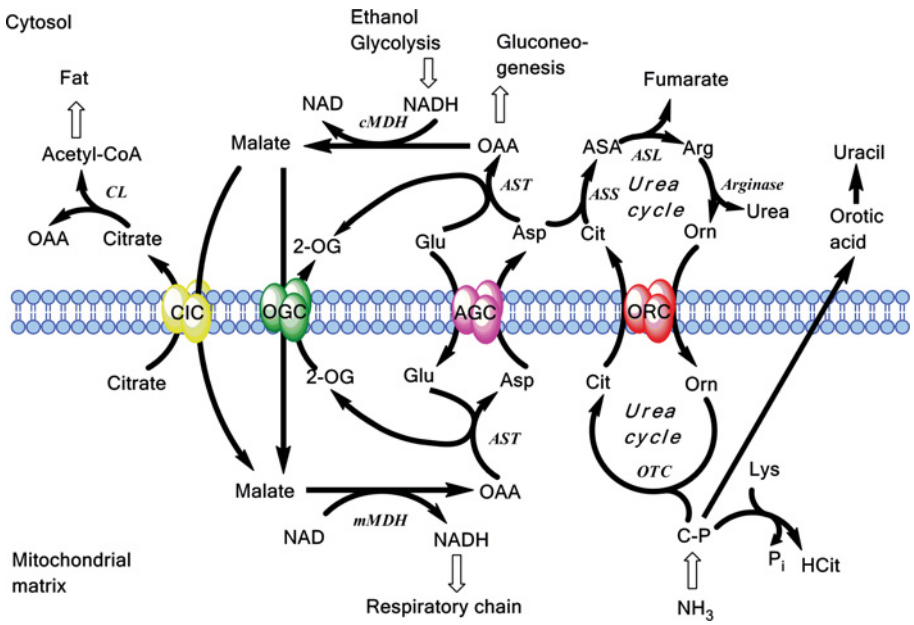

Figure 4 Relationship between metabolic pathways and mitochondrial transporters in liver

The emphasis is on pathways affected in HHH syndrome and AGC2 deficiency. AGC2 (SLC25A13) is the only AGC isoform expressed in liver, and ORC1 (SLC25A15) is the prevalent ORC isoform expressed in liver. Other mitochondrial transporters are: CIC, citrate carrier (SLC25A1); OGC, oxoglutarate carrier (SLC25A11). For explanations see the text. Metabolites: ASA, arginosuccinic acid; Asp, L-aspartate; Cit, citrulline; C-P, carbamoyl phosphate; Glu, L-glutamate; HCit, homocitrulline; Lys, L-lysine; OAA, oxaloacetic acid; Orn, L-ornithine; 2-OG, 2-oxoglutarate. Enzymes: ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; AST, aspartate aminotransferase, CL, ATP citrate lyase; cMDH, cytosolic malate dehydrogenase; mMDH, mitochondrial malate dehydrogenase; OTC, ornithine transcarbamoylase.

Biochemistry of cystinosin

Cystinosin is a protein with seven putative transmembrane helices that is weakly related to G-protein-coupled receptors. Two isoforms are known, one of 400 amino acids and the other of 367 amino acids. Functional studies suggest that it is a transporter specific for L-cystine and L-cysteine, with a K_m of ~0.3 mM for the former [2]. The transporter has a C-terminal sorting motif (GYDQL), which directs the protein to the lysosomes. Removal of the motif has been used to study the transporter in the plasma membrane of transfected cells. Uptake of radiolabelled cystine into these cells was increased at acidic pH, suggesting that the transporter releases cystine from the lysosome in co-transport with protons.

AMINO ACID TRANSPORTERS INVOLVED IN RENAL AND INTESTINAL DISORDERS

SLC1A1 and dicarboxylic aminoaciduria

Dicarboxylic aminoaciduria [OMIM (Online Mendelian Inheritance in Man) 222730] is a rare autosomal-recessive disorder affecting glutamate and aspartate transport in the kidney, intestine and brain [67]. A massive increase in glutamate and, to a smaller extent, aspartate in the urine is the hallmark of the disorder. Kidney stones have been reported in one case [68]. It is caused by mutations of the neuronal glutamate transporter EAAT3 (SLC1A1) [68], which takes up glutamate and aspartate in the intestine and reabsorbs them from the kidney glomerular filtrate. Two mutations have been identified, namely an R445W replacement and a deletion of Ile^{395}. Arg^{445} is located in TM8 close to the substrate-binding site (Figure 1B). Both mutations inactivate the transporter. In the brain, EAAT3 is widely expressed in the cortex, particularly in the hippocampus, basal ganglia and the olfactory bulb, where it is thought to be involved in GABA and glutathione biosynthesis [68]. Surprisingly, there is little evidence to suggest that dicarboxylic aminoaciduria has a significant neural component in humans [68], other than an association with OCD (obsessive–compulsive disorder; see the section 'Amino acid transporters involved in neurological disorders'). Although some cases with mental retardation have been reported [67,69], these were diagnosed with dicarboxylic aminoaciduria retrospectively. Similar to other aminoacidurias, urine-screening programmes have identified a number of individuals with highly elevated glutamate and aspartate in the urine, who never had clinical symptoms [70]. Careful examination of aged *Slc1a1* nullizygous mice showed signs of neurodegeneration [71]. There has been no examination of older individuals with dicarboxylic aminoaciduria to allow verification of these results in humans. Interestingly, no defect in intestinal glutamate transport was reported in a study by Melancon et al. [72], which would be inconsistent with a mutation in *SLC1A1* in this particular case.

SLC3A1, SLC7A9 and cystinuria

Cystinuria is the most common primary inherited aminoaciduria (OMIM 220100) [73]. Hyperexcretion of cystine and dibasic amino acids into the urine is the hallmark of the disease. It is caused by the defective transport of cystine and dibasic amino acids across the apical membrane of epithelial cells of the renal proximal tubule and the small intestine [74–76], mediated by the rBAT/$b^{0,+}$AT heterodimer (*SLC3A1* and *SLC7A9* genes) (Figure 3). Mutations in both *SLC3A1* and *SLC7A9* cause cystinuria [77,78]. The poor solubility of cystine results in the formation of renal calculi, which can cause obstruction, infection

and ultimately chronic kidney disease [79]. Cystinuria causes 1–2 % of all cases of renal stone formation and even 6–8 % in paediatric patients [80]. Cystinuria is usually considered to be an autosomal-recessive disorder, requiring two mutated alleles for the disease to occur, but two 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 [79]. Some individuals carrying a single non-type-I allele also produce cystine calculi [81]. Non-type-I cystinuria should, therefore, be considered an autosomal-dominant disease, where a single mutated allele suffices to cause disease. However, it would be considered to be of incomplete penetrance with regard to the cystine lithiasis trait, because only some individuals will produce kidney stones. Association of type I cystinuria with hypotonia-related syndromes (OMIM 606407) is due to the combined deletion of *SLC3A1* and contiguous genes [82–85].

Worldwide, 133 mutations have been reported in *SLC3A1* (in a total of 579 alleles) and 95 mutations have been reported in *SLC7A9* (in a total of 436 mutated alleles) [79]. Only ~13 % of the studied cystinuria alleles have not been identified, leaving little room for other cystinuria-causing genes [79]. The unexplained cystinuria alleles might correspond to mutations in unexplored regions of *SLC3A1* and *SLC7A9* genes. Alternatively, it has been proposed that partially inactivating (hypomorphic) *SLC7A9* polymorphisms contribute to the cystinuria phenotype [86,87]. Type I cystinuria is usually caused by mutations in *SLC3A1* encoding rBAT, with <15 % of the mutant alleles involving *SLC7A9* [81]. Some of these type-I-associated *SLC7A9* alleles carry hypomorphic mutations [81,88]. Similarly, mice harbouring the missense mutation D140G in *Slc7a9* recapitulate type I cystinuria [89]. In contrast, non-type I cystinuria is usually caused by mutations in *SLC7A9* encoding $b^{0,+}$AT, with <4 % having mutations in *SLC3A1* [79]. Accordingly, *Slc7a9*-deficient mice recapitulate non-type-I cystinuria [90]. This lack of a complete genotype–phenotype correlation instigated a classification of cystinuria based on genetics [91]: type A (mutations in *SLC3A1* alleles) and B (mutations in *SLC7A9* alleles). Double heterozygotes (AB) have been identified, but this is a very rare genotype among patients with cystinuria and these individuals do not produce cystine calculi [79,92]. Thus digenic inheritance of cystinuria has been ruled out as a significant mechanism for the disease.

Functionally studied rBAT (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 [93]. Two different mechanisms underlie the trafficking defects. The mutation L89P, located in the TM segment, fails to assemble efficiently with $b^{0,+}$AT, but the small amounts that reach the plasma membrane have mature glycosylation and form heterotetramers. In contrast, mutations in the extracellular domain of rBAT efficiently assemble with $b^{0,+}$AT to form disulfide-linked heterodimers but remain core-glycosylated, fail to oligomerize and are degraded, most probably via the proteasome [93]. All cystinuria-specific mutations in $b^{0,+}$AT studied so far cause loss of function. Similar to other transporters, the molecular causes include a lack of protein expression (mutation G105R) [94], defective trafficking to the plasma membrane (mutation A182T) [94] and defective transport function (mutations A354T and P482L) [13,95]. The crystal structure of the prokaryotic AdiC and ApcT transporters provides further insight into the molecular defects of $b^{0,+}$AT mutations. Gly^{105}, for instance, is located just after the conserved intracellular loop 1 (IL1) between the TM2 and TM3 segments (see Figure 2), suggesting that the G105R mutation causes protein misfolding and degradation. The position of residue Ala^{182} in the TM5 segment suggests an interaction with the TM segment of rBAT, but experimental evidence is missing. There are no clues why mutations A354T (TM9) and P482L (C-terminus) are transport defective. Since L-arginine binds to residues in TMs 1, 3, 6, 8 and 10 of the structural homologue AdiC [20] (PDB codes 3NCY and 3L1L), it is not expected that these mutations affect binding directly, but rather affect the conformational changes necessary for the translocation of the substrate. Interestingly, mutation T123M causes a very rare type of cystinuria characterized by urinary hyperexcretion of cystine alone (i.e. isolated cystinuria) [88,94]. Thr^{123} is located in TM3 within the putative substrate cavity of $b^{0,+}$AT, suggesting a role of this residue in cystine recognition.

SLC6A19 and Hartnup disorder

Hartnup disorder (OMIM 234500) is an autosomal-recessive inherited disorder of neutral amino acid transport [96], which is caused by mutations in the neutral amino acid transporter SLC6A19 (B^0AT1) [97,98]. It affects neutral amino acid transport across the apical membrane in the intestine and kidney (Figure 3). The disorder was initially described in 1956 by Baron et al. [99], who defined the major clinical symptoms: pellagra-like skin rash, a lack of co-ordinated muscle movement (ataxia), psychotic behaviour and neutral aminoaciduria. Subsequent to the initial report, urine-screening programmes have shown that the clinical symptoms are not regularly observed [100]. The aminoaciduria is the defining hallmark of the disorder. A skin rash in younger individuals is frequently observed, but the ataxia and psychotic behaviour are rare features of the disease. Cases with all clinical symptoms have been reported in Japan and China [101,102]. The clinical symptoms are thought to arise from a lack of tryptophan supplementation [103]. Tryptophan is the immediate precursor of serotonin and levels of the latter correlate with tryptophan levels in blood plasma. This could explain the psychotic behaviour observed in Hartnup patients, but the relationship with the ataxia is less clear. The skin rash has been described as pellagra-like, and in fact responds to niacin (nicotinamide and nicotinic acid) supplementation. Both tryptophan and niacin are precursors for NADPH synthesis [104]. Both metabolites are also important for skin metabolism and a lack of these compounds could cause the observed symptoms.

To date, 21 different mutations have been identified in *SLC6A19* causing Hartnup disorder [103]. All of them have been shown to abolish transport function. Mutated Gly^{284}, for instance, is located in helix 6 of the transporter (Figure 2). Owing to the flexibility afforded by its lacking side chain, Gly^{284} allows unwinding of the helix in this area. Mutation of Arg^{57}, located in helix 1, disrupts a proposed extracellular gate of the transporter. This gate is essential for the transport mechanism, closing the pore by interacting with Asp^{486}. Mutation of Arg^{57} to a neutral residue abolishes this interaction. Mutation R240Q only abolishes function when the protein is co-expressed with collectrin or ACE2, but does not affect transport activity when B^0AT1 is expressed alone [43]. As a result, it has been suggested that this residue is likely to interact with collectrin and ACE2 (Figure 2). No mutations have so far been identified in collectrin or ACE2. Collectrin-deficient mice appear to be generally healthy and normal, but display neutral aminoaciduria [42]. ACE2 nullizygous mice have a more complex phenotype, including cardiac deficiencies and glomerulosclerosis, but exhibit normal urine amino acid levels [105].

SLC7A7 and LPI (lysinuric protein intolerance)

LPI (OMIM 222700) is a very rare (~200 patients reported) primary inherited aminoaciduria with a recessive mode of inheritance [106,107]. Patients with LPI are usually asymptomatic while being breast-fed. LPI symptoms appear after weaning and may include vomiting, diarrhoea, failure to thrive, hepatosplenomegaly, bone-marrow abnormalities, osteoporosis, episodes of coma, mental retardation, lung involvement, altered immune response and chronic renal disease. Mutations in the *SLC7A7* gene encoding the HAT light-chain y^+LAT1 cause LPI [108,109]. The transporter is highly expressed in kidney, small intestine, placenta, spleen and macrophages [110,111]. In epithelial cells, the transporter has a basolateral location [112] (Figure 3). The second system-y^+L isoform (4F2hc/y^+LAT2; also called SLC3A2/SLC7A6) is widely expressed, but at much lower levels in the kidney and small intestine compared with 4F2hc/y^+LAT1. As a result, 4F2hc/y^+LAT2 cannot replace 4F2hc/y^+LAT1 in the kidney and intestine, but it explains why y^+L activity is not deficient in fibroblasts and erythrocytes from LPI patients [113,114]. Under physiological conditions, the high extracellular Na^+ concentration drives the electroneutral efflux of cationic amino acids in exchange for neutral amino acids. This mode of exchange explains why mutations in y^+LAT1 cause urine hyperexcretion and intestinal malabsorption of dibasic amino acids only.

Transport of dibasic amino acids across the basolateral membrane of epithelial cells in kidney and small intestine is defective in LPI [115,116] (Figure 3). Impairment of intestinal absorption and renal re-absorption of dibasic amino acids cause 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 (and orotic aciduria) and protein aversion (see also the subsection 'Amino acid transporters involved in liver metabolism'). In contrast with disorders of apical amino acid transporters (Hartnup disorder and cystinuria), the basolateral location of the LPI transporter 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). Thus patients fail to thrive normally. Treatment based on a low-protein diet and citrulline supplementation ameliorates urea-cycle dysfunction, but it is not sufficient to prevent other severe complications of the disease. The pathogenic mechanisms of these complications (e.g. alveolar proteinosis, a diffuse lung disorder characterized by lipoprotein deposits derived from surfactant, and chronic renal disease) are still unknown [117]. Similarly, mice nullizygous for *Slc7a7* that survive the massive neonatal lethality display identical metabolic derangement to LPI patients [118].

In *SLC7A7*, 49 LPI-specific mutations in a total of 141 patients from 107 independent families have been described (for a mutation update, see [81,119]). These known mutations explain >95% of the studied alleles. No LPI mutations have been identified in the heavy subunit *SLC3A2* (4F2hc). Indeed, 4F2hc serves to traffic six amino acid transporter subunits [10], is necessary for proper β1 integrin function [120,121] (see the section 'Amino acid transporters involved in complex and acquired diseases') and its knockout in mice is lethal [122]. All patients with identified *SLC7A7* mutations present with aminoaciduria, whereas other symptoms vary widely, even when harbouring the same mutation (e.g. the Finnish founder splice-site mutation 1181–2A>T) [123]. This precludes the establishment of genotype–phenotype correlations.

Of the approximately 20 LPI point mutations, only ten have been studied and shown to cause loss of function [108,123–125]. Four mutations (E36del, G54V, F152L and L334R) reach the plasma membrane and show defective system-y^+L transport activity in heterologous expression systems [123–125]. The crystal structures of the prokaryotic transporter AdiC [18–20] (PDB codes 3NCY and 3L1L) offer clues to the molecular defects underlining some of these mutants. Gly^{54} corresponds to the third residue in the highly conserved unwound segment GS/AG in TM1 of the 5+5 inverted repeat fold (Figure 2). The α-carboxy group of the substrate interacts with this unwound segment in amino acid transporters with this protein fold, such as $LeuT_{Aa}$ (PDB code 2A65) and AdiC [20,22]. Thus defective substrate binding appears probable in the G54V mutant. Residue Leu^{334}, in TM8, is in close proximity to the conserved α-helical IL1 (intracellular loop 1) located between TM2 and TM3. IL1 is one of the key structural elements that blocks diffusion of the substrate to the cytosol in the outward-facing conformation of transporters with this fold [126]. Thus L334R might block the transporter in the inward-facing conformation, subsequently preventing the translocation of the substrate. In contrast, there are no clear clues for the molecular defects of F152L and E36del. Residue Phe^{152} is located just after Cys^{151} (in loop TM3–TM4), which constitutes the disulfide bridge with 4F2hc. F152L is a hypomorphic mutant [125]. Accordingly, a partial reduction in the amount of the heterodimer 4F2hc/y^+LAT1-F152L reaching the plasma membrane might explain the partial loss of function associated with this mutant. Interestingly, mutation E36del causes a dominant-negative effect when co-expressed in *Xenopus* oocytes with wild-type y^+LAT1 or even y^+LAT2 [125], suggesting that 4F2hc/y^+LAT1 has a multiheteromeric structure including y^+LAT2. However, biochemical evidence for an oligomerization of the heterodimers is lacking [26]. The dominant-negative effect of E36del is more probably caused by titration of the co-expressed 4F2hc in oocytes. In this scenario, E36del might have higher affinity for 4F2hc than does wild-type y^+LAT1, compromising the full expression of wild-type heterodimers at the plasma membrane. Glu^{36} is located two residues before the N-terminus of TM1 and might interact with the intracellular N-terminus of 4F2hc.

SLC36A2 and iminoglycinuria

Iminoglycinuria (OMIM 242600) is an autosomal disorder expressed in homozygotes or combinations of mutated alleles associated with excessive amounts of proline, hydroxyproline and glycine in the urine [127]. It is caused by several autosomal alleles, some of which are partially expressed in heterozygotes. Although a variety of clinical symptoms, such as hypertension, kidney stones, mental retardation, deafness and blindness, have been described in cases of iminoglycinuria, these are likely to represent ascertainment bias. Large urine-screening studies have shown that iminoglycinuria is a benign condition, and the aminoaciduria was only detected retrospectively [128]. Iminoglycinuria is, however, relevant from a genetic point of view, because it provides mechanistic explanations for genetic variability, such as reduced penetrance and modifiers.

In general, iminoglycinuria is the recessive phenotype, whereas glycinuria appears to be present in many, but not all, heterozygotes and thus can present as a dominant trait [129]. Further complexity arises from investigation of intestinal transport. In some families intestinal proline (but not glycine) transport is affected, whereas in other families intestinal transport is unaffected [127].

Proline and glycine transport in mammalian cells is mediated by a variety of transporters [130,131]. Owing to significant species differences, it is often difficult to compare studies and this has

caused confusion in the field. In general, joint transporters for both amino acids have been identified as well as specific carriers. Two proton–amino acid co-transporters serve as joint carriers, namely PAT1 (SLC36A1) and PAT2 (SLC36A2) [61] (Figure 3). In the kidney, the apical proline and glycine transporter is PAT2, which is not expressed in the intestine. It is mainly expressed in the early segment of the proximal tubule (segment S1) [41]. PAT1 appears to be located in a vesicular compartment below the brush border [41], but is in the apical membrane in the intestine [62]. In addition, both glycine and proline have specific transporters. SLC6A20 (IMINO) is a specific proline transporter that correlates with the functionally defined 'Imino system' [132]. The transporter is stereospecific, preferring L- over D-amino acids, and only accepts amino acids with a secondary amino group such as proline, hydroxyproline, MeAIB, sarcosine and betaine. In contrast with PAT2, it is expressed in both the kidney and intestine. In the kidney, it is found in the S3 segment of the proximal tubule [41]. A specific glycine transporter has been described in a variety of functional studies from different species, but its molecular identity is difficult to ascertain. SLC6A18 has recently been identified as a neutral amino acid transporter and named B^0AT3 [41,133]. The transporter prefers glycine and alanine, but also transports a variety of neutral amino acids with the exception of proline. More specific glycine transporters are found in the brain (GlyT1 and GlyT2) (see the section 'Amino acid transporters involved in neurological disorders'), but do not appear to be expressed in the kidney in significant amounts. Similar to SLC6A20 (IMINO), SLC6A18 (B^0AT3) is mainly expressed in the S3 segment of the proximal tubule [41]. The transporter is not found in the intestine.

In individuals with iminoglycinuria, mutations are found in *SLC36A2*, *SLC6A18* and *SLC6A20* [134]. The major gene involved in homozygous cases of iminoglycinuria appears to be *SLC36A2*. However, it appears that two types of mutations occur. First, a splice mutation (IVS1+1G>A) that fully inactivates the transporter by introducing a premature stop codon; and secondly, a nonsense mutation (G87V) that partially compromises transport. Genetic and functional analysis suggests that a homozygous G87V mutation only causes iminoglycinuria in combination with an additional mutated allele of the specific imino transporter *SLC6A20*. Consistent with this notion the G87V mutation caused an increase in the K_m, which affects glycine transport more than proline transport [134]. A similar K_m-variant in a family with iminoglycinuria was described previously by Greene et al. [135]. Whether this family carried the G87V mutation is unknown. In the imino transporter, a T199M mutation causes an almost complete inactivation of the transporter. Because of the difference in tissue expression, cases of iminoglycinuria resulting from inactivation of SLC36A2 would have a renal phenotype only, whereas cases of iminoglycinuria that have a combination of SLC36A2 (G87V) and SLC6A20 (T199M) alleles would also have an intestinal phenotype.

The genetics of iminoglycinuria become even more complicated when considering mutations in *SLC6A18*. Several mutations occur at high frequency in *SLC6A18* and are listed in the SNP (single nucleotide polymorphism) database (http://www.ncbi.nlm.nih.gov/snp). The stop codon Y318X occurs with a frequency of 0.44 and the nonsense mutation L478P is found with a frequency of 0.39 [134]. Thus a considerable proportion of the human population carries a non-functional B^0AT3 transporter. Interestingly, although the mouse transporter is active when expressed together with collectrin or ACE2, a functional human transporter has not yet been reported. In the rat, proline uptake into kidney brush-border-membrane vesicles shows properties of system Imino (SLC6A20), whereas in the rabbit it has the properties of the imino acid carrier (i.e. SLC36A1 or SLC36A2). It is tempting to speculate that in mouse and rat IMINO and B^0AT3 carry out the bulk of proline and glycine transport, whereas in rabbit and humans it is PAT2 and IMINO.

Cystinosin and cystinosis

Lysosomes degrade all cellular components into building blocks. Breakdown of proteins in lysosomes must be accompanied by the release of the resulting amino acids into the cytosol [136]. To date, two amino acid transporters have been identified in lysosomal membranes, namely PAT1 and cystinosin. Cystine, the disulfide of cysteine, is generated in lysosomes because of the oxidative environment in this organelle. Cystine has a low solubility and therefore causes precipitates if not transported into the cytoplasm, where it is reduced by the glutathione system. CTNS (encoding cystinosin) was identified by a positional cloning strategy as the gene mutated in cystinosis [137]. Cystinosis (OMIM 219750 and 219800) is the most common inherited cause of the renal Fanconi syndrome, a general dysfunction of renal proximal tubular transport. The most severe form, infantile cystinosis, manifests generally between 6 and 12 months of age as fluid and electrolyte loss, aminoaciduria, glycosuria, phosphaturia, renal tubular acidosis, rickets and growth retardation [138]. These symptoms are largely explained by reduced proximal tubular transport, resulting in the loss of metabolites, ions and water; the loss of phosphate results in rickets. There are two less severe forms, which are caused by different alleles affecting the same protein. It appears that there is a correlation between the extent of protein inactivation and the severity of the disease. Administration of cysteine-dimethylester to mice replicates tubular dysfunction, but cystinosin-deficient mice develop Fanconi syndrome only in certain genetic backgrounds. How lysosomal cystine accumulation causes general epithelial dysfunction is still unknown [139].

AMINO ACID TRANSPORTERS INVOLVED IN NEUROLOGICAL DISORDERS

SLC1A1 and OCD

OCD is a disabling and chronic anxiety disorder. It is clinically defined by the presence of obsessions (intrusive, recurrent thoughts or impulses) and compulsions (ritualistic and repetitive patterns of behaviour) [140]. Several independent studies have consistently shown an association of the gene locus of *SLC1A1* with early-onset OCD [141–143]. The involvement of the neurotransmitter glutamate appears surprising, given that OCD is usually treated with serotonin re-uptake inhibitors [140]. However, significant numbers of patients do not respond to this treatment. Immunohistochemical and functional data from brain slices suggest that the bulk of neurotransmitter glutamate is taken up by the glial transporters EAAT1 and EAAT2 [144]. The density of EAAT3 in the brain appears to be significantly lower than that of EAAT1 or EAAT2. In neurons, most of the immunoreactivity is found on intracellular compartments, where its function is unknown. When in the plasma membrane, the transporter is found on the soma and the perisynaptic membrane of dendrites. Because of its low abundance and subcellular localization, the role of the neuronal glutamate transporter EAAT3 in glutamate re-uptake at the synapse is unclear. The transporter is, however, also found in GABAergic neurons. As a result, it is thought that EAAT3 may play a role in providing glutamate as a precursor for GABA and glutathione biosynthesis [71]. During brain development,

EAAT3 appears to be expressed earlier than the glial glutamate transporters, pointing to an important role in brain development. In OCD, the level of glutamate in the cerebrospinal fluid is elevated, suggesting glutamatergic hyperactivity. Accordingly, antiglutamatergic agents have been shown to reduce the severity of OCD symptoms. In addition, the metabolic rate is increased in the brain of OCD patients. Greenberg et al. [145] reported decreased intracortical inhibition in OCD patients, which could result from reduced GABA levels caused by lack of EAAT3. Wang et al. [143] reported screening of OCD-related alleles in 378 OCD-affected individuals. One non-synonymous coding SNP (c.490A>G, T164A) and three synonymous SNPs were identified. There was no difference in the genotype frequency between OCD cases and controls for the common synonymous SNPs. The rare variant T164A was found in one family only and was not functionally analysed. However, the residue is located in a loop region between transmembrane helices 3 and 4 of EAAT3 and is not conserved between species or isoforms (Figure 1B). In OCD patients, a volume reduction in the anterior cingulated cortex and the orbitofrontal cortex was noted, whereas the thalamic volume, in contrast, was increased. It is important to note that parts of the thalamus have intensive connections to these cortical areas, which appear to be overactive in OCD patients. Functionally, these areas are important for emotional processing, conflict monitoring and error detection [140]. Reduced inhibition by a lack of GABA could cause an imbalance in these areas, resulting in the mis-judgment of emotional processing. There is evidence that individuals with mutations in EAAT3 resulting in dicarboxylic aminoaciduria (see the section 'Amino acid transporters involved in renal and intestinal disorders') can also have OCD [68].

SLC1A3 and EA (episodic ataxia)

EA is a heterogenous syndrome characterized by episodes of inco-ordination and imbalance [146,147]. Related neural dysfunctions are hemiplegic migraine and seizures. Mutations in three genes have been identified in EA. Mutations in the K^+ channel Kv1.1 cause EA type 1, and mutations in the Ca^{2+} channel $Ca_v2.1$ cause EA type 2 [146,147]. Mutations in the Ca^{2+} channels are also associated with familial hemiplegic migraine. Another gene involved in hemiplegic migraine is the α_2-subunit of the Na^+/K^+-ATPase (*ATP1A2*). Two different mutations in EAAT1 (*SLC1A3*), namely a P290R and a C186S replacement, were identified in patients with EA who did not have mutations in the above-mentioned genes [146,147]. Both mutations were heterozygous, i.e. the mutation was found on one chromosome only, leaving one intact gene copy. Pro^{290} sits in a critical region of helix 5 of the EAAT1, where it induces a kink in the helix (Figure 1B). Replacement by an arginine residue is likely to interfere with folding of the protein. Accordingly, the transport activity of the mutant, when expressed in COS7 cells was close to untransfected controls. To explain how this heterozygous mutation could cause transporter malfunction, the authors tested whether the mutation would have a dominant effect in a glutamate transporter trimer [147]. Cells co-transfected with mutated and wild-type transporter showed a reduced uptake, supporting this notion. Surface localization studies further showed that the mutated transporter failed to reach the plasma membrane. When co-expressed, plasma membrane levels of the wild-type transporter were also reduced. The second mutation is more mysterious: C186S has little effect on transport activity in heterologous systems. The residue is located in a loop between helix 4a and helix 4b (Figure 1B). The corresponding part in the prokaryotic transporter is missing and therefore cannot be modelled. The mutation occurred in heterozygosity, and in a cohort of 20 patients that did not have mutations in the Ca^{2+} channel $Ca_v2.1$ only one individual was found to have a mutation in EAAT1 [146]. Moreover, in the same family an asymptomatic carrier of the mutation exists. Further evidence is required to support an involvement of EAAT1 in certain forms of EA.

SLC6A5 and hyperekplexia

Hyperekplexia (OMIM 149400) is a rare neurological disorder characterized by neonatal muscle stiffness and an exaggerated startle reflex induced by noise or touch [148]. The disorder can have serious consequences, including brain damage and/or sudden infant death due to episodes of apnoea. The disorder is caused by defects of the glycinergic system [148]. Several different genes have been found to be affected in hyperekplexia, namely the α_1-subunit and the β-subunit of the glycine receptor, the receptor-clustering protein gephyrin and the glycine transporter GlyT2 (SLC6A5). Glycine together with GABA is the most important inhibitory neurotransmitter in the brain [149]. GlyT1 (SLC6A9) is expressed in astrocytes throughout the brain. Its substrate and ion gradients are close to equilibrium under physiological conditions. It is hence thought to act as a buffer for extracellular glycine, possibly modulating excitatory NMDA (*N*-methyl-D-aspartate) receptors through their glycine-binding sites [36]. GlyT2 is expressed mainly in the spinal cord in glycinergic neurons, where it resides in the pre-synaptic membrane recapturing released neurotransmitter. Blocking of this transporter would extend the time of glycine in the synaptic cleft, thereby activating glycine receptors more strongly. This in turn would enhance the inhibition of synapses, explaining the stiffness observed after startle. Homology modelling of the transporter along the structure of $LeuT_{Aa}$ has provided further insight into the mechanism of some mutations. Mutation N509S, for instance, changes a critical ligand for Na^+-binding site 1 in the glycine transporter [148] (Figure 2). Replacement of asparagine by serine increases the co-ordination bond length, thereby reducing Na^+ affinity. Because Na^+ binding and substrate binding are interlinked in this family, the mutation also affects the substrate affinity [150]. Another intriguing case is the combination of two mutations (L306V and N509S) in one individual (compound heterozygote). Mutation L306V by itself does not change the transport activity of GlyT2. When expressed together with GlyT2(N509S), however, no activity was observed, suggesting that both mutants form heterodimers that are inactive [150]. Dimer formation of glycine transporters has been demonstrated previously [151]. Similarly, mutation S510R caused hyperekplexia even when only one copy of the gene is affected. Expression of this mutant alone resulted in intracellular localization of the protein. When expressed together with the wild-type transporter, all transporters were trapped in the cytosol confirming the dominance of the mutation.

SLC17A8 and non-syndromic deafness

Mice lacking *Slc17a8* (VGLUT3) are profoundly deaf due to the absence of glutamate release from inner hair cells in the auditory pathway [152,153]. VGLUT3 fills ribbon-synapse vesicles with glutamate in this cell type. The mice also show degeneration of some cochlear ganglion neurons, suggesting a role of *Slc17a8* in development. They further exhibit cortical seizures as evidenced by EEG (electroencephalogram) recordings; surprisingly, these have limited behavioural consequences [152]. Autosomal-dominant sensorineural hearing loss in humans (OMIM 605583) was mapped to chromosome region 12q21-q24, which contains

SLC17A8. A heterozygous A211V mutation was identified in two families with the disorder [153]. No functional results of this mutation have been reported, but the residue is conserved between species. The deafness in humans has a variable onset and penetrance is not complete (i.e. 17 of 22 individuals over 20 years of age with the mutation have deafness). The mutation is rare and was not found in 267 control individuals. Heterozygous mice have normal hearing, which is at variance with dominant inheritance in humans. As a result, the human mutation might have a dominant-negative effect on the protein produced by the wild-type allele. Transgenic mice with a replication of the human mutation have not been reported.

SLC25A12 and global cerebral hypomyelination

Aspartate/glutamate transporter 1 (AGC1) deficiency has been reported in a child who showed global cerebral hypomyelination, severe hypotonia, arrested psychomotor development and seizures beginning at a few months of age (OMIM 612949) [154]. Isolated mitochondria from a skeletal-muscle biopsy of the patient showed a drastic reduction in ATP production only when glutamate plus succinate or glutamate plus malate were used as substrates. This suggested the impaired function of AGC1 in muscle, because of the role of this transporter in the malate–aspartate shuttle (Figure 4). SLC25A12 mutational analysis revealed a homozygous Q590R missense mutation. AGC1 (Q590R) is expressed normally and localized to mitochondria in fibroblasts of the patient, but it is unable to transport aspartate or glutamate when reconstituted in liposomes.

The AGC1 deficiency case offers clues to the role of AGC1 in the central nervous system. AGC1 expression in the central nervous system is restricted to neurons and, as a component of the malate–aspartate shuttle, is thought to be required for mitochondrial oxidation of cytosolic NADH in the brain [155,156]. The fact that the patient had no substantial accumulation of lactate indicates that the malate–aspartate shuttle may not be bioenergetically essential in the central nervous system, because the glycerol 3-phosphate cycle might compensate for the defective malate–aspartate cycle. Neuronal aspartate is, however, a substrate for the formation of *N*-acetylaspartate by aspartate-N-acetyltransferase in mitochondria and microsomes [157,158]. *N*-acetylaspartate produced in neurons undergoes transaxonal transfer to oligodendrocytes, where it supplies acetyl groups for the synthesis of myelin lipids [159,160]. This readily explains the global cerebral hypomyelination of the patient. Indeed, defective myelination and reduced *N*-acetylaspartate levels in brain in *slc25a12*-deficient mice delineates the mechanisms underlining the cerebral hypomyelination in AGC1 deficiency [161,162]: defective aspartate transport from mitochondria to the cytosol via AGC1 in neurons prevents myelin formation by failing to provide *N*-acetylaspartate to oligodendrocytes.

The structural model of AGC1 based on the crystal structure of the ADP/ATP carrier (SLC25A4) [60] (PDB code 1OKC) reveals that residue Gln^{590} is exposed to the internal cavity of the transporter [154] just above the proposed general substrate-binding site for SLC25 transporters [163]. Docking of substrate (aspartate or glutamate) in the wild-type AGC1 model suggests an interaction on top of the salt-bridge network (Asp^{348}–Lys^{451}, Glu^{448}–Lys^{543} and Asp^{540}–Lys^{35}) that closes the cavity at the mitochondrial-matrix side of the molecule [154]. In contrast, the Q590R mutant showed a misplacement of the substrate, which interacts directly with Arg^{590} and is located at a distance from the salt-bridge network. Formation and disruption of this network is expected to be relevant for the conformational changes that the transporter undergoes during the transport cycle [164]. Thus this analysis suggests that an arginine residue in position 590 results in the trapping of the substrate at the binding site, impeding its translocation.

SLC25A22 and neonatal myoclonic epilepsy

Neonatal myoclonic epilepsy (OMIM 609304) is a neuromuscular dysfunction with a characteristic EEG pattern [165] caused by mutations in GC1 (mitochondrial glutamate carrier 1). Two homozygous mutations have been found in GC1, namely P206L (four affected children in one family) and G236W (one affected child) [165,166]. The symptoms of the affected children in both families were similar: spontaneus muscle contractions, hypotonia, unusually small head size, abnormal optic nerve conduction and rapid evolution into general brain dysfunction and spasticity. P206L and G236W mutations abolish glutamate transport in reconstituted liposomes [165,166]. In parallel, mitochondrial glutamate oxidation, but not succinate oxidation, in fibroblasts from patients was strongly defective [166]. These results provided evidence that, despite normal oxidative phosphorylation, impaired mitochondrial glutamate import/metabolism leads to an alteration of neuronal excitability [56]. The mechanisms explaining myoclonic epilepsy in GC1 deficiency are not yet clear. GC1 has a higher expression in astrocytes than in neurons, specifically within brain areas that contribute to the genesis and control of myoclonic seizures [166], and astrocytes are devoid of aspartate/glutamate carrier activity (AGC isoforms) [155]. In this scenario, a plausible working hypothesis is that defective mitochondrial transport of glutamate in astrocytes leads to accumulation of glutamate in the cytosol and then to glutamate release. This release could result in neuronal synchronicity contributing to the generation of epileptic-like discharges [165]. There are no clues why P206L and G236W mutations abolish GC1 transport function [56].

AMINO ACID TRANSPORTERS INVOLVED IN LIVER METABOLISM

SLC7A7 and LPI

See the section ‘Amino acid transporters involved in renal and intestinal disorders’.

SLC25A15 and HHH (hyperammonaemia–hyperornithinaemia–homocitrullinuria) syndrome

HHH syndrome (OMIM 238970) is caused by mutations in ornithine–citrulline carrier 2 [56,167]. The disease can present at any age, but it usually manifests in early childhood. The most common symptoms are episodes of confusion, lethargy and coma due to hyperammonaemia, and neurological features such as mental retardation, learning difficulties, dysfunction of lower limbs due to neuronal lesions, lack of co-ordinated muscle movement and seizures. Patients often present with abnormal liver function, leading to hepatitis-like attacks, and coagulation problems. The disease has an autosomal-recessive mode of inheritance and a pan-ethnic distribution with a higher proportion of cases reported in Canada, Italy and Japan [56]. More than 30 *SLC25A15* mutations have been described in HHH patients [56,168]. Deletion of Phe^{188} is the founder mutation in Canadian patients [53], whereas a premature stop codon at position 180 is the most common mutation among Japanese patients [169]. Since its first description [170], approximately 75 cases have been reported and >97 % of the affected alleles have been explained by mutations in *SLC25A15* [56,171]. The small

number of reported cases is most probably due to misdiagnosis. Indeed, genotyping analysis for the founder French-Canadian *ORC1* mutation revealed a relatively high incidence (one in 1550 live births) in isolated communities in northern Canada [171]. No clear genotype–phenotype correlation of the syndrome regarding the onset and severity, the long-term evolution and the neurological and hepatic presentations at onset has been established [168,172,173]. This suggests a significant impact of genetic background and/or environment on the severity of the syndrome. Neither mitochondrial DNA haplotypes affecting oxidative metabolism nor sequence variants in ORC2 have been demonstrated to modify the severity of the disease [168]. In any case, it is believed that the expression of the ornithine/citrulline isoform ORC2 in liver can compensate to some extent for the defect in ORC1, resulting in a phenotype milder than those associated with defective enzymes of the urea cycle. Recently, another transporter of this family (SLC25A29) has been reported to rescue the impaired ornithine transport in mitochondria of cultured fibroblasts from patients with HHH syndrome [53]. SLC25A29 is expressed at higher levels in the central nervous system than in the liver and kidney. The role of SLC25A29 in the urea cycle remains to be determined.

The symptoms hyperammonaemia, hyperornithinaemia and homocitrullinuria can be explained by the physiological role of ORC1 (Figure 4). Defective ORC1 reduces the mitochondrial import of ornithine from the cytosol, compromising the condensation of carbamoyl phosphate and ornithine to form citrulline by ornithine transcarbamoylase, which results in impairment of the urea cycle and hyperammonaemia. Reduction in the hepatic metabolism of ornithine causes hyperornithinaemia. Carbamoyl phosphate accumulates in liver mitochondria and either condenses with lysine to form homocitrulline or enters the pyrimidine biosynthetic pathway in the cytosol to form orotic acid and uracil. Thus HHH syndrome is characterized by homocitrullinuria and increased urinary excretion of orotic acid and uracil. Hyperammonaemia is treated by protein restriction in the diet, supplementation with arginine or citrulline and urine elimination of nitrogen by treatment with sodium benzoate or sodium phenylbutyrate. This treatment improves hyperammonaemia, and in some cases the neurological and liver function at onset [174], but not the long-term progression of the disease [168]. This scenario is similar to that of LPI, in which reduced availability of the urea-cycle intermediates (e.g. arginine and ornithine) compromises the cycle, resulting in hyperammonaemia, homocitrullinuria and orotic acid excretion [117]. The pathogenic mechanisms underlying the neurological symptoms, hepatitis-like attacks and coagulation defects in HHH syndrome are not well understood [175].

Functional studies have shown that HHH-syndrome-specific ORC1 mutations result in loss of function. The common French-Canadian Phe^{188} deletion results in an unstable protein with little (<10 %) residual activity [53,55]. Eleven missense mutations have been studied for their capacity to complement ornithine transport in mitochondria from fibroblasts of HHH patients [53,173] or upon production in bacteria and reconstitution in proteoliposomes [55,168]. Reconstituted mutants G27R, M37R, E180K and R275Q showed no transport activity, whereas mutants L71Q, P126R, T272I and L283F retained some activity (4–10 %). Three mutants, T32R (<20 %), M273K (<20 %) and G190D (<40 %), retained significant activity, but proper trafficking to the mitochondrial inner membrane has only been tested for T32R. The impact of these mutations has been analysed in the light of homology models of the human ORC1 [168] based on the crystal structure of the ADP/ATP carrier (SLC25A4) [60]. Mutations R275Q and E180K involve residues in the proposed ornithine-binding site, suggesting defective substrate interaction with the transporter [56]. One of the most interesting mutations is M37R (in loop TM1–TM2 located towards the mitochondrial matrix), which may interfere with network salt bridges Asp^{31}–Lys^{131} and Lys^{34}–Asp^{231} [168]. Formation and disruption of this network is thought to be relevant for the conformational changes that the transporter undergoes during the transport cycle [164]. This scenario suggests a translocation defect for the M337R mutant.

SLC25A13 (AGC2) deficiency

Aspartate/glutamate transporter 2 (AGC2) deficiency causes two age-dependent phenotypes: NICCD [neonatal intrahepatic cholestasis (bile-flow obstruction) caused by citrin (AGC2) deficiency; OMIM 605814] and adult-onset CTLN2 (type II citrullinaemia; OMIM 603471). Patients with CTLN2 have citrullinaemia, hypoproteinaemia, recurrent episodes of hyperammonaemia, neuropsychiatric symptoms, fatty liver and, in some cases, hyperlipidaemia [176]. The hyperammonaemia causes encephalopathy. Patients also show distaste for carbohydrates and preference for fat and protein, and an incapacity to consume alcohol [177]. The neonatal phenotype (NICCD) has only been recognized within the last decade [178,179]. Affected children commonly show a variety of symptoms, such as transient bile-flow obstruction, fatty and enlarged liver, growth retardation, citrullinaemia, hyperaminoacidaemias (methionine, threonine, tyrosine, phenylalanine, lysine and arginine), an increased threonine/serine ratio, galactosaemia, ketotic hypoglycaemia and hypoproteinaemia. Some patients present with hepatitis, jaundice, decreased coagulation factors, increased bleeding, haemolytic anaemia and hypoproteinaemia, all of which are signs of liver dysfunction. The neonatal phenotype of AGC2 deficiency is usually benign and symptoms disappear by the age of 12 months. Affected individuals continue to display aversion to carbohydrates and a preference for protein and fat in the diet. A propensity to bleed and the absence of bile flow have also been reported in infants [180]. At 20–50 years of age, some AGC2-deficient individuals develop CTLN2. In contrast with urea-cycle defects, a diet high in protein and lipid content and low in carbohydrates is highly recommended in AGC2 deficiency to prevent hyperammonaemia and resolve growth retardation in children [181,182]. In any case, CTLN2 is progressive and patients usually die from hyperammonaemic encephalopathy and complications of brain oedema, which can be prevented by partial liver transplantation [183,184].

The physiological role of AGC2 and the pathophysiological mechanisms of AGC2 deficiency explain each other (for reviews see [52,56,185]) (Figure 4). The major function of AGC transporters is to supply aspartate to the cytosol, which is particularly relevant in hepatocytes that have a negligible capacity to take up aspartate from the blood. Because AGC2 is the only isoform of this carrier expressed in liver, the lack of its function explains many symptoms of the disease. The cytosolic urea-cycle enzyme ASS (argininosuccinate synthetase) condenses aspartate and citrulline to produce argininosuccinate. Reduced availability of cytosolic aspartate results in citrullinaemia and hyperammonaemia. In addition, CTLN2 patients present variable deficiency in ASS (protein and activity, but not mRNA levels) [186], suggesting a role of aspartate in the stability of the enzyme. Cytosolic aspartate is also relevant for the oxidation of cytosolic NADH, since AGC2 is a key component of the malate–aspartate shuttle that transfers the reducing equivalents of NADH from the cytosol into mitochondria in liver. The inhibition of cytosolic NADH oxidation results in an increased $NADH/NAD^+$ ratio, which reduces the glycolytic flux and the metabolism of alcohol,

and explains the distaste for carbohydrates and the incapacity to consume alcohol. Alternative removal of the increased cytosolic NADH by the citrate–malate shuttle would increase the transfer of acetyl-coenzyme A from the mitochondrial matrix to the cytosol and hence fatty acid synthesis. This is the likely cause of the fatty liver phenotype. Finally, cytosolic aspartate is a precursor for gluconeogenesis and protein synthesis. Thus low availability of aspartate in the cytosol would also explain hypoglycaemia and hypoproteinaemia. These mechanisms of pathophysiology have been demonstrated in AGC2-deficient mice. Liver from *Slc25a13*-deficient mice exhibited a marked decrease in mitochondrial aspartate transport, ureagenesis, malate–aspartate shuttle activity and gluconeogenesis from lactate, and showed an increased cytosolic NADH/NAD^+ ratio [187]. However, the mice did not manifest hyperammonaemia and changes in amino acid levels, even following administration of protein-rich diets, nor did they show hypoglycaemia upon fasting. Compensation of the defective malate–aspartate shuttle activity by the NAD^+-regenerating glycerol 3-phosphate cycle, which is highly active in mice, is a plausible hypothesis for the lack of some phenotypic characteristics of AGC2 deficiency in *Slc25a13*-deficient mice. Indeed, the double knockout of AGC2 and mitochondrial glycerol-3-phosphate dehydrogenase paralleled most of the human AGC2-deficiency phenotype [188], namely low aspartate content, increased cytosolic NADH/NAD^+ ratio, hyperammonaemia, citrullinaemia, hypoglycaemia upon fasting, fatty liver and reduction in growth rate.

AGC2 deficiency has an autosomal-recessive mode of inheritance, with a carrier prevalence of approximately one in 70 individuals in East Asia, suggesting that over 80 000 East Asians are homozygotes with two mutated *SLC25A13* alleles [189]. In contrast, only ~400 cases, almost all from East Asia (mainly Japan), but also from Israel, the U.S. and the U.K., have been reported [190,191], indicating misdiagnosis. *SLC25A13* is the only known gene to be associated with AGC2 deficiency, but no significant correlation has been observed between *SLC25A13* mutation types and NICCD and CTLN2 phenotypes or the age of CTLN2 onset. More than 30 mutations in *SLC25A13* have been reported [192]. Apparently only the AGC2 missense mutation R588Q has been functionally studied [190]. This mutant showed low residual activity (<10 %) when reconstituted in proteoliposomes. Arg^{588} belongs to the proposed common substrate-binding site together with Arg^{492}, Glu^{404} and Lys^{405}. Arg^{588} is a well-conserved residue in SLC25 family members and, indeed, mutation of the homologous residue in human ORC1 (R275Q) causes HHH syndrome [56]. Docking of aspartate in the proposed AGC2-binding site, based on the crystal structure of the ADP/ATP carrier [60] (PDB code 1OKC), suggested that mutation R388Q altered the substrate location in such a way that it is not interacting with the salt-bridge network (characteristic of SLC25A members), and thus causing loss of substrate translocation [56].

AMINO ACID TRANSPORTERS INVOLVED IN COMPLEX AND ACQUIRED DISEASES

SLC3A2, SLC7A5, SLC1A5 and cancer

It is believed that tumour cells adapt to the local microenvironment by expressing a set of amino acid transporters to support tumour metabolism and trigger cell growth and survival. The expression levels of the heterodimer SLC3A2/SLC7A5 (4F2hc/LAT1) is elevated in a wide spectrum of primary human cancers [193] and metastasis [194]. Frequently, this overexpression is co-ordinated with high expression of SLC1A5 [also called ASCT2 (neutral amino acid transporter 2)] [193]. The mechanisms involved in the role of 4F2hc/LAT1 and ASCT2 in tumour cells is beginning to be delineated.

Human 4F2hc/LAT1 exchanges large neutral amino acids with high affinity ($K_m \leqslant 50\ \mu M$) and glutamine and asparagine with lower affinity (K_m ~2 mM) [195]. Human ASCT2 mediates the electroneutral exchange of neutral amino acids and Na^+ ions [196]. Mouse ASCT2 has high affinity for small neutral amino acids, asparagine and glutamine (K_m ~20 μM) and lower affinity for some large neutral amino acids, including some of the 4F2hc/LAT1 substrates (leucine, isoleucine, phenylalanine, methionine, histidine, tryptophan and valine) [197]. In contrast with ASCT2, LAT1 substrate affinities are lower for intracellular substrates than for extracellular substrates, suggesting that the exchange velocity of 4F2hc/LAT1 is modulated by the intracellular amino acid concentration [198]. ASCT2 mediates net uptake of glutamine to drive cell growth and survival [199]. Fuchs and Bode [193] proposed that in tumour cells LAT1 uses intracellular ASCT2 substrates to adjust the essential amino acid concentrations for metabolic demands and signalling to the kinase mTOR (mammalian target of rapamycin). The co-ordinated action of both transporters in mTOR activation has been demonstrated in cultured cells [200]. Moreover, there is a reciprocal regulatory connection between mTOR signalling, ASCT2 expression and 4F2hc/LAT1 expression at the cell surface [193]. mTOR integrates signalling from growth factors [through PI3K (phosphoinositide 3-kinase) and Akt], energy metabolism [through AMPK (AMP-dependent kinase)] and nutrients to control cell growth and cell-cycle progression [201]. LAT1, ASCT2 and the lactate transporters MCT (monocarboxylate transporter) 1 (also called SLC16A1) and MCT4 (SLC16A3) associate in a metabolic activation-related CD147–CD98 complex [202]. The complex also includes EpCAM (epithelial cell adhesion molecule), a regulator of cell proliferation. CD147 (also named basigin or EMMPRIN) acts as an ancillary protein carrying MCT1 and MCT4 to the cell surface [203]. Cell-surface-exposed CD147 also promotes the production of matrix metalloproteinases and hyaluronan, and is emerging as a prognostic marker in a wide range of tumours [204]. Knockdown experiments showed a strong positive association between the CD147–CD98 complex and cell proliferation, and a negative association with the metabolic-sensing AMPK [202], one of the signal pathways integrated by mTOR [205,206]. Thus the CD147–CD98 complex might co-ordinate the transport of different nutrients to fulfill the metabolic needs and survival signals of tumour cells. Another mechanism giving an advantage to tumour cells is the tryptophan (influx)/kynurenine (efflux) exchange via 4F2hc/LAT1, which might represent a tumour immune-escape mechanism [207]. This exchange protects tumour cells with enhanced tryptophan catabolism from accumulation of the catabolite kynurenine, which in turn induces apoptosis in T-cells.

In addition to the role in amino acid transport, 4F2hc mediates β1- and β3-integrin signalling [120,121,208]. Indeed, 4F2hc is necessary for the integrin-dependent rapid proliferation of B-lymphocytes during clonal growth and for growth of vascular smooth muscle cells after arterial injury [121,209]. The mechanism of 4F2hc-mediated β-integrin signalling is not known. It seems to be independent of the transport activity of the 4F2hc-associated transporters [210], but requires interaction with β-integrins [211]. Cross-linking of 4F2hc with antibodies promotes β-integrin clustering and signalling, and anchorage-independent growth [212,213]. This suggests that association (e.g. clustering) of 4F2hc heterodimers at the cell surface is a necessary trigger for β-integrin signalling. The factors modulating this association are not known. The 4F2hc heterodimers interacting

with β-integrins have not been characterized. On the basis of the high expression of LAT1 in proliferative and tumour cells, we speculate that 4F2hc/LAT1 participates in β-integrin signalling in tumour cells.

SLC6A14 and obesity

In Finnish families, an association was found between obesity and the chromosome region Xq22-24 [214]. In a subsequent study, this was refined and an association was detected for two SNPs in intron 12 and the 3′-UTR (untranslated region) of the *SLC6A14* gene [215]. One particular haplotype (a group of alleles that are inherited together because they are close to each other) containing both SNPs showed a significantly different frequency in obese compared with control subjects. In an independent study in a French-Caucasian cohort, the association was confirmed [216]. It should be noted, however, that the occurrence of the obesity-associated alleles is not vastly different. For example, SNP 20649C>T occurs with a frequency of 0.72 in obese individuals, compared with 0.68 in normal individuals. Similarly SNP 22510C>G was found with a frequency of 0.64 in obese individuals compared with 0.56 in control individuals. The association was found to be stronger in women than in men. Although statistically sound, because of the relatively large cohort, the result suggests little influence of *SLC6A14* on the obesity phenotype in general. Obesity is a multifactorial condition and many genes could have small impacts on the development of this phenotype [217,218].

There are several scenarios that could explain obesity arising from malfunction of the $ATB^{0,+}$ transporter, which is expressed in the lung, colon, pituitary gland, salivary gland and mammary gland [37]. Owing to its expression in the pituitary gland, $ATB^{0,+}$ may have a role in appetite regulation. It could feed amino acids to an amino acid sensor, which then regulates appetite together with other nutrients. Alternatively, it could provide tryptophan for serotonin production, a neurotransmitter involved in appetite control. Another scenario relates to the function of $ATB^{0,+}$ in fetal development [219]. Reduced amino acid provision to the fetus could result in epigenetic programming, which in turn could foster inappropriate eating behaviour later in life. To address one aspect of this question, eating habits were scored in the study by Durand et al. [216] and compared with SNP frequencies. Obese women with a BMI (body mass index) of 30–40 showed an association between SNP22510 and eating behaviour. No association was detected in the total adult obese group or in the male adult obese group. It has to be pointed out that the SNPs identified in the coding region of *SLC6A14* did not alter its amino acid sequence. Thus any malfunction of the transporter would be a result of splicing problems, altered post-transcriptional regulation or epigenetic mechanisms. Further testing is required to establish the role of *SLC6A14* in obesity.

SLC6A18 and SLC6A19 and hypertension

Both *SLC6A18* and *SLC6A19* have been implicated in hypertension. In favour of such a role, *Slc6a18*-deficient mice were shown to have hypertension [220]. However, in a subsequent study, differences in blood pressure were only found under stress-inducing conditions [133]. In humans, 46 % of the normal population carries a stop codon at position 318 in the SLC6A18 protein and ~16 % are homozygous for this inactivating mutation. In a cohort of 1004 Japanese individuals, no evidence for elevated blood pressure was found, and this lack of association was also confirmed in a Korean population [221,222]. A study has reported the association of a specific microsatellite marker in the *SLC6A19* gene with essential hypertension [223]. The underlying physiology is unclear as other microsatellites in the *SLC6A19* gene did not show this association. Currently, there is no convincing mechanism linking blood pressure regulation to neutral amino acid transport in the kidney.

SLC36 family, hair colour and cell growth

The champagne coat colour is controlled by a single autosomal-dominant gene locus in horses. A T63R mutation in SLC36A1 was found to be completely associated with this trait in 85 horses and was not found in 97 horses of different colour [224]. This much-valued feature of horse coat colour suggests that the transporter plays a role in skin and hair pigmentation. It is unknown whether the mutation, which occurs in a transmembrane helix, inactivates the transporter. No lysosomal-storage disease has been associated with the transporter. In the fruit fly *Drosophila*, a homologue of SLC36 appears to be important for growth by modulating the mTOR pathway [225]. These results were later confirmed in HEK (human embryonic kidney)-293 cells, where PAT1 is located on endosomal membranes and may regulate tumour growth together with PAT4 [226]. Further research is required to substantiate the role of these two transporters in amino acid signalling and cell growth.

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