Diseases associated with general amino acid transporters of the solute carrier 6 family (SLC6).

Stefan Bröer

ResearchSchool of Biology
AustralianNationalUniversity
Canberra, ACT 0200, Australia
Phone: +61-2-6125-2540
email: Stefan.broer@anu.edu.au

Abbreviations: SLC solute carrier families; SNP, single nucleotide polymorphism; TM, transmembrane domain

Key words: Hartnup disorder, iminoglycinuria, obesity, diabetes, cancer

Abstract

Amino acid transporters of the SLC6 family mediate the Na⁺-dependent uptake of neutral amino acids into neurons and epithelial cells of the intestine, kidney and other organs. They are integral parts of amino acid homeostasis in the whole body and the brain. In the intestine they are involved in protein absorption, while in the kidney they regulate plasma amino acid concentrations through reabsorption. The metabolic role of SLC6 amino acid transporters in the brain is less clear and most likely related to anaplerosis of the TCA cycle. Mutations in these transporters cause rare inherited disorders such as Hartnup disorder and iminoglycinuria. They may also play a role in complex traits such as depression, anxiety, obesity, diabetes and cancer. The review does not cover the transport of neurotransmitter amino acids.

Introduction

The SLC6 family, also known as the neurotransmitter transporter family, is comprised of four branches. These are the GABA transporter branch, the monoamine transporter branch and the amino acid transporter branches (I) and (II) (Fig. 1) [1]. Transporters of the SLC6 family are involved in a variety of simple and complex disorders. This review will focus on diseases associated with general amino acid transporters and will also summarise features of mouse models of these transporters. It excludes diseases associated with the transport of the neurotransmitter glycine. Amino acid transporters of the SLC6 family are mainly expressed in two different types of cells: SLC6A7, SLC6A15, SLC6A16 and SLC6A17 are expressed in neurons, whereas SLC6A14, SLC6A18, SLC6A19 and SLC6A20 are expressed in epithelial cells.

Structure of SLC6 amino acid transporters

The structure of all transporters in the SLC6 family is closely related to the structure of the leucine transporter LeuT from *Aquifex aeolicus* [2]. LeuT is characterised by the presence of 12 transmembrane helices, 10 of which form the core of the transporter. The 10 core helices are arranged in an inverted repeat fold, called the 5+5 inverted repeat (Fig. 2). In this repeat, helices 1-5 and 6-10 are arranged in a pseudo two-fold symmetric pattern. This symmetry is thought to underlie the alternative access of substrates to the transporter [3]. LeuT has been crystallised in a conformation where the substrate is occluded in the center of the membrane, but where nevertheless a translocation pathway from the outside is clearly discernible [2]. Using mutations to destabilise the Na⁺-binding site 2 allowed crystallisation of LeuT in an inside-open conformation [4]. It is now thought that LeuT and structurally related transporters undergo a series of steps during the transport cycle [5] (Fig. 3). Initially, the substrate binding site is fully accessible from the outside

(open-out) (Fig. 3A). Once substrate and cosubstrates bind (Fig. 3B) they are occluded by small conformational changes of nearby residues (Fig. 3C). In the LeuT structure Phe253 interacts with the substrate and at the same time encloses it (occluded-out). The next step is a larger conformational change, following the symmetry of the inverted repeat, which results in a conformation that is fully occluded from both sides (Fig. 3D). This translocation is facilitated by interactions of the substrate and cosubstrates with the transporter. Subsequently the translocation pathway opens to the inside, but the substrate is still locked (occluded-in, Fig. 3E). Finally, small conformational changes of nearby residues including Phe259 allow release of the substrate and cosubstrates to the inside (open-in, Fig. 3F). The empty transporter (Fig. 3G) can then change conformation back to the original state (Fig. 3H).

Small competitive inhibitors bind to the substrate binding site and are translocated, while more bulky competitive inhibitors bind to the substrate binding site, but cannot be enclosed thereby preventing transporter turnover. This is exemplified by the binding of tryptophan to LeuT, which results in an open-out conformation with tryptophan in the center of the membrane [6]. A second binding site in the outer vestibule has been identified in LeuT, but its role in substrate translocation or as an inhibitor binding site remains controversial [7, 8].

LeuT shows two binding sites for Na^+ -ions. The Na1-site is coordinated by the carboxyl-group of the substrate, side-chain oxygens of N27, T254 and N286, and backbone carbonyls of A22 and T254 (Table 1) [2]. The Na2 site is coordinated by side-chain oxygens of T354 and S355 and backbone carbonyls of G20, V23 and A351. The secondary structure of helix 1 and 6 is interrupted in the center [2]. This allows backbone residues to make contact with the Na^+ -ions, instead of engaging in hydrogen-bonding within the α -helix. Comparison with other transporters of similar architecture suggests that the Na1-site is specific to the neurotransmitter transporter family, while the Na2-site also occurs in the glucose transporter vSGLT1 and the hydantoin transporter Mhp1 [9]. The critical residues involved in substrate and ion binding are found in helix 1, 3, 6, 7 and 8 [2].

When analysing the structure of LeuT in the two alternating access conformations it appears that helices 1,2,6 and 7 undergo significant conformational changes, while helices 3,4,5,8,9 and 10 are rather immobile [3]. The two groups of helices have been referred to as the hash (because of the arrangement of helices 3,4,8,9 in a # shape) and the bundle (helices 1,2,6,7) [10]. The movement of the bundle against the hash scaffold allows the movement of the substrate through the transporter. Helix 1, in particular bends around a hinge in the center of the helix, thereby allowing release of the substrate in the inside-open conformation [4]. The precise making and breaking of bonds during this conformational change is unclear, but it is thought to involve interactions between Arg5 (TM1), Arg

30 (TM1), Ser267 (TM6), Tyr268 (TM6), Asp369 (TM8) and Asp404 (TM10). In particular the charge pair between Arg5 (bundle) and Asp369 (hash) on the intracellular side, must be broken to allow access from the inside. This energy could be balanced by the formation of a similar charge pair, involving Arg 30 (bundle) and Asp404 (hash) on the outside (Fig. 3). It is assumed that helix 11 and 12 would be part of the scaffold. The sequence similarity of the mammalian SLC6 transporters to LeuT is high enough to align the residues of helix 1-10 with good precision. This allows the generation of homology models, which are thought to be quite reliable in the transmembrane domains [11, 12] (Table 1). The larger loops and helices 11 and 12, by contrast, cannot be modelled with similar precision.

Substrate transport in mammalian SLC6 transporters is frequently Cl⁻-dependent. The LeuT structure contains a peripheral chloride ion, which is unlikely to be of functional relevance [2]. Comparison of mammalian homology structures with the LeuT structure, however, revealed a buried glutamate residue (E290), which was replaced by a neutral residue in the mammalian transporter [11, 12]. In addition there was a cavity in the homology structures, which was flanked by residues that would allow Cl⁻ binding. Subsequent structure-function studies revealed that this site is the functional chloride binding site in mammalian SLC6 transporters.

The LeuT structure and its proposed different conformations thus provide a framework for the analysis of structure-function studies and for the analysis of mutations occurring in rare diseases. Residues critical for the function of LeuT and their homologues in the SLC6 amino acid transporters are listed in table 1.

In the following, individual transporters will be described in more detail including their role in human disorders. The common name will be used when referring to the transport protein, the SLC nomenclature will be used when referring to the gene. The transporters are subdivided into two groups: (i) epithelial amino acid transporters affecting whole body homeostasis and (ii) brain amino acid transporters affecting brain metabolism.

SLC6A14 (alias ATB^{0,+})

Cloning and distribution: ATB^{0,+} was identified by database screening as a novel member of the SLC6 family [13]. The transporter is expressed in the lung, salivary gland, mammary gland, stomach, pituitary gland, eye and colon. In the colon, lung and retina pigment epithelium the transporter is localised to the luminal membrane of epithelial cells [14, 15].

Biochemistry of ATB^{0,+}: ATB^{0,+} transports all neutral and cationic amino acids, but has no affinity for anionic amino acids. Proline has a much lower affinity for the transporter than other amino acids.

The order of preference is Ile>Leu=Met=Phe=Trp>Val=Ser>Tyr=Ala=His=Gly=Cys=Lys=Arg>Asn=Thr>Gln>>Pro [13]. Similar to other members of the SLC6 family, it co-transport substrates together with 2Na^+ and 1Cl^- , although this has only been tested with neutral amino acids and may be different for cationic substrates. The physiological role of the transporter has not been fully resolved. It could be important in regulating bacterial growth in the colon and preventing bacterial growth in the lung [16]. $\text{ATB}^{0,+}$ also has an important role in carnitine transport and has been implicated in regulating development of early embryos at the blastocyst stage [17, 18].

SLC6A14 and obesity: In Finnish and French populations an association between obesity and chromosomal region Xq22-24 was identified. The linkage was refined and found to be maximal for two SNPs in the 3'-UTR of the SLC6A14 gene [19-21]. The frequency of the obesity associated alleles was slightly higher in the obese cohort, suggesting that $\text{ATB}^{0,+}$ plays only a relatively minor role as a factor in the development of obesity. Owing to its expression in the pituitary gland the transporter might be involved in appetite regulation. Alternatively, it could contribute to development of the embryo thereby contributing to the development of obesity later in life [17].

$\text{ATB}^{0,+}$ and cancer: Due to its broad specificity $\text{ATB}^{0,+}$ can provide cells with all essential amino acids. Accordingly, it is upregulated in cancers derived from tissues that express the transporter endogenously, such as colon cancer and cervical cancer [22, 23]. It is also upregulated in breast cancer cell lines that are estrogen receptor positive. Accordingly, the SLC6A14 promoter is responsive to estrogen receptor activation [24]. An inhibitor of $\text{ATB}^{0,+}$ α -methyl-DL-tryptophan reduces growth of tumor cells and their ability to form colonies [25]. Interestingly, α -methyl-DL-tryptophan also inhibits the system L transporter LAT1, which has been implicated in cancer growth [26]. However, the inhibitor only inhibited cell growth in $\text{ATB}^{0,+}$ over-expressing cancer cells and not in others, while all cell lines expressed LAT1 at similar levels. As a result α -methyl-DL-tryptophan has the potential to inhibit certain forms of cancer.

SLC6A19 (alias $\text{B}^0\text{AT1}$)

Cloning and distribution: SLC6A19 was identified by screening open reading frames on chromosome 5p15 for genes that might be involved in Hartnup disorder [27]. The transporter is expressed mainly in the kidney and intestine. In the intestine it mediates the uptake of neutral amino acids after protein and peptide hydrolysis, while in the kidney it serves as the major mechanism to reabsorb neutral amino acids (Fig. 4). Smaller amounts are found in the skin and pancreas. The transporter is located in the apical membrane of the S1 and S2 segments in the kidney proximal tubule [28, 29]. In the intestine expression increases along the crypt villus axis towards the tips.

Biochemistry of B⁰AT1: The SLC6A19 gene encodes a transporter for a broad (B) range of neutral (0) amino acids and was hence named B⁰AT1 [30, 31]. The protein cotransports amino acids together with 1Na⁺ in an electrogenic transport mechanism. There is no obvious binding order as indicated by a reciprocal influence of Na⁺-concentration on substrate binding and of substrate concentration on Na⁺-binding [32]. The voltage-sensitive step in the transport cycle appears to be the step where substrate and cosubstrate are translocated through the membrane [30]. In view of the structure of the SLC6 family this charge is most likely the positively charged amino group of the substrate amino acid, because the negatively charged carboxyl-group forms an ion pair with Na⁺. Branched-chain amino acids and methionine are the preferred substrates of the transporter being translocated with a K_m of about 1 mM. Smaller and more polar amino acids bind with lower affinity and are transported with K_m-values of up to 5 mM. Histidine, proline and glycine are very weak substrates of the transporter. B⁰AT1 expression at the cell surface requires coexpression of a trafficking subunit. In the kidney the type I transmembrane protein collectrin (TMEM27) binds to B⁰AT1 allowing surface expression [33, 34]. In the intestine this function is mediated by angiotensin converting enzyme 2 (ACE2) [35, 36]. Purification of B⁰AT1 from renal BBMV and reconstitution in proteoliposomes has yielded functional transporter in the absence of collectrin, suggesting that the trafficking subunits are not required for catalytic activity [32]. This also indicates that the interaction between collectrin and B⁰AT1 is fairly weak although they can be co-immunoprecipitated. The transporter is sensitive to sulfhydryl-reactive agents, such as mersalyl and HgCl₂. The CXXC motif Cys200-Cys203 has been proposed as the binding site for methylmercury and HgCl₂, while Cys45 or Cys49 have been proposed as residues for mersalyl binding [32].

Human disorders associated with SLC6A19: Hartnup disorder (OMIM234500) is an autosomal recessive disorder occurring with a frequency of about 1:30,000 in European populations [37]. It affects neutral amino acid transport in kidney and intestine and potentially other tissues such as skin. The hallmark of the disorder is a constant neutral aminoaciduria [38]. In some individuals additional clinical symptoms can be observed, such as a photosensitive skin rash, cerebellar ataxia, diarrhoea and psychotic behaviour. These symptoms usually occur in younger individuals and disappear in adults. This is most likely related to the increased amount of protein required during growth. Most of the symptoms are thought to arise from tryptophan deficiency. There are two lines of evidence supporting this. First, tryptophan-ethyl ester, a lipid soluble tryptophan derivative, was found to alleviate most symptoms of Hartnup disorder [39]. Second, the skin rash, often described as pellagra-like, responds to niacin supplementation [40]. Pellagra is caused by niacin deficiency (referring to either nicotinamide or nicotinic acid). In the human body about 1/60th of tryptophan is converted to NADH, the remainder is synthesized from niacin [41]. As a result tryptophan can

contribute more to NADH biosynthesis than niacin depending on the tryptophan content of the food. In Hartnup disorder the lack of tryptophan absorption would need to be replaced by niacin supplementation. Urine screening programs in Canada and Australia have shown that clinical symptoms are not developed by every individual with Hartnup disorder [37, 40].

To date more than 20 mutations in SLC6A19 have been described in patients with Hartnup disorder (Table 2). All of these change the peptide sequence of the transporter, no regulatory mutation has been identified. Neither have mutations been identified in collectrin or ACE2 that lead to Hartnup disorder. The homology model of B⁰AT1 can shed light on the molecular mechanism of some of the mutations [42]. Mutation Arg57Cys for example affects the formation of the charge pair with Asp486, which is involved in the gating process. Mutation Gly66Arg affects a narrow turn between helix 1 and 2, which is incompatible with a bulky side-chain. Mutation Gly284Arg is located at the beginning of the unwound alpha-helical structure in helix 6, which is essential for providing contacts with substrate and ions. Mutations D173N and R240Q affect the interaction between the trafficking subunits and the transporter and thus may be involved in protein-protein interactions [43]. An inherited disease that indirectly leads to aminoaciduria is MODY3 (maturity onset diabetes of the young type 3). MODY3 is caused by mutations in the nuclear hepatic factor 1 α (HNF1 α) [44]. HNF1 α in turn is a transcription factor that drives expression of collectrin in the kidney [45]. Thus mutations in HNF1 α cause collectrin deficiency, which in turn causes B⁰AT1 deficiency in the kidney. Deletion of HNF1 α in mice causes a number of symptoms including renal Fanconi syndrome [46], a general defect of proximal tubular reabsorption including amino acids.

Mouse models of B⁰AT1 deficiency: Four mouse models have been created, which shed light on the physiology of B⁰AT1 deficiency: B⁰AT1-deficient mice [47], collectrin-deficient mice [33, 34], ACE2-deficient mice [48] and hph2 mice [49]. B⁰AT1-deficient mice lack the transporter in any cell type. Mice nullizygous for collectrin will lack surface expression of B⁰AT1 in collectrin expressing tissues, most notably the kidney. ACE2 nullizygous mice will have a lack of B⁰AT1 expression in the intestine and possibly to some extent in the kidney. Lastly hph2 mice show a similar aminoaciduria as in Hartnup disorder, but the mutation is unknown. Mice nullizygous for B⁰AT1 replicate most features of Hartnup disorder [47]. The mice have highly elevated levels of most neutral amino acids in the urine. Adult mice are 10-20% smaller than heterozygous and wildtype littermates. In some mice dermatitis was observed. Experiments with inverted sections of mouse intestine and renal BBMV showed that for most neutral amino acids B⁰AT1 is the only transporter in the apical membrane. While B⁰AT1 is the only transporter for glutamine in the intestine an additional transport activity was found in the kidney. B⁰AT1 mediates about 50% of proline uptake and 2/3 of glycine uptake in the

human kidney [50]. The residual proline uptake is mediated by the IMINO transporter (SLC6A20), the residual glycine uptake by B⁰AT3 (SLC6A18). Western blotting indicated signs of amino acid malnutrition in the intestine, as ascertained by down-regulation of the mTOR pathway and up-regulation of the GCN2/ATF4 pathway. The latter pathway is switched on by unloaded tRNAs, which appears to occur despite the presence of basolateral transporters. The mice also showed signs of altered tight-junction permeability as indicated by reduced claudin2 and occludin expression. Surprisingly, the study revealed that mice nullizygous for B⁰AT1 have severe problems in controlling body weight on diets of different protein content. When adapted to a 20% protein diet, B⁰AT1 deficient mice rapidly lost weight on both a 6% protein diet and a 40% protein diet. Significant fat loss was observed on the 40% protein diet, which is most likely caused by the reduced carbohydrate content in this diet.

Collectrin-deficient mice show similar aminoaciduria as B⁰AT1-deficient mice, and also show reduced body weight [33, 51]. Insulin secretion is reduced in both B⁰AT1 and collectrin-deficient mice. In collectrin-deficient mice this has been attributed to the interaction of collectrin with vesicular SNARE proteins, such as snapin, which regulate fusion of insulin-containing vesicles with the plasma membrane [45, 52]. However, it is less clear how this would occur in B⁰AT1-deficient mice unless the transporter is also expressed in β -cells. Both B⁰AT1 and B⁰AT3 require collectrin as a trafficking subunit to reach the apical membrane in the kidney [29, 48]. As a result it is expected that glycinuria is more prominent in collectrin-deficient mice than in B⁰AT1-deficient mice. Fractional excretion reaches 81% in collectrin-deficient mice, but the fractional excretion in Hartnup mice is unknown. Collectrin is not expressed in enterocytes, where this function is mediated by ACE2. ACE2-deficient mice do not show aminoaciduria consistent with a very marginal overlap of ACE2 and B⁰AT1 expression in the kidney, but have a number of additional symptoms caused by the lack of ACE2 activity [35, 48].

HPH2 mice were created by random ENU mutagenesis combined with urine screening in an attempt to identify the gene for Hartnup disorder [53]. The mutation was mapped to a locus on chromosome 7, close to the gene of fibroblast growth factor 3. Interestingly, there is no obvious candidate gene within 0.8 cM of fgf3.

SLC6A18 (alias XT2, XTRP2, ROSIT, B⁰AT3)

Cloning and distribution: The B⁰AT3 transporter is closely related to B⁰AT1 [1]. The genes encoding both transporters are next to each other on chromosome 5 in humans and share significant sequence homology. B⁰AT3 was identified by homology cloning of SLC6 transporters from the kidney [54]. Initial studies failed to express the transporter functionally. In mouse the transporter is

expressed in six splice variants, many of which are severely truncated and unlikely to be functional [54]. While B⁰AT1 is expressed in both kidney and intestine, B⁰AT3 is largely confined to the kidney. Under normal conditions the transporter is expressed in the cortex, more precisely the S3 segment of the proximal tubule [28, 29]. Hypernatremic conditions cause strong up-regulation in both cortex and the outer medulla [55], while ischemia causes downregulation [56].

Biochemistry of B⁰AT3: B⁰AT3, like B⁰AT1 requires ACE2 or collectrin for surface expression, explaining the failure to express the transporter functionally in earlier studies [29, 48]. The first coexpression experiments suggested that only ACE2 could promote migration of B⁰AT3 to the cell surface [48]. This was surprising given that ACE2 expression in the proximal tubule is limited, while collectrin expression is strong. Subsequently, it was shown that both ACE2 and collectrin support surface expression [29]. In the presence of collectrin or ACE2 the transporter can be characterised in heterologous expression systems. Glycine and alanine are the main substrates of the transporter although it also mediates the uptake of a variety of neutral amino acids, particularly branched-chain amino acids and methionine. The transporter is both Na⁺- and Cl⁻-dependent. There is an apparent discrepancy between two studies with regard to the electrogenicity of the transporter. Singer *et al.*, [48] reported the transporter to be electroneutral, while Vanslambrouck *et al.* [29] showed it to be electrogenic. In contrast to B⁰AT1 and B⁰AT2, B⁰AT3 is not pH-dependent. It is tempting to speculate that the Cl⁻-binding site in the former transporters is occupied by a OH⁻ ion, which is not translocated. At acidic pH, the transporter loses activity due to lack of hydroxyl ions. Slightly different kinetic parameters were reported by the two groups. In the presence of collectrin the K_M-values for glycine and alanine were 2.3 mM and 0.9 mM, respectively. These amino acids induced the biggest currents in B⁰AT3 expressing oocytes. In the presence of ACE2 glycine was transported with a K_M = 0.5 mM and isoleucine with a K_M = 0.2 mM. The maximum velocity for isoleucine was twice as high as for glycine, whereas the opposite was observed in the case of collectrin.

Mouse models of B⁰AT3 deficiency: In an attempt to understand B⁰AT3 function an SLC6A18 nullizygous mouse was generated by Quan *et al.* [57]. The main phenotype was a three-fold increased glycine to creatinine ratio in the urine of B⁰AT3 deficient mice. Autoradiography confirmed reduced glycine uptake in the outer medulla. A high-affinity component of glycine transport was missing in brush-border membrane vesicles (BBMV) derived from kidney. The blood pressure of the mice was slightly elevated. In a subsequent study SLC6A18-nullizygous mice were fully back-crossed to the C57BL/6J background [48]. Urine analysis of these mice showed increased fractional excretion of Gly (51%), Met (33%), Leu (12%) and His (10%); values in parentheses indicate the amount of filtered blood appearing in the urine. ACE2-deficient mice have no increased fractional excretion.

Transport studies in renal BBMV derived from B⁰AT1-deficient mice, suggested that B⁰AT3 has a significant contribution to glycine transport, but not to leucine transport [47].

Human diseases associated with B⁰AT3: In humans glycinuria (OMIM 138500) and iminoglycinuria (IG, OMIM 242600) have been reported [50, 58]. Both are rare inherited disorders that seem to be caused by a combination of different alleles [59] (Table 3). In some pedigrees, glycinuria appears to be caused by haploinsufficiency of a major allele that in homozygosity causes iminoglycinuria (i.e. glycinuria caused by a mutation on one chromosome, iminoglycinuria caused by mutations in both chromosomes). In other cases glycinuria appears to be caused by separate alleles. In mice lack of B⁰AT3 causes glycinuria and alaninuria, consistent with the preference of the transporter [48]. In humans this does not seem to be the case, because a stop codon (Y319X) in SLC6A18 occurs in about 40% of the population [60]. As a result 16% of the population are expected to be homozygous for this mutation, far more than the reported incidence frequency of glycinuria [61]. In a recent study on iminoglycinuria one individual in a cohort of 29 persons had a homozygous stop codon in the presence of normal alleles for all other proline and glycine transporters [50]. The urine amino acid levels were found to be normal. In human kidney the bulk of glycine is transported by the proton-amino acid symporter PAT2 and the general neutral amino acid transporter B⁰AT1 [50] (Fig. 4). Haploinsufficiency of PAT2 causes glycinuria and thus is considered to be the major cause of glycinuria in humans [50].

Both B⁰AT1 and B⁰AT3 have been implicated in hypertension in line with the phenotype observed in SLC6A18-nullizygous mice [57]. However, in a subsequent study differences in blood pressure were only found under stress-inducing conditions [48]. In a cohort of 1004 Japanese individuals no evidence for elevated blood pressure was found and this lack of association was also confirmed for the Korean population [60, 62]. A recent study reported the association of a specific microsatellite marker in the *SLC6A19* gene with essential hypertension [63]. 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 [64].

SLC6A20 (IMINO, XTRP3, XT3, SIT1, rb21a)

Cloning and distribution: SLC6A20 was initially identified as a novel member of the SLC6 family [54, 65]. Initial attempts to reveal its function were unsuccessful. After the identification of the amino acid transporter (II) family, two independent studies demonstrated that SLC6A20 encodes the system IMINO transporter [66, 67]. In mouse and rat two genes are found at the SLC6A20 locus, while only one gene remains in humans [1]. These are now referred to as SLC6A20a (IMINOa) and

SLC6A20b (IMINO_b). Only IMINO_a carries out active transport, IMINO_b appears to be non-functional [67]. The IMINO_a mRNA and protein is found in the brain and in the kidney, while IMINO_b is found in the kidney only. In the brain the transporter is expressed in the choroid plexus and the pia mater [66, 67]. In the kidney the transporter is expressed throughout the proximal tubule, but expression increases towards the S3 segment. In the developing mouse embryo it is upregulated a few hours after fertilisation remaining high until the 2-cell stage [68]. During this time the transporter accumulates and retains betaine, which is known to improve development of preimplantation embryos. Expression of SLC6A20 is also developmentally regulated in newborn mice and humans [29]. Newborn humans show iminoglycinuria, which recedes after about 3 months. This is caused by an increased expression of SLC6A20 and of the proline/glycine transporter PAT2 (SLC36A2). Newborn mice have a more general aminoaciduria, which is remediated by increasing expression of SLC6A18, SLC6A19 and SLC6A20 in the first three weeks. Collectrin expression also increases over the same period.

Biochemistry of IMINO: System Imino is a 2Na^+ and Cl^- proline cotransporter ($K_M = 0.13 \text{ mM}$) [66, 67]. In addition it also transports the physiological substrates hydroxyproline ($K_M = 0.19 \text{ mM}$), betaine ($K_M = 0.2 \text{ mM}$) and sarcosine ($K_M = 3.2 \text{ mM}$) and the amino acid analogues MeAIB, pipecolic acid ($K_M = 0.09 \text{ mM}$), nipecotic acid ($K_M = 2.8 \text{ mM}$). The transporter is weakly stereospecific. The $K_{0.5}$ for Na^+ is 22 mM , chloride binds with higher affinity ($K_{0.5} = 2 \text{ mM}$). The chloride dependence can be rationalised by the presence of a conserved chloride binding site close to the Na^+ binding site [69] (Table 1). When expressed in oocytes the transporter induces a leak current, the physiological role of which is unknown [69]. The Na^+ -affinity increases with increasing membrane potential consistent with the Na^+ -binding site being buried in the center of the transporter.

Human diseases associated with SLC6A20: Iminoglycinuria (IG, OMIM 242600) is a rare disorder associated with increased secretion of glycine, proline and hydroxyproline in the urine [59]. The condition is benign but has helped to understand amino acid reabsorption in the kidney. Developmental IG is a normal phenomenon in humans, dogs and rodents, whereby newborns release increased amounts of proline, hydroxyproline and glycine into the urine [70]. In humans prolinuria ceases after about 3 months, while glycinuria persists up to 6 months. Proline and glycine reabsorption is species specific, which has caused confusion in the interpretation of data [42]. In humans the main transporter for proline and glycine is the proton amino acid transporter PAT2 (SLC36A2). Complete inactivation of this transporter by a homozygous mutation is sufficient to cause IG [50] (Table 3). PAT2 is not expressed in the intestine and therefore causes a purely renal iminoglycinuria. In addition IG can be caused by partially inactivating mutations of SLC36A2 in

combination with mutations in SLC6A20 (Table 3). This explains a known heterogeneity of IG, in which some individuals do have intestinal malabsorption of proline, while others do not [59]. In the mutated IMINO transporter Thr199 is replaced by methionine [69]. There are numerous additional non-synonymous SNPs in SLC6A20, which have not been functionally assessed. The combined PAT2/IMINO cases have reduced uptake of proline in the intestine due to reduced activity of the IMINO transporter. In mice proline and glycine transport is mediated by B⁰AT3 and IMINO, while PAT2 expression is minimal [29]. In fact PAT2 activity cannot be observed in mouse renal BBMV. Thus it appears PAT2 has replaced B⁰AT3 in humans.

SLC6A20 and diabetes: In the urine of rhesus macaques with early signs of type 2 diabetes elevated levels of pipecolic acid, glycine betaine and proline were detected [71]. These metabolites are substrates of IMINO and were also been found in the db/db mouse model of type 2 diabetes and in humans. The increased metabolite levels were paralleled by a reduced level of IMINO in the kidney of macaques and mice. Other transporters such as SLC36A1 and the trafficking subunit collectrin were unaffected, suggesting that the reduction was not caused by general proximal tubular degeneration. As a result IMINO substrates may serve as an early indicator of type 2 diabetes in humans.

SLC6A15 (alias v7-3, B⁰AT2)

Cloning and localisation: The B⁰AT2 transporter was cloned from mouse brain, showing widespread expression in neurons across the cerebral cortex, hippocampus, cerebellum, midbrain and olfactory bulb [72, 73]. The mRNA for the transporter was detected in a variety of neurons, such as dopaminergic neurons in the substantia nigra, serotonergic neurons in the raphe nuclei, noradrenergic neurons in the locus coeruleus, glutamatergic neurons in the hippocampus and the olfactory bulb and cholinergic motor neurons [73, 74]. B⁰AT2 is expressed at the plasma membrane, while its closest homologue NTT4 has a similar tissue and cellular distribution, but resides largely on vesicular compartments [74, 75] (Fig. 5). Outside the brain the transporter is found in the lung and kidney in relatively small amounts.

Biochemistry of B⁰AT2: B⁰AT2 is a neutral amino acid transporter preferring branched-chain neutral amino acids and proline. At saturating concentrations (causing the transporter be at V_{max}) the order of the transport-induced currents is Pro (K_M=200 μM) > Cys = Ala (K_M=670 μM) = Thr = Val = Ser = Met (K_M=40 μM) = Ile (K_M=58 μM) = Leu (K_M=81 μM) > Asn > Phe (K_M=1 mM) > Gln (K_M=5.3 mM) [76, 77]. The K_M-values do not coincide with the order of the maximum currents, suggesting that V_{max} and K_M are determined by different aspects of the substrate. Substrate uptake is Na⁺- but not Cl⁻-dependent and electrogenic; 1 charge (Na⁺) per substrate is translocated. The transporter is strongly pH-

dependent, being almost inactive at acidic pH, but is affected only by the extracellular pH. The substrate K_M decreases when the Na^+ -concentration is increased and vice versa, suggesting that there is no strong preference in the binding order of substrate and cosubstrate [76, 77]. The interdependence of substrate and co-substrate affinity suggests that the Na^+ -binding site is functional in $\text{B}^0\text{AT}2$. The proposed chloride binding site is not fully conserved (Ala309 instead of Ser, see Table 1), which is consistent with a lack of chloride dependence. One of the major functions of branched-chain amino acids in the brain is to serve as anaplerotic metabolites in neurons [78] and provide amino-groups for neurotransmitter biosynthesis [79] (Fig. 5). Neurotransmitter biosynthesis, particularly glutamate and GABA removes intermediates of the TCA cycle, which need to be replenished. Valine, threonine, isoleucine and methionine degradation generates succinyl-CoA, which can be converted to α -ketoglutarate. Neurons lack pyruvate carboxylase, which is the major anaplerotic enzyme in most cell types [80].

Human diseases associated with SLC6A15: Depression is caused largely by environmental factors, but the predisposition to succumb to environmental factors has a heritable component. For instance, a Leu255Met mutation in the serotonin transporter SLC6A4 has been associated with depression [81]. In a large genome-wide association study a SNP 287kb downstream of the SLC6A15 gene was recently found to be associated with major depression [82]. The odds ratio for homozygous carriers of the risk allele was 1.42 in a cohort of more than 6000 individuals. Whether this region has any regulatory influence on SLC6A15 expression is unknown. Analysis of $\text{B}^0\text{AT}2$ expression levels in brain samples from individuals with temporal lobe epilepsy showed a correlation between the SNP rs1545843 and $\text{B}^0\text{AT}2$ mRNA expression levels. Risk genotype carrier status was associated with slightly reduced transcript levels. Whether this reduction is sufficient to influence brain metabolism remains to be shown.

Mouse model of $\text{B}^0\text{AT}2$ deficiency: $\text{B}^0\text{AT}2$ deficient mice are viable and fertile and do not show any obvious phenotype [83]. The animals gain weight at the same rate as wildtype littermates. Posture, gait, reflexes and general behaviour are normal. Initial tests suggested reduced olfactory sensitivity in $\text{B}^0\text{AT}2$ deficient mice, consistent with its high expression in the olfactory bulb, however, these results could not be repeated with a different cohort. To test for a role of $\text{B}^0\text{AT}2$ in hippocampus and cerebellum, spatial memory and locomotor coordination was tested; without observing significant differences. Interestingly, mature female $\text{B}^0\text{AT}2$ -deficient mice could hold balance on a rotating rod for longer time, than wildtype control mice. The bulk of proline transport in brain synaptosomes appears to be mediated by the specific proline transporter PROT, while about 20% of proline uptake

capacity appeared to be mediated by B⁰AT2. Similarly, about 50% of Na⁺-dependent leucine uptake was abolished in B⁰AT2 deficient mice.

In the same study that investigated the association of B⁰AT2 with major depression [82], it was also found that stress-susceptible mice had significantly reduced levels of B⁰AT2 mRNA compared to stress-resilient mice. These results are at variance with the results from B⁰AT2 deficient mice, which showed no difference to wildtype mice in anxiety assessment after induction of stress.

SLC6A17 (alias NTT4, XT1)

Cloning and distribution: The NTT4 transporter was initially identified as an unusual member of the neurotransmitter transporter family [72, 84, 85]. The main difference was an extended extracellular loop between helix 7 and 8. In situ hybridisation revealed that the distribution of NTT4 and B⁰AT2 are largely overlapping [74]. The highest concentration of NTT4 mRNA was found in the olfactory bulb, cerebral cortex, hippocampus, habenular and pontine nuclei, and cerebellum, lower expression was widespread. NTT4 mRNA in general was more abundant than B⁰AT2 mRNA. NTT4 is expressed in neurons, such as pyramidal cells, granular cells, Purkinje cells and motor neurons. The cellular distribution largely follows glutamatergic pathways, but neurons using other neurotransmitters also express NTT4. Immunohistochemistry revealed that NTT4 is found in axon terminals of glutamatergic and some GABAergic neurons. In transfected cells and in neurons NTT4 is mainly expressed in synaptic vesicles [75]. The transporter is expressed highly during development, when protein synthesis is high [86, 87].

Biochemistry of NTT4: Two studies have investigated the functional properties of NTT4 in different expression systems, coming to slightly different conclusions. Zaia and Reimer [88] exchanged the carboxy-terminus of NTT4 with that of B⁰AT2, thereby achieving expression in the plasma membrane of HEK293 cells. Functional characterisation showed the transport to be similar to B⁰AT2. Preferred substrates were leucine ($K_i=0.3$ mM), methionine, proline ($K_M= 0.4$ mM), cysteine, alanine and glutamine ($K_M= 5.2$ mM). Transport was Na⁺-dependent ($K_M= 23$ mM), but not Cl⁻-dependent and electrogenic. Uptake is pH-dependent and is barely detectable at pH 5.5. In transfected cells the wildtype transporter was largely located in intracellular compartments, but still induced a 2.4-fold increase of proline uptake. This suggests that a fraction of the transporters is located at the cell surface. Induction of exocytosis by ionomycin further increased the transport activity. Parra *et al.* [89], by contrast used vesicles from PC12 cells, which express NTT4 endogenously and compared vesicular transport to cells where NTT4 had been silenced. Preferred substrates determined by this method were leucine, glycine ($K_M=1.7$ mM), proline ($K_M= 0.86$ mM) and alanine. Further experiments used the increase of vesicular transport in transfected CHO cells. These results suggested that NTT4

is neither Na⁺-dependent nor Cl⁻-dependent [89]. Vesicular proline uptake was almost abolished in the presence of bafilomycin or the absence of ATP, both manipulations inhibiting the V-type ATPase, which acidifies vesicular compartments. Further experiments using nigericin and valinomycin suggested that the pH-gradient was more important as a driving force compared to the membrane potential. The discrepancy between the two studies is difficult to resolve. The structure of the SLC6-type transporters suggests that an exchange of the carboxy-terminus is unlikely to affect ion binding in the transporter. In the study by Parra *et al.*, significant background activity had to be subtracted in all experiments, while there was significantly less background in the study by Zaia and Reimer. Sequence conservation also suggests functional Na⁺-binding sites to be present in NTT4 (Table 1). The lack of chloride dependence is consistent with a partially conserved Cl⁻ binding site (A308 instead of Thr). Overall it appears more likely that the transporter is Na⁺-dependent rather than H⁺-dependent. The pH-dependence would render the transporter inactive on synaptic vesicles, whereas the study by Parra *et al.* suggests that the transporter is active in this compartment.

There is no mouse model of NTT4 deficiency nor are there human mutations associated with the SLC6A17 gene. Three non-synonymous SNP's are listed in the database (<http://www.ncbi.nlm.nih.gov/snp>): Ala57Thr/Pro; Val184Ile and Lys679Arg. These replacements are either conservative or do not occur in sensitive regions of the transporter.

SLC6A16 (alias NTT5)

Little is known about the physiology and biochemistry of NTT5. It was identified through database searches as a member of the SLC6 family [90]. It is mainly expressed in testis, and at lower levels in other peripheral organs such as lung, liver, prostate, pancreas and uterus. Expression of the myc-tagged NTT5 in CHO cells suggested a predominantly intracellular/vesicular localisation. Attempts to express NTT5 in heterologous systems have been unsuccessful (unpublished data), as a result there are no functional data. Inspection of the critical residues involved in Na⁺-binding show that NTT5 has cysteine in position 125. The equivalent position in all other transporters is occupied by a conserved asparagine.

There is no mouse model of NTT5 deficiency nor are there human mutations associated with the SLC6A16 gene. Four non-synonymous SNP's are listed in the database (<http://www.ncbi.nlm.nih.gov/snp>): Ser108Arg; Phe233Val; Leu278Val; Arg459Gly. These replacements are either conservative and do not occur in sensitive regions of the transporter.

SLC6A7 (alias PROT)

Cloning and distribution: The brain-specific proline transporter PROT was isolated by using degenerate primers to amplify conserved regions of SLC6 related genes. The transporter is strongly expressed in the cortex, hippocampus and midbrain, weaker in other areas [91]. More detailed investigation showed the transporter to be expressed in a subset of glutamatergic neurons, such as pyramidal cells of the cerebral, entorhinal and piriform cortex and mitral cells of the olfactory bulb [92]. In the hippocampus the transporter is found in the molecular layer of the dentate gyrus and specific layers of the CA1 and CA3 regions. The transporter is confined to synaptic vesicles in terminals apposing NMDA and AMPA receptors [93]. Exocytosis would then result in transient expression of the transporter in the presynaptic membrane.

Biochemistry of PROT: The transporter is specific for L-proline ($K_M = 9.7 \mu\text{M}$) and is inhibited by its analogues pipecolic acid ($K_i = 14 \mu\text{M}$) and sarcosine ($K_i = 30 \mu\text{M}$), but not by hydroxyproline, which discriminates it from IMINO [94]. Some amino acids such as phenylalanine and histidine inhibit the transporter, but do not seem to be substrates. The transporter is Na^+ - and Cl^- -dependent, which are typical features of a plasma membrane transporter. The activity of the transporter on synaptic vesicles is unknown. Several high-affinity inhibitors of PROT have been identified namely enkephalin peptides (K_i between $0.2 \mu\text{M}$ and $5 \mu\text{M}$), benztropin ($K_i = 7.5 \mu\text{M}$) and a compound called LP-403812 ($K_i = 0.12 \mu\text{M}$) [95, 96].

The physiological role of PROT in the brain is unclear. Proline is a potent neuromodulator, which can potentiate glutamatergic neurotransmission in the hippocampus. Synaptosomes can synthesize glutamate from proline, but the synaptosomal localisation of the transporter is at variance with a role as precursor uptake mediator (Fig. 5).

No mouse models or human diseases have been reported that could elucidate PROT function.

Conclusion

Sodium dependent amino acid transporters of the SLC6 family play an essential role in amino acid homeostasis in epithelial tissues and the brain. They provide building blocks for protein synthesis and serve as precursors for neurotransmitter metabolism. They regulate plasma amino acid levels and serve still unknown roles as metabolites in synaptic vesicles. Human diseases and mouse models illustrate these functions and lack of understanding prevails for transporters, where neither is known.

Acknowledgements:

Work in the laboratory of the author has been supported by grants of the Australian Research Council (ARC) and the National Health and Medical Research Council (NHMRC).

References

- [1] Broer, S. The SLC6 orphans are forming a family of amino acid transporters. *Neurochem Int.* 2006, **48** (6-7), 559-567.
- [2] Yamashita, A.; Singh, S. K.; Kawate, T.; Jin, Y.; Gouaux, E. Crystal structure of a bacterial homologue of Na(+)/Cl(-)-dependent neurotransmitter transporters. *Nature.* 2005, **437** 215-223.
- [3] Forrest, L. R.; Rudnick, G. The rocking bundle: a mechanism for ion-coupled solute flux by symmetrical transporters. *Physiology (Bethesda).* 2009, **24** 377-386.
- [4] Krishnamurthy, H.; Gouaux, E. X-ray structures of LeuT in substrate-free outward-open and apo inward-open states. *Nature.* 2012, **481** (7382), 469-474.
- [5] Forrest, L. R.; Kramer, R.; Ziegler, C. The structural basis of secondary active transport mechanisms. *Biochim Biophys Acta.* 2011, **1807** (2), 167-188.
- [6] Singh, S. K.; Piscitelli, C. L.; Yamashita, A.; Gouaux, E. A competitive inhibitor traps LeuT in an open-to-out conformation. *Science.* 2008, **322** (5908), 1655-1661.
- [7] Shi, L.; Quick, M.; Zhao, Y.; Weinstein, H.; Javitch, J. A. The mechanism of a neurotransmitter:sodium symporter--inward release of Na⁺ and substrate is triggered by substrate in a second binding site. *Mol. Cell.* 2008, **30** (6), 667-677.
- [8] Piscitelli, C. L.; Krishnamurthy, H.; Gouaux, E. Neurotransmitter/sodium symporter orthologue LeuT has a single high-affinity substrate site. *Nature.* 2010, **468** (7327), 1129-1132.
- [9] Abramson, J.; Wright, E. M. Structure and function of Na(+)-symporters with inverted repeats. *Curr Opin Struct Biol.* 2009, **19** (4), 425-432.
- [10] Shimamura, T.; Weyand, S.; Beckstein, O.; Rutherford, N. G.; Hadden, J. M.; Sharples, D.; Sansom, M. S.; Iwata, S.; Henderson, P. J.; Cameron, A. D. Molecular basis of alternating access membrane transport by the sodium-hydantoin transporter Mhp1. *Science.* 2010, **328** (5977), 470-473.
- [11] Forrest, L. R.; Tavoulari, S.; Zhang, Y. W.; Rudnick, G.; Honig, B. Identification of a chloride ion binding site in Na⁺/Cl⁻-dependent transporters. *Proc Natl Acad Sci U S A.* 2007, **104** (31), 12761-12766.
- [12] Zomot, E.; Bendahan, A.; Quick, M.; Zhao, Y.; Javitch, J. A.; Kanner, B. I. Mechanism of chloride interaction with neurotransmitter:sodium symporters. *Nature.* 2007, **449** (7163), 726-730.

- [13] Sloan, J. L.; Mager, S. Cloning and functional expression of a human Na⁽⁺⁾ and Cl⁽⁻⁾-dependent neutral and cationic amino acid transporter B⁽⁰⁺⁾. *J Biol Chem.* 1999, **274** (34), 23740-23745.
- [14] Hatanaka, T.; Haramura, M.; Fei, Y. J.; Miyauchi, S.; Bridges, C. C.; Ganapathy, P. S.; Smith, S. B.; Ganapathy, V.; Ganapathy, M. E. Transport of amino acid-based prodrugs by the Na⁺- and Cl⁽⁻⁾-coupled amino acid transporter ATB⁰⁺ and expression of the transporter in tissues amenable for drug delivery. *J Pharmacol Exp Ther.* 2004, **308** (3), 1138-1147.
- [15] Sloan, J. L.; Grubb, B. R.; Mager, S. Expression of the amino acid transporter ATB⁰⁺ in lung: possible role in luminal protein removal. *Am J Physiol Lung Cell Mol Physiol.* 2003, **284** (1), L39-49.
- [16] Mager, S.; Sloan, J. Possible role of amino acids, peptides, and sugar transporter in protein removal and innate lung defense. *Eur J Pharmacol.* 2003, **479** (1-3), 263-267.
- [17] Van Winkle, L. J.; Tesch, J. K.; Shah, A.; Campione, A. L. System B⁰⁺ amino acid transport regulates the penetration stage of blastocyst implantation with possible long-term developmental consequences through adulthood. *Hum Reprod Update.* 2006, **12** (2), 145-157.
- [18] Nakanishi, T.; Hatanaka, T.; Huang, W.; Prasad, P. D.; Leibach, F. H.; Ganapathy, M. E.; Ganapathy, V. Na⁺- and Cl⁽⁻⁾-coupled active transport of carnitine by the amino acid transporter ATB^(0,+) from mouse colon expressed in HRPE cells and *Xenopus* oocytes. *J Physiol.* 2001, **532** (Pt 2), 297-304.
- [19] Durand, E.; Boutin, P.; Meyre, D.; Charles, M. A.; Clement, K.; Dina, C.; Froguel, P. Polymorphisms in the amino acid transporter solute carrier family 6 (neurotransmitter transporter) member 14 gene contribute to polygenic obesity in French Caucasians. *Diabetes.* 2004, **53** (9), 2483-2486.
- [20] Suviolahti, E.; Oksanen, L. J.; Ohman, M.; Cantor, R. M.; Ridderstrale, M.; Tuomi, T.; Kaprio, J.; Rissanen, A.; Mustajoki, P.; Jousilahti, P.; Vartiainen, E.; Silander, K.; Kilpikari, R.; Salomaa, V.; Groop, L.; Kontula, K.; Peltonen, L.; Pajukanta, P. The SLC6A14 gene shows evidence of association with obesity. *J Clin Invest.* 2003, **112** (11), 1762-1772.
- [21] Ohman, M.; Oksanen, L.; Kaprio, J.; Koskenvuo, M.; Mustajoki, P.; Rissanen, A.; Salmi, J.; Kontula, K.; Peltonen, L. Genome-wide scan of obesity in Finnish sibpairs reveals linkage to chromosome Xq24. *J Clin Endocrinol Metab.* 2000, **85** (9), 3183-3190.
- [22] Gupta, N.; Miyauchi, S.; Martindale, R. G.; Herdman, A. V.; Podolsky, R.; Miyake, K.; Mager, S.; Prasad, P. D.; Ganapathy, M. E.; Ganapathy, V. Upregulation of the amino acid transporter ATB⁰⁺ (SLC6A14) in colorectal cancer and metastasis in humans. *Biochim Biophys Acta.* 2005, **1741** (1-2), 215-223.

- [23] Gupta, N.; Prasad, P. D.; Ghamande, S.; Moore-Martin, P.; Herdman, A. V.; Martindale, R. G.; Podolsky, R.; Mager, S.; Ganapathy, M. E.; Ganapathy, V. Up-regulation of the amino acid transporter ATB(0,+) (SLC6A14) in carcinoma of the cervix. *Gynecol. Oncol.* 2006, **100** (1), 8-13.
- [24] Karunakaran, S.; Ramachandran, S.; Coothankandaswamy, V.; Elangovan, S.; Babu, E.; Periyasamy-Thandavan, S.; Gurav, A.; Gnanaprakasam, J. P.; Singh, N.; Schoenlein, P. V.; Prasad, P. D.; Thangaraju, M.; Ganapathy, V. SLC6A14 (ATB0,+) protein, a highly concentrative and broad specific amino acid transporter, is a novel and effective drug target for treatment of estrogen receptor-positive breast cancer. *J Biol Chem.* 2011, **286** (36), 31830-31838.
- [25] Karunakaran, S.; Umapathy, N. S.; Thangaraju, M.; Hatanaka, T.; Itagaki, S.; Munn, D. H.; Prasad, P. D.; Ganapathy, V. Interaction of tryptophan derivatives with SLC6A14 (ATB0,+) reveals the potential of the transporter as a drug target for cancer chemotherapy. *Biochem J.* 2008, **414** (3), 343-355.
- [26] Oda, K.; Hosoda, N.; Endo, H.; Saito, K.; Tsujihara, K.; Yamamura, M.; Sakata, T.; Anzai, N.; Wempe, M. F.; Kanai, Y.; Endou, H. L-type amino acid transporter 1 inhibitors inhibit tumor cell growth. *Cancer Sci.* 2010, **101** (1), 173-179.
- [27] Broer, A.; Klingel, K.; Kowalczyk, S.; Rasko, J. E.; Cavanaugh, J.; Broer, S. Molecular Cloning of Mouse Amino Acid Transport System B0, a Neutral Amino Acid Transporter Related to Hartnup Disorder. *J Biol Chem.* 2004, **279** (23), 24467-24476.
- [28] Romeo, E.; Dave, M. H.; Bacic, D.; Ristic, Z.; Camargo, S. M.; Loffing, J.; Wagner, C. A.; Verrey, F. Luminal kidney and intestine SLC6 amino acid transporters of B0AT-cluster and their tissue distribution in *Mus musculus*. *Am J Physiol Renal Physiol.* 2006, **290** (2), F376-383.
- [29] Vanslambrouck, J. M.; Broer, A.; Thavyogarah, T.; Holst, J.; Bailey, C. G.; Broer, S.; Rasko, J. E. Renal imino acid and glycine transport system ontogeny and involvement in developmental iminoglycinuria. *Biochem J.* 2010, **428** (3), 397-407.
- [30] Bohmer, C.; Broer, A.; Munzinger, M.; Kowalczyk, S.; Rasko, J. E.; Lang, F.; Broer, S. Characterization of mouse amino acid transporter B0AT1 (slc6a19). *Biochem J.* 2005, **389** (Pt 3), 745-751.
- [31] Camargo, S. M.; Makrides, V.; Virkki, L. V.; Forster, I. C.; Verrey, F. Steady-state kinetic characterization of the mouse B(0)AT1 sodium-dependent neutral amino acid transporter. *Pflugers Arch.* 2005, **451** (2), 338-348.
- [32] Oppedisano, F.; Pochini, L.; Broer, S.; Indiveri, C. The B(0)AT1 amino acid transporter from rat kidney reconstituted in liposomes: Kinetics and inactivation by methylmercury. *Biochim Biophys Acta.* 2011, **1808** (10), 2551-2558.

- [33] Danilczyk, U.; Sarao, R.; Remy, C.; Benabbas, C.; Stange, G.; Richter, A.; Arya, S.; Pospisilik, J. A.; Singer, D.; Camargo, S. M.; Makrides, V.; Ramadan, T.; Verrey, F.; Wagner, C. A.; Penninger, J. M. Essential role for collectrin in renal amino acid transport. *Nature*. 2006, **444** (7122), 1088-1091.
- [34] Malakauskas, S. M.; Quan, H.; Fields, T. A.; McCall, S. J.; Yu, M. J.; Kourany, W. M.; Frey, C. W.; Le, T. H. Aminoaciduria and altered renal expression of luminal amino acid transporters in mice lacking novel gene collectrin. *Am J Physiol Renal Physiol*. 2007, **292** (2), F533-544.
- [35] Kowalczyk, S.; Broer, A.; Tietze, N.; Vanslambrouck, J. M.; Rasko, J. E.; Broer, S. A protein complex in the brush-border membrane explains a Hartnup disorder allele. *Faseb J*. 2008, **22** (8), 2880-2887.
- [36] Camargo, S. M.; Singer, D.; Makrides, V.; Huggel, K.; Pos, K. M.; Wagner, C. A.; Kuba, K.; Danilczyk, U.; Skovby, F.; Kleta, R.; Penninger, J. M.; Verrey, F. Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations. *Gastroenterology*. 2009, **136** (3), 872-882.
- [37] Auray-Blais, C.; Giguere, R.; Lemieux, B. Newborn urine screening programme in the province of Quebec: an update of 30 years' experience. *J Inherit Metab Dis*. 2003, **26** (4), 393-402.
- [38] Baron, D. N.; Dent, C. E.; Harris, H.; Hart, E. W.; Jepson, J. B. Hereditary pellagra-like skin rash with temporary cerebellar ataxia, constant renal aminoaciduria and other bizarre biochemical features. *Lancet*. 1956, **268** 421-428.
- [39] Jonas, A. J.; Butler, I. J. Circumvention of defective neutral amino acid transport in Hartnup disease using tryptophan ethyl ester. *J Clin Invest*. 1989, **84** (1), 200-204.
- [40] Wilcken, B.; Yu, J. S.; Brown, D. A. Natural history of Hartnup disease. *Arch Dis Child*. 1977, **52** (1), 38-40.
- [41] Lindner, M. C. Nutrition and metabolism of vitamins. In *Nutritional Biochemistry and Metabolism* (Lindner, M. C., ed.). 1991, pp. 111-189, Elsevier, New York.
- [42] Broer, S. Apical transporters for neutral amino acids: physiology and pathophysiology. *Physiology (Bethesda)*. 2008, **23** 95-103.
- [43] Broer, S. The role of the neutral amino acid transporter B0AT1 (SLC6A19) in Hartnup disorder and protein nutrition. *IUBMB Life*. 2009, **61** (6), 591-599.

- [44] Yamagata, K.; Oda, N.; Kaisaki, P. J.; Menzel, S.; Furuta, H.; Vaxillaire, M.; Southam, L.; Cox, R. D.; Lathrop, G. M.; Boriraj, V. V.; Chen, X.; Cox, N. J.; Oda, Y.; Yano, H.; Le Beau, M. M.; Yamada, S.; Nishigori, H.; Takeda, J.; Fajans, S. S.; Hattersley, A. T.; Iwasaki, N.; Hansen, T.; Pedersen, O.; Polonsky, K. S.; Turner, R. C.; Velho, G.; Chevre, J. C.; Froguel, P.; Bell, G. I. Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature*. 1996, **384** (6608), 455-458.
- [45] Fukui, K.; Yang, Q.; Cao, Y.; Takahashi, N.; Hatakeyama, H.; Wang, H.; Wada, J.; Zhang, Y.; Marselli, L.; Nammo, T.; Yoneda, K.; Onishi, M.; Higashiyama, S.; Matsuzawa, Y.; Gonzalez, F. J.; Weir, G. C.; Kasai, H.; Shimomura, I.; Miyagawa, J.; Wollheim, C. B.; Yamagata, K. The HNF-1 target collectrin controls insulin exocytosis by SNARE complex formation. *Cell Metab*. 2005, **2** (6), 373-384.
- [46] Pontoglio, M.; Barra, J.; Hadchouel, M.; Doyen, A.; Kress, C.; Bach, J. P.; Babinet, C.; Yaniv, M. Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. *Cell*. 1996, **84** (4), 575-585.
- [47] Broer, A.; Juelich, T.; Vanslambrouck, J. M.; Tietze, N.; Solomon, P. S.; Holst, J.; Bailey, C. G.; Rasko, J. E.; Broer, S. Impaired Nutrient Signaling and Body Weight Control in a Na⁺ Neutral Amino Acid Cotransporter (Slc6a19)-deficient Mouse. *J Biol Chem*. 2011, **286** (30), 26638-26651.
- [48] Singer, D.; Camargo, S. M.; Huggel, K.; Romeo, E.; Danilczyk, U.; Kuba, K.; Chesnov, S.; Caron, M. G.; Penninger, J. M.; Verrey, F. Orphan transporter SLC6A18 is renal neutral amino acid transporter B0AT3. *J Biol Chem*. 2009, **284** (30), 19953-19960.
- [49] Symula, D. J.; Shedlovsky, A.; Guillery, E. N.; Dove, W. F. A candidate mouse model for Hartnup disorder deficient in neutral amino acid transport. *Mamm Genome*. 1997, **8** (2), 102-107.
- [50] Broer, S.; Bailey, C. G.; Kowalczyk, S.; Ng, C.; Vanslambrouck, J. M.; Rodgers, H.; Auray-Blais, C.; Cavanaugh, J. A.; Broer, A.; Rasko, J. E. Iminoglycinuria and hyperglycinuria are discrete human phenotypes resulting from complex mutations in proline and glycine transporters. *J Clin Invest*. 2008, **118** (12), 3881-3892.
- [51] Malakauskas, S. M.; Kourany, W. M.; Zhang, X. Y.; Lu, D.; Stevens, R. D.; Koves, T. R.; Hohmeier, H. E.; Muoio, D. M.; Newgard, C. B.; Le, T. H. Increased insulin sensitivity in mice lacking collectrin, a downstream target of HNF-1alpha. *Mol Endocrinol*. 2009, **23** (6), 881-892.
- [52] Akpınar, P.; Kuwajima, S.; Krutzfeldt, J.; Stoffel, M. Tmem27: a cleaved and shed plasma membrane protein that stimulates pancreatic beta cell proliferation. *Cell Metab*. 2005, **2** (6), 385-397.
- [53] Symula, D. J.; Shedlovsky, A.; Dove, W. F. Genetic mapping of hph2, a mutation affecting amino acid transport in the mouse. *Mamm Genome*. 1997, **8** (2), 98-101.

- [54] Nash, S. R.; Giros, B.; Kingsmore, S. F.; Kim, K. M.; el-Mestikawy, S.; Dong, Q.; Fumagalli, F.; Seldin, M. F.; Caron, M. G. Cloning, gene structure and genomic localization of an orphan transporter from mouse kidney with six alternatively-spliced isoforms. *Receptors Channels*. 1998, **6** (2), 113-128.
- [55] Baba, T.; Nonoguchi, H.; Itoh, K.; Nakayama, Y.; Kohda, Y.; Inoue, T.; Tomita, K. Gene regulation of renal-osmotic stress-induced Na-CL organic solute cotransporter. *Nephron Exp Nephrol*. 2004, **96** (3), e89-96.
- [56] Obermuller, N.; Kranzlin, B.; Verma, R.; Gretz, N.; Kriz, W.; Witzgall, R. Renal osmotic stress-induced cotransporter: expression in the newborn, adult and post-ischemic rat kidney. *Kidney Int*. 1997, **52** (6), 1584-1592.
- [57] Quan, H.; Athirakul, K.; Wetsel, W. C.; Torres, G. E.; Stevens, R.; Chen, Y. T.; Coffman, T. M.; Caron, M. G. Hypertension and impaired glycine handling in mice lacking the orphan transporter XT2. *Mol Cell Biol*. 2004, **24** (10), 4166-4173.
- [58] Chesney, R. W. Iminoglycinuria. In *The Metabolic & Molecular Bases of Inherited Diseases* (Scriver, C. H., Beaudet, A. L., Sly, W. S.; Valle, D., eds.). 2001, pp. 4971-4982, McGraw-Hill, New York.
- [59] Broer, S.; Chesney, R. W. Iminoglycinuria. In *The Online Metabolic & Molecular Bases of Inherited Disease* ed.)^eds.). 2010, p.^pp. Chapter 194, McGraw-Hill
- [60] Eslami, B.; Kinboshi, M.; Inoue, S.; Harada, K.; Inoue, K.; Koizumi, A. A nonsense polymorphism (Y319X) of the solute carrier family 6 member 18 (SLC6A18) gene is not associated with hypertension and blood pressure in Japanese. *Tohoku J Exp Med*. 2006, **208** (1), 25-31.
- [61] Procopis, P. G.; Turner, B. Iminoaciduria: a benign renal tubular defect. *J Pediatr*. 1971, **79** (3), 419-422.
- [62] Yoon, Y. H.; Seol, S. Y.; Heo, J.; Chung, C. N.; Park, I. H.; Leem, S. H. Analysis of VNTRs in the solute carrier family 6, member 18 (SLC6A18) and lack of association with hypertension. *DNA Cell Biol*. 2008, **27** (10), 559-567.
- [63] Seol, S. Y.; Lee, S. Y.; Kim, Y. D.; Do, E. J.; Kwon, J. A.; Kim, S. I.; Chu, I. S.; Leem, S. H. Minisatellite polymorphisms of the SLC6A19: susceptibility in hypertension. *Biochem Biophys Res Commun*. 2008, **374** (4), 714-719.
- [64] Broer, S.; Palacin, M. The role of amino acid transporters in inherited and acquired diseases. *Biochem J*. 2011, **436** (2), 193-211.

- [65] Wasserman, J. C.; Delpire, E.; Tonidandel, W.; Kojima, R.; Gullans, S. R. Molecular characterization of ROSIT, a renal osmotic stress-induced Na(+)-Cl(-)-organic solute cotransporter. *Am J Physiol.* 1994, *267* (4 Pt 2), F688-694.
- [66] Takanaga, H.; Mackenzie, B.; Suzuki, Y.; Hediger, M. A. Identification of Mammalian Proline Transporter SIT1 (SLC6A20) with Characteristics of Classical System Imino. *J Biol Chem.* 2005, *280* (10), 8974-8984.
- [67] Kowalczyk, S.; Broer, A.; Munzinger, M.; Tietze, N.; Klingel, K.; Broer, S. Molecular cloning of the mouse IMINO system: an Na⁺- and Cl⁻-dependent proline transporter. *Biochem J.* 2005, *386* (Pt 3), 417-422.
- [68] Anas, M. K.; Lee, M. B.; Zhou, C.; Hammer, M. A.; Slow, S.; Karmouch, J.; Liu, X. J.; Broer, S.; Lever, M.; Baltz, J. M. SIT1 is a betaine/proline transporter that is activated in mouse eggs after fertilization and functions until the 2-cell stage. *Development.* 2008, *135* (24), 4123-4130.
- [69] Broer, A.; Balkrishna, S.; Kottra, G.; Davis, S.; Oakley, A.; Broer, S. Sodium translocation by the iminoglycinuria associated imino transporter (SLC6A20). *Mol Membr Biol.* 2009, *26* (5), 333-346.
- [70] Brodehl, J.; Gellissen, K. Endogenous renal transport of free amino acids in infancy and childhood. *Pediatrics.* 1968, *42* (3), 395-404.
- [71] Patterson, A. D.; Bonzo, J. A.; Li, F.; Krausz, K. W.; Eichler, G. S.; Aslam, S.; Tigno, X.; Weinstein, J. N.; Hansen, B. C.; Idle, J. R.; Gonzalez, F. J. Metabolomics reveals attenuation of the SLC6A20 kidney transporter in nonhuman primate and mouse models of type 2 diabetes mellitus. *J Biol Chem.* 2011, *286* (22), 19511-19522.
- [72] Uhl, G. R.; Kitayama, S.; Gregor, P.; Nanthakumar, E.; Persico, A.; Shimada, S. Neurotransmitter transporter family cDNAs in a rat midbrain library: 'orphan transporters' suggest sizable structural variations. *Brain Res Mol Brain Res.* 1992, *16* (3-4), 353-359.
- [73] Inoue, K.; Sato, K.; Tohyama, M.; Shimada, S.; Uhl, G. R. Widespread brain distribution of mRNA encoding the orphan neurotransmitter transporter v7-3. *Brain Res Mol Brain Res.* 1996, *37* (1-2), 217-223.
- [74] Masson, J.; Pohl, M.; Aidouni, Z.; Giros, B.; Hamon, M.; el Mestikawy, S. The two orphan Na⁺/Cl⁻-dependent transporters Rxt1 and V-7-3-2 have an overlapping expression pattern in the rat central nervous system. *Receptors Channels.* 1996, *4* (4), 227-242.
- [75] Masson, J.; Riad, M.; Chaudhry, F.; Darmon, M.; Aidouni, Z.; Conrath, M.; Giros, B.; Hamon, M.; Storm-Mathisen, J.; Descarries, L.; El Mestikawy, S. Unexpected localization of the Na⁺/Cl⁻-dependent-like orphan transporter, Rxt1, on synaptic vesicles in the rat central nervous system. *Eur J Neurosci.* 1999, *11* (4), 1349-1361.

- [76] Takanaga, H.; Mackenzie, B.; Peng, J. B.; Hediger, M. A. Characterization of a branched-chain amino-acid transporter SBAT1 (SLC6A15) that is expressed in human brain. *Biochem Biophys Res Commun.* 2005, **337** (3), 892-900.
- [77] Broer, A.; Tietze, N.; Kowalczyk, S.; Chubb, S.; Munzinger, M.; Bak, L. K.; Broer, S. The orphan transporter v7-3 (slc6a15) is a Na⁺-dependent neutral amino acid transporter (B0AT2). *Biochem J.* 2006, **393** (Pt 1), 421-430.
- [78] Yudkoff, M.; Daikhin, Y.; Nelson, D.; Nissim, I.; Erecinska, M. Neuronal metabolism of branched-chain amino acids: flux through the aminotransferase pathway in synaptosomes. *J Neurochem.* 1996, **66** (5), 2136-2145.
- [79] Yudkoff, M.; Nissim, I.; Kim, S.; Pleasure, D.; Hummeler, K.; Segal, S. [15N] leucine as a source of [15N] glutamate in organotypic cerebellar explants. *Biochem Biophys Res Commun.* 1983, **115** (1), 174-179.
- [80] Shank, R. P.; Bennett, G. S.; Freytag, S. O.; Campbell, G. L. Pyruvate carboxylase: an astrocyte-specific enzyme implicated in the replenishment of amino acid neurotransmitter pools. *Brain Res.* 1985, **329** (1-2), 364-367.
- [81] Di Bella, D.; Catalano, M.; Balling, U.; Smeraldi, E.; Lesch, K. P. Systematic screening for mutations in the coding region of the human serotonin transporter (5-HTT) gene using PCR and DGGE. *Am J Med Genet.* 1996, **67** (6), 541-545.
- [82] Kohli, M. A.; Lucae, S.; Saemann, P. G.; Schmidt, M. V.; Demirkan, A.; Hek, K.; Czamara, D.; Alexander, M.; Salyakina, D.; Ripke, S.; Hoehn, D.; Specht, M.; Menke, A.; Hennings, J.; Heck, A.; Wolf, C.; Ising, M.; Schreiber, S.; Czisch, M.; Muller, M. B.; Uhr, M.; Bettecken, T.; Becker, A.; Schramm, J.; Rietschel, M.; Maier, W.; Bradley, B.; Ressler, K. J.; Nothen, M. M.; Cichon, S.; Craig, I. W.; Breen, G.; Lewis, C. M.; Hofman, A.; Tiemeier, H.; van Duijn, C. M.; Holsboer, F.; Muller-Myhsok, B.; Binder, E. B. The Neuronal Transporter Gene SLC6A15 Confers Risk to Major Depression. *Neuron.* 2011, **70** (2), 252-265.
- [83] Drgonova, J.; Liu, Q. R.; Hall, F. S.; Krieger, R. M.; Uhl, G. R. Deletion of v7-3 (SLC6A15) transporter allows assessment of its roles in synaptosomal proline uptake, leucine uptake and behaviors. *Brain Res.* 2007, **1183** 10-20.
- [84] Liu, Q. R.; Mandiyan, S.; Lopez-Corcuera, B.; Nelson, H.; Nelson, N. A rat brain cDNA encoding the neurotransmitter transporter with an unusual structure. *FEBS Lett.* 1993, **315** (2), 114-118.
- [85] el Mestikawy, S.; Giros, B.; Pohl, M.; Hamon, M.; Kingsmore, S. F.; Seldin, M. F.; Caron, M. G. Characterization of an atypical member of the Na⁺/Cl⁻-dependent transporter family: chromosomal localization and distribution in GABAergic and glutamatergic neurons in the rat brain. *J Neurochem.* 1994, **62** (2), 445-455.

- [86] Masson, J.; Gaspar, P.; Aidouni, Z.; Ezan, P.; Giros, B.; Hamon, M.; El Mestikawy, S. Ontogeny of Rxt1, a vesicular "orphan" Na⁺/Cl⁻-dependent transporter, in the rat. *Neuroscience*. 2000, *96* (3), 627-637.
- [87] Jursky, F.; Nelson, N. Developmental expression of the neurotransmitter transporter NTT4. *J Neurosci Res*. 1999, *55* (1), 24-35.
- [88] Zaia, K. A.; Reimer, R. J. Synaptic Vesicle Protein NTT4/XT1 (SLC6A17) Catalyzes Na⁺-coupled Neutral Amino Acid Transport. *J Biol Chem*. 2009, *284* (13), 8439-8448.
- [89] Parra, L. A.; Baust, T.; El Mestikawy, S.; Quiroz, M.; Hoffman, B.; Haflett, J. M.; Yao, J. K.; Torres, G. E. The orphan transporter Rxt1/NTT4 (SLC6A17) functions as a synaptic vesicle amino acid transporter selective for proline, glycine, leucine, and alanine. *Mol Pharmacol*. 2008, *74* (6), 1521-1532.
- [90] Farmer, M. K.; Robbins, M. J.; Medhurst, A. D.; Campbell, D. A.; Ellington, K.; Duckworth, M.; Brown, A. M.; Middlemiss, D. N.; Price, G. W.; Pangalos, M. N. Cloning and characterization of human NTT5 and v7-3: two orphan transporters of the Na⁺/Cl⁻-dependent neurotransmitter transporter gene family. *Genomics*. 2000, *70* (2), 241-252.
- [91] Fremeau, R. T., Jr.; Caron, M. G.; Blakely, R. D. Molecular cloning and expression of a high affinity L-proline transporter expressed in putative glutamatergic pathways of rat brain. *Neuron*. 1992, *8* (5), 915-926.
- [92] Renick, S. E.; Kleven, D. T.; Chan, J.; Stenius, K.; Milner, T. A.; Pickel, V. M.; Fremeau, R. T., Jr. The mammalian brain high-affinity L-proline transporter is enriched preferentially in synaptic vesicles in a subpopulation of excitatory nerve terminals in rat forebrain. *J Neurosci*. 1999, *19* (1), 21-33.
- [93] Crump, F. T.; Fremeau, R. T.; Craig, A. M. Localization of the brain-specific high-affinity L-proline transporter in cultured hippocampal neurons: molecular heterogeneity of synaptic terminals. *Mol Cell Neurosci*. 1999, *13* (1), 25-39.
- [94] Shafqat, S.; Velaz-Faircloth, M.; Henzi, V. A.; Whitney, K. D.; Yang-Feng, T. L.; Seldin, M. F.; Fremeau, R. T., Jr. Human brain-specific L-proline transporter: molecular cloning, functional expression, and chromosomal localization of the gene in human and mouse genomes. *Mol Pharmacol*. 1995, *48* (2), 219-229.
- [95] Fremeau, R. T., Jr.; Velaz-Faircloth, M.; Miller, J. W.; Henzi, V. A.; Cohen, S. M.; Nadler, J. V.; Shafqat, S.; Blakely, R. D.; Domin, B. A novel nonopioid action of enkephalins: competitive inhibition of the mammalian brain high affinity L-proline transporter. *Mol Pharmacol*. 1996, *49* (6), 1033-1041.

- [96] Yu, X. C.; Zhang, W.; Oldham, A.; Buxton, E.; Patel, S.; Nghi, N.; Tran, D.; Lanthorn, T. H.; Bomont, C.; Shi, Z. C.; Liu, Q. Discovery and characterization of potent small molecule inhibitors of the high affinity proline transporter. *Neurosci Lett*. 2009, **451** (3), 212-216.
- [97] Notredame, C.; Higgins, D. G.; Heringa, J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol*. 2000, **302** (1), 205-217.
- [98] Kleta, R.; Romeo, E.; Ristic, Z.; Ohura, T.; Stuart, C.; Arcos-Burgos, M.; Dave, M. H.; Wagner, C. A.; Camargo, S. R.; Inoue, S.; Matsuura, N.; Helip-Wooley, A.; Bockenhauer, D.; Warth, R.; Bernardini, I.; Visser, G.; Eggermann, T.; Lee, P.; Chairoungdua, A.; Jutabha, P.; Babu, E.; Nilwarangkoon, S.; Anzai, N.; Kanai, Y.; Verrey, F.; Gahl, W. A.; Koizumi, A. Mutations in SLC6A19, encoding B0AT1, cause Hartnup disorder. *Nat Genet*. 2004, **36** (9), 999-1002.
- [99] Azmanov, D. N.; Kowalczyk, S.; Rodgers, H.; Auray-Blais, C.; Giguere, R.; Rasko, J. E.; Broer, S.; Cavanaugh, J. A. Further evidence for allelic heterogeneity in Hartnup disorder. *Hum Mutat*. 2008, **29** (10), 1217-1221.
- [100] Seow, H. F.; Broer, S.; Broer, A.; Bailey, C. G.; Potter, S. J.; Cavanaugh, J. A.; Rasko, J. E. Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter SLC6A19. *Nat Genet*. 2004, **36** (9), 1003-1007.
- [101] Cheon, C. K.; Lee, B. H.; Ko, J. M.; Kim, H. J.; Yoo, H. W. Novel mutation in SLC6A19 causing late-onset seizures in Hartnup disorder. *Pediatr. Neurol*. 2010, **42** (5), 369-371.

Figure legends:

Figure 1: Dendrogram of the human SLC6 family.

Reference peptide sequences of the human SLC6 family were aligned using T-coffee [97]. Distances were plotted as a dendrogram. The GABA transporter branch is highlighted in red, the monoamine transporter branch in orange, the amino acid transporter branch (I) in purple and the amino acid transporter branch (II) in green.

Figure 2: Depiction of the 5+5 inverted repeat fold of the SLC6 family.

(A) Transmembrane helices 1-10 of LeuT shown in blue (1-5) and yellow (6-10) to emphasize the inverted repeat fold. (B,C) Helices 1-5 were rotated to show the similarity of the two repeats. Helix 1 and 6 can be identified by the interruption of the helix in the center. (D) The rotated repeats were merged to show the close overlap. Note the deviation between helix 1,2 and 6,7.

Figure 3: Putative Transport cycle of SLC6 family transporters

The transporter is open to the outside (A) allowing the substrate to bind (B). Binding of substrate causes small conformational changes of neighboring residues, which enclose the substrate (C). These changes trigger a large conformational change, which occludes the substrate in the center (D). Subsequently, the transporter opens to the inside, while the substrate is still enclosed (E). Small conformational changes of nearby residues (F) allow the release of the substrate (G). The transporter returns to its original conformation without substrate (H). Ionic interactions between mobile parts of the protein are important to facilitate the conformational change.

Figure 4: Neutral amino acid transport in the kidney

The bulk of neutral amino acids in mouse and human kidney is transported by B⁰AT1. Surface expression requires interaction with collectrin. In mouse kidney B⁰AT3 transports small neutral amino acids and IMINO transports proline. IMINO also requires collectrin for surface expression. In humans B⁰AT3 is mutated and its function is replaced by PAT2. Glycinuria in mice is caused by lack of B⁰AT3, while it is caused by PAT2 haploinsufficiency in humans. Iminoglycinuria is caused by lack of PAT2 or combined mutations in PAT2 and IMINO.

Figure 5: The role of SLC6 amino acid transporters in the brain

Branched-chain amino acids (BCAA) and proline are taken up into neurons by B⁰AT2. Proline can be converted to glutamate or is sequestered into synaptic vesicles by PROT and NTT4. Branched-chain amino acids are converted into TCA cycle intermediates or used to produce acetyl-CoA.

Table 1: Residues involved in ion binding in the SLC6 amino acid transporters.

Residues that could disrupt ion-binding site function are indicated in grey. Atoms involved in binding are shown in the last row.

Transporter	Na ₁	Na ₁	Na ₁	Na ₁	Na ₂	Na ₂	Na ₂	Na ₂	Na ₂	Cl	Cl	Cl	Cl	Cl
LeuT	A22	N27	T254	N286	G20	V23	A351	T354	S355	Y47	Q250	T254	N286	E290
B ⁰ AT1	C49	N54	S278	N310	G47	V50	L427	S430	S431	F74	Q274	S278	N310	S314
B ⁰ AT2	S78	N83	A309	N341	G76	V79	L470	G473	S474	Y103	Q305	A309	N341	S345
B ⁰ AT3	A35	N40	S264	N296	G33	V36	L413	S416	T417	Y60	Q260	S264	N296	S300
ATB ^{0,+}	A53	N58	S322	N354	G51	V54	L419	D422	S423	Y78	Q318	S322	N354	S358
NTT4	S77	N82	A308	N340	G75	V78	L469	G472	S473	Y102	Q304	A308	N340	S344
NTT5	S120	C125	N349	N381	G118	M121	M508	G511	S512	Y145	Q345	N349	N381	L385
IMINO	A22	N27	S251	N283	S20	V23	L402	G405	S406	Y47	Q247	S251	N283	S287
PROT	C54	N59	S298	N330	G52	V55	L395	D398	S399	Y79	Q294	S298	N330	S334
Atom	O	O δ	O,O γ	O δ	O	O	O	O γ	O γ	O ϵ -H	N ϵ -H	O γ -H	N δ -H	O γ -H

Table 2: Mutations associated with Hartnup disorder

Mutation (DNA)	Mutation (protein)	Frequency	Reference
Missense			
169C>T	R57C	n.r.	[98]
196G>A	G66R	<0.001	[99]
205G>A	A69T	n.r.	[36]
277G>A	G93R	<0.001	[99]
517G>A	D173N	0.004-0.007	[100]
532C>T	R178X	<0.001	[99]
719G>A	R240Q	<0.001	[100]
725T>C	L242P	n.r.	[100]
794C>T	P265L	n.r.	[36]
850G>A	G284R	<0.001	[99]
908C>T	S303L	n.r.	[101]
982C>T	R328C	<0.001	[99]
1213G>A	E405K	<0.001	[99]
1501G>A	E501K	n.r.	[100]
1550A>G	D517G	<0.001	[99]
1735C>T	P579L	n.r.	[36]
Nonsense			
682-683AC>TA	T228X	n.r.	[98]
718C>T	R240X	0.001	[100]
Deletions			
340delC	L114fsX114	n.r.	[98]
c884_885delTG	V295fsX351	n.r.	[98]
Insertions			
1787_1788insG	Thr596fsX73	n.r.	[101]
Splice site			
IVS8 + 2G	Aberrant splicing	<0.01	[100]
IVS11 + 1A	Aberrant splicing	n.r.	[100]

n.r.: not reported

Table 3: Mutations and polymorphisms associated with Iminoglycinuria (IG) and Glycinuria (G)

Mutation (DNA)	Gene	Mutation (Protein)	Frequency	Reference	Comment
IVS1+1G>A	SLC36A2	Protein truncation	0.004	[50]	IG (homozygous), G(heterozygous)
260G>T	SLC36A2	G87V	0.012	[50]	IG in combination with T199M
596C>T	SLC6A20	T199M	0.075	[50]	IG in combination with G87V
235G>A	SLC6A18	G79S	n.r.	[50]	Role unclear
957C>G	SLC6A18	Y318X	0.44	[50]	Role unclear
1433T>C	SLC6A18	L478P	0.39	[50]	Role unclear
1486G>A	SLC6A18	G496R	0.003	[50]	Role unclear

n.r.: not reported

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5