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SLC17: A functionally diverse family of organic anion transporters

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Abstract

Molecular studies have determined that the SLC17 transporters, a family of nine proteins initially implicated in phosphate transport, mediate the transport of organic anions. While their role in phosphate transport remains uncertain, it is now clear that the transport of organic anions facilitated by this family of proteins is involved in diverse processes ranging from the vesicular storage of the neurotransmitters, to urate metabolism, to the degradation and metabolism of glycoproteins.

Keywords

SLC17; type I phosphate transporter; vesicular glutamate transporter; sialin; neurotransmitter transporter; vesicular nucleotide transporter; organic anion transport

1. Phylogeny of the SLC17 family

The SLC17 family of transporters is a group of nine structurally related proteins (Figure 1) that mediate the transmembrane transport of organic anions. The first characterized members of this family, the type I phosphate transporters (SLC17A1–4), were initially identified as Na⁺-dependent inorganic phosphate (P_i) transporters. More recent work has, however, determined the type I phosphate transporters are involved in the transport of organic anions. Other identified mammalian members of this family include a lysosomal acidic sugar transporter (sialin; SLC17A5), vesicular glutamate transporters (VGLUT1–3; SLC17A7, SLC17A6, and SLC17A8 respectively), and a vesicular nucleotide transporter (VNUT; SLC17A9). These proteins are integral membrane proteins with 12 predicted transmembrane domains that reside on synaptic vesicles (the VLGUTs and VNUT), lysosomes (sialin) and the plasma membrane (type I phosphate transporters).

The SLC17 family is part of the larger group of transporters found in prokaryotes and eukaryotes that make up the Anion:Cation Symporter Family within the Major Facilitator Superfamily (TCID 2.A.1.14; see <http://www.tcdb.org/>). The SLC17 proteins are not structural similar to the other vesicular neurotransmitter transporter families, other lysosomal transport proteins, other phosphate transporters, or other organic anion transporters. The Anion:Cation Symporter Family includes bacterial proteins that are

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putative transporters for organic anions including anionic sugars and phthalate and transporters in plants and fungi including the *Arabidopsis thaliana* anion transporter 1 ANTR1.

2. SLC17A1–4: The type I phosphate transporters

2.1. History of Identification and Cloning

The first SLC17 family member to be characterized, SLC17A1, was identified through a functional screen for Na⁺-dependent inorganic phosphate (P_i) transporters (Murer et al., 2000). The screen led to isolation of a sequence encoding a 465 amino acid protein that was designated NaP_i-1. A human orthologue of NaP_i-1 and three other closely related proteins have been identified through genomic analysis, and designated NPT1 (SLC17A1/NaP_i-1), NPT3 (SLC17A2), NPT4 (SLC17A3), and NPT5 (SLC17A4) (Miyamoto et al., 2011). NPT2 is actually a type II transporter (SLC34A1) and structurally unrelated to the SLC17 proteins.

2.2. Transport and Pharmacology

Although NaP_i-1 was cloned in a screen for P_i transport, it is unlikely that this is its primary function (Murer et al., 2000). The measured affinity for P_i transport by heterologously expressed SLC17A1 (~1mM) is much lower than for transport by native tissues (~0.1 mM). Two other structurally unrelated families of phosphate transporters (type II and type III) that mediate phosphate uptake with higher affinity have been identified and are considered the primary mammalian Na⁺ dependent phosphate transporters *in vivo* (Murer et al., 2000). Further, the type I phosphate transporters have been shown to transport organic anions (Miyamoto et al., 2011). In oocytes expressing SLC17A1 the transport of probenecid and penicillin, but not P_i, correlate directly with expression of the recombinant protein. The transport of organic anions is electrogenic with affinities in the submillimolar range. In a reconstituted system, proteoliposomes containing SLC17A1 have been shown to exhibit Cl⁻ dependent transport of *p*-aminohippurate and urate that is competitively inhibited by salicylate and acetylsalicylate (aspirin). SLC17A1 has also been shown to mediate an inorganic anion conductance with the apparent selectivity of I⁻>Br⁻>Cl⁻ (Murer et al., 2000). This inorganic ion conductance is inhibited by the organic ion transport substrates with high affinity - for benzylpenicillin, the EC₅₀ is 10-fold lower than the apparent K_m for transport, suggesting that organic ion transport negatively regulates the inorganic ion conductance. Recently, SLC17A3/NPT4 has been shown to mediate voltage dependent transport a number of organic anions including urate (Jutabha et al., 2010). The loop diuretics bumetanide and furosemide cause a dose dependent inhibition of SLC17A3-mediated urate uptake (Jutabha et al., 2010). As of yet, SLC17A2 and SLC17A4 have not been functionally characterized.

2.3. Expression and Regulation

Expression of the SLC17A1–4 proteins is relatively restricted. SLC17A1 is expressed in the brush border membrane of the proximal tubule in the kidney and to a lesser extent the sinusoidal membrane of hepatocytes in the liver (Biber et al., 1993; Yabuuchi et al., 1998). SLC17A3 is also expressed in the kidney and localized to the apical side of renal tubules (Jutabha et al., 2010). *SLC17A2* mRNA is expressed in muscle, liver, and kidney and SLC17A4 is expressed in kidney, livers, stomach and intestine (Sreedharan et al., 2010). Hepatocyte nuclear factor 1 alpha (HNF1α) has been shown to upregulate *SLC17A1* and *SLC17A3* expression and to alter their splicing patterns (Cheret et al., 2002). For *SLC17A3* the shorter splice variant appears to be non-functional (Jutabha et al., 2010), but the physiological significance has not been determined for *SLC17A1* splicing.

2.4. Physiology

The role of the SLC17A1–4 transporters remains only partially understood. This reflects, in part, the lack of functional data for SLC17A2 and SLC17A4. SLC17A1 and SLC17A3 appear to have a role in the transport of organic anions, in particular, urate and *para*-aminohippurate. The localization of SLC17A1 at the sinusoidal membrane of hepatocytes and SLC17A1 and SLC17A3 to the apical surfaces of renal tubule epithelium and the electrogenic nature of their organic anion transport activities suggest that they have a role in hepatic and renal clearance of organic anions including urate. Although other transporters also recognize urate, the association of polymorphisms in the *SLC17A1* and *SLC17A3* genes with elevated blood urate levels and gout (see below) indicates a crucial role for these transporters in this process. Additionally, these transporters may have roles in metabolism and secretion of drugs including aspirin and β -lactam antibiotics (Iharada et al., 2010; Jutabha et al., 2010).

2.5. Genetics and animal models

The four related genes (*SLC17A1–4*) all localize to 6p21.3-p23, which is part of the extended major histocompatibility complex (MHC) region. Polymorphisms in the *SLC17A1* and *SLC17A3* genes have been associated with risk for gout (Dehghan et al., 2008; Kolz et al., 2009). A recent study indicates that common polymorphisms within the *SLC17A1–4* region are associated with schizophrenia, but the significance is unclear (Shi et al., 2009). There are no reported animal models for deficiency of any of the SLC17A1–4 proteins.

3. SLC17A5: Sialin

3.1. History of Identification, Genetics, and Cloning

The identification of free sialic acid as the material accumulated in the enlarged lysosomes of individuals with sialic acid storage disorders (Salla Disease and infantile sialic acid storage disease (ISSD)) led to the characterization of a transport system that recognizes sialic acids and several additional acidic sugars including glucuronic acid (Aula, 2001). Genetic linkage studies of patients with Salla Disease identified a gene associated with the disease on chromosome 6q14-q15 encoding a single protein. The protein, designated sialin, is ubiquitously expressed. Over a dozen disease-causing mutations have now been identified in the sialin gene (*SLC17A5*). The most common mutation, R39C, causes Salla Disease, which is characterized by developmental delay, ataxia and marked cognitive impairment with intelligence quotients of 20–30, but life expectancy into adulthood. The other identified mutations are associated with dysmorphic features, more severe neurologic symptoms and death by 2 years of age.

3.2. Transport and Pharmacology

The native lysosomal sialic acid transport activity saturates with an apparent K_m of ~0.24 mM and depends on the transmembrane pH gradient (Aula, 2001). Transport is not influenced by the membrane potential or any inorganic ion gradients, suggesting a H^+ coupled electroneutral process with a 1:1 (sialic acid: H^+) stoichiometry. In addition to sialic acid, monocarboxylic acids, including lactate, glucuronic acid and gluconate, but not neutral sugars such as glucose and mannose are recognized by the transporter. Although the initial assays measured by the uptake of radiolabelled sialic acid into lysosomes, the biologically relevant process is thought to be efflux, with the H^+ electrochemical gradient ($\Delta\mu H^+$) generated by the lysosomal H^+ -ATPase as the driving force. Studies using whole cell uptake assays with the recombinantly expressed sialin targeted to the plasma membrane have demonstrated transport with characteristics similar to those of the activity described in

lysosomes (Morin et al., 2004; Wreden et al., 2005). Specific inhibitors for sialin mediated sugar transport have not been reported.

Recent studies have suggested that sialin also transports aspartate and glutamate (Miyaji et al., 2008). The detected transport activity is dependent on the electrical gradient. This finding suggests that sialin mediates uptake of glutamate and aspartate into synaptic vesicles as well as transporting sialic acid and glucuronic acid out of lysosomes. However, these studies have been carried out on purified and reconstituted recombinantly expressed sialin, and whether sialin mediates transport of aspartate and/or glutamate into synaptic vesicles remains unclear.

3.3. Expression, Trafficking and Regulation

Sialin is expressed in all tissues (Aula, 2001). Targeting to lysosomes requires a di-leucine based motif in the amino-terminus of the protein that is similar to motifs required for adaptor protein mediated targeting of a number of lysosomal proteins (Morin et al., 2004; Wreden et al., 2005). Sialin is part of a coordinated transcriptional network of lysosomal proteins that is regulated by the transcription factor TFEB (Sardiello et al., 2009) and in cell culture experiments, hypoxia upregulates sialin (Yin et al., 2006).

3.4. Physiology

Sialic acids and glucuronic acid are incorporated in O-linked chains and complex N-linked chains of glycoproteins and are present in glycolipids (Alberts et al., 2008). After these acidic sugars are removed in the lysosome as part of an intrinsic turnover process of cellular macromolecules, sialin mediates their transport out of the lysosome and into the cytoplasm where they can be further metabolized or re-incorporated into newly synthesized glycoproteins and glycolipids. Loss of sialin function leads to lysosomal accumulation of the sugars and a defect in lysosomal maturation and function (Prolo et al., 2009).

3.5. Animal model

Mice lacking sialin recapitulate the primary characteristics of the human lysosomal free sialic acid storage disorders (Prolo et al., 2009). The *Slc17a5*^{-/-} mice exhibit poor coordination, seizures, and premature death. Prominent clear cytoplasmic vacuoles similar to those seen in the human disease are present in neurons in the brains of the *Slc17a5*^{-/-} mice. There is also a loss of the central nervous myelin formation that appears to be due to a defect in maturation of cells in the oligodendrocyte lineage.

4. SLC17A6–8: The vesicular glutamate transporters

4.1. History of Identification and Cloning

Glutamate is the primary excitatory neurotransmitter in the mammalian nervous system. Release of glutamate at synapses requires its transport into synaptic vesicles that fuse with the plasma membrane and release their contents during synaptic transmission. *SLC17A7* encoding VGLUT1 was identified in a screen for cDNAs upregulated in cerebellar granule cells in response to subtoxic levels of the glutamate receptor agonist NMDA (Ni et al., 1994) and *SLC17A6* encoding VGLUT2 was isolated in a screen for cDNAs upregulated during differentiation of rat pancreatic AR42J cells to a neuroendocrine phenotype (Aihara et al., 2000). The structural similarity to SLC17A1/NaPi-1 (~32% identity) led to an initial focus on phosphate transport for both proteins. Although heterologous expression of these proteins in *Xenopus* oocytes increases Na⁺-dependent phosphate uptake, both proteins localize exclusively to synaptic vesicles in glutamatergic neurons and transport glutamate into vesicles indicating that vesicular glutamate transport is their primary physiological role (Reimer and Edwards, 2004). A third protein, VGLUT3 (encoded by *SLC17A8*) with 78%

identity to VGLUT1 and 74% identity to VGLUT2 was identified by homology screens and also shown to mediate vesicular glutamate transport.

4.2. Transport and Pharmacology

The synaptic vesicle glutamate transport activity and the activities of three VGLUT isoforms when heterologously expressed exhibit have K_m values of ~1 mM and are driven primarily by the electrical component ($\Delta\Psi$) of the proton electrochemical gradient across the vesicle membrane (Reimer and Edwards, 2004). Residual transport in the absence of $\Delta\Psi$ (but presence of $\Delta\pi H$) suggests that transport can be coupled to proton exchange. Transport is absolutely dependent upon Cl^- , however, Cl^- also dissipates $\Delta\Psi$ in synaptic vesicles creating a biphasic dependence. VGLUT1 has also been shown to mediate a chloride conductance, but the role of Cl^- is an allosteric modulator and substrate remains unclear (Schenck et al., 2009; Juge et al., 2010).

The VGLUTs have a striking substrate preference. Unlike the Na^+ -dependent plasma membrane excitatory amino acid transporters, the VGLUTs do not recognize aspartate and have a marked preference for L-glutamate over D-glutamate. Inhibition studies have identified Evans Blue and Chicago Sky Blue, and the fluorescein derivative Rose Bengal as vesicular glutamate transport inhibitors (Shigeri et al., 2004). Other inhibitors that have been identified include several glutamate analogues and substituted quinoline-2,4-dicarboxylic acids and the anion channel blocker DIDS. Two endogenous factors have been shown to influence vesicular glutamate transport - a calpain-derived fragment of α -fodrin that has been designated IPF for inhibitory protein factor (Ozkan et al., 1997) and ketone bodies (Juge et al., 2010). The very low IC_{50} (26 nM) for IPF suggests a biological role, but because IPF also inhibits GABA transport into synaptic vesicles this role is not specific to regulation of VGLUTs. The ketone bodies acetoacetate and β -hydroxybutyrate block the activation of VGLUTs by chloride at physiological concentrations suggesting a specific physiologically relevant effect on VGLUT function (Juge et al., 2010).

4.3 Expression

VGLUT1 and VGLUT2 exhibit a complementary distribution that includes nearly all glutamatergic neurons (Reimer and Edwards, 2004). All cerebral cortical layers express VGLUT1, whereas only layer IV of frontal and parietal cortex and layers IV and VI of temporal cortex express VGLUT2. In the hippocampus, dentate gyrus granule cells contain only *SLC17A7* mRNA whereas pyramidal neurons from CA1 through CA3 also express abundant *SLC17A7*, and low levels of *SLC17A6*. In the amygdala, the medial and central nuclei contain abundant mRNA for *SLC17A6*, whereas the lateral and basolateral nuclei express *SLC17A7*. The thalamus expresses much more VGLUT2 than VGLUT1, but certain thalamic nuclei express VGLUT1. The predominant isoform expressed by brainstem and deep cerebellar nuclei is VGLUT2, whereas cerebellar cortex expresses VGLUT1. Both VGLUT1 and VGLUT2 are also expressed in some ACh and GABA neurons and VGLUT2 is also expressed in the dopaminergic neurons of the ventral tegmental area (El Mestikawy et al., 2011). Co-storage of glutamate appears to increase the capacity for dopamine storage in the synaptic vesicles (Hnasko et al., 2010).

VGLUT3 expression extends to neurons not classically considered glutamatergic (Reimer and Edwards, 2004). Inhibitory cells in layer II of the parietal cortex and stratum radiatum of CA1-CA3 of the hippocampus express VGLUT3 as do dopaminergic cells in the substantia nigra pars compacta and ventral tegmental area, serotonergic cells in the dorsal raphe, neurons in the pontine raphe nuclei and olivary nuclei, and cholinergic interneurons in the dorsal striatum express. In the cerebellum, the granule cell layer labels most strongly for VGLUT3, but the molecular layer also contains scattered hybridizing cells. Interestingly,

immunoelectron microscopy also demonstrates that VGLUT3 occurs in astrocytes, on both processes surrounding synapses and endfeet abutting capillaries.

All three VGLUTs are expressed outside the central nervous system (Reimer and Edwards, 2004). VGLUT2 has been identified in intrinsic and extrinsic primary afferent neurons of the gut, α and β cells in the pancreatic islets, and pinealocytes. VGLUT1 has been identified in β cells of the pancreas. The expression of VGLUT1 and VGLUT2 on secretory vesicles and the regulation of transport activity and VGLUT2 mRNA by glucose levels in cultured pancreatic cells is consistent with a role for glutamate in intercellular signaling in the pancreas and glucose regulation. VGLUT3 is expressed in the liver and to a lesser extent the kidney.

4.4. Trafficking and Regulation

All VGLUT isoforms localize to synaptic vesicles, but each appears to have a different distribution among other cell membranes (Voglmaier and Edwards, 2007). VGLUT1 segregates most closely with synaptic vesicle markers and to a lesser extent the plasma membrane. VGLUT2 cofractionates with synaptic vesicle and plasma membrane markers as well as other light membranes lighter. In addition to localizing to synaptic vesicles, VGLUT3 is present in dendrites. Dendritic localization of VGLUT3 is thought to be crucial for retrograde signaling at some synapses. Part of the mechanism regulating trafficking of VGLUT1 has been uncovered. A poly-proline domain unique to the carboxy-terminus of VGLUT1 mediates an interaction with endophilin and regulates vesicle recycling and release (Voglmaier and Edwards, 2007).

4.5. Animal models, physiology, and human genetics

Invertebrate and vertebrate animals models have demonstrated that the VGLUTs are required for vesicular accumulation and synaptic release of glutamate and that their expression can influence quantal size. Although *Slc17a7* knockout mice typically live only until weaned, they can be kept alive longer with aggressive care, but surviving animals are blind (Freneau et al., 2004). *Slc17a6* knockout mice die at birth, but studies of heterozygous *Slc17a6* knockouts and conditional *Slc17a6* knockouts have demonstrated roles for VGLUT2 in memory, pain, itch, and reward (Wallen-Mackenzie et al., 2010). *Slc17a8* knockout mice are deaf and have seizures as well as altered pain sensation (Ruel et al., 2008; Seal et al., 2008; Seal et al., 2009).

The genes encoding the three VGLUTs are segregated on different human chromosomes – *SLC17A6* (VGLUT2) is localized to 11p14.3, *SLC17A7* (VGLUT1) to 19q13, and *SLC17A8* (VGLUT3) to 12q23.1. No association to human disease has been confirmed for the *SLC17A7* or *SLC17A6* genes, but mutations in *SLC17A8* (VGLUT3) have been associated with nonsyndromic deafness at the *DFNA25* locus (Ruel et al., 2008). A single mutation predicted to cause a substitution of a highly conserved alanine at residue 211 with a valine has been identified in *DFNA25* deafness. Interestingly, the disease is inherited in an autosomal dominant fashion and mice heterozygous for deletion of *Slc17a8* have normal hearing, suggesting that the mutation is behaving in a dominant negative fashion (Ruel et al., 2008; Seal et al., 2008).

5. SLC17A9: The Vesicular Nucleotide Transporter (VNUT)

5.1. History of Identification, Genetics, and Cloning

Studies demonstrating uptake of ATP into chromaffin granule vesicles and synaptic vesicles indicated that vesicular exocytosis is a primary mechanism for release of purines as signaling molecules. Although the vesicular nucleotide transport activity had been studied

for several decades, the protein mediating this activity was only identified in 2008 when a genomic DNA database screen for sequences encoding proteins with structural similarity to other SLC17 family members identified *SLC17A9* on chromosome 20 (Sawada et al., 2008). A role for SLC17A9 in vesicular ATP transport was predicted by its expression in chromaffin granule cells in the adrenal gland and confirmed by functional characterization of the recombinant protein.

5.2. Transport and pharmacology

Biochemical studies of chromaffin granules and synaptic vesicles identified a specific concentrative transport activity for purines (Luqmani, 1981; Gualix et al., 1999). A similar activity has shown to be mediated by purified VNUT reconstituted into proteoliposomes (Sawada et al., 2008). With the reconstituted protein ATP has an apparent K_m in the low millimolar range and is inhibited by GTP, UTP, ITP and ATP γ S, as well as DIDS, Evans Blue, and atractyloside. Transport is also dependent upon Cl^- with saturating effect at ~4 mM.

5.3. Expression

VNUT is expressed in several tissues in mammals, most prominently in the chromaffin cells of the adrenal gland, brain, and thyroid gland (Sawada et al., 2008). In the brain it is present in synaptic vesicles of neurons with highest levels in cerebellar cortex, the olfactory bulb, and the hippocampus (Larsson et al., 2012). T lymphocytes and biliary epithelial cells have also been shown to express VNUT and release ATP (Tokunaga et al.; Sathe et al.).

6. Transport models

The SLC17 family members share a similar structure. They are predicted to have 12 transmembrane segments with the amino and carboxy termini located in the cytoplasm. The greatest divergence among the proteins occurs in the cytoplasmic termini and the first luminal loop. Homology modeling with the glycerol phosphate transporter GIPT and site directed mutagenesis have been used to identify residues that are likely involved in transport process in VGLUT1, VGLUT2, NaPi-1, and sialin (Juge et al., 2006; Almqvist et al., 2007; Courville et al., 2010; Iharada et al., 2010). A number of titrable residues within transmembrane segments are highly conserved within the family, suggesting some conservation of the overall transport process. Analysis of mutations have demonstrated that an Arg residue in TM4 is required for both VGLUT2 and sialin, and that a His residue in TM2 that is unique to the VGLUTs is required for VGLUT2 function. A GXXXG motif in TM4 is conserved throughout the family and has been shown to be required for sialin, suggesting that tight helix-helix packing involving TM4 is common to all members of the family. Comparison of all SLC17 sequences across vertebrate species indicates that there are a number of additional residues that are absolutely conserved (Figure 2). Many of these residues reside within the predicted membranous segments.

The unifying function of the SLC17 family of transporters is organic anion transport. The coupling of organic ion transport to inorganic ions appears to be variable. Sialin uses proton co-transport to drive transmembrane movement of acidic sugars, while urate transport by SLC17A1 and SLC17A3, glutamate transport by the VGLUTs, and ATP transport by VNUT. The characterized transporters all exhibit dependence on Cl^- appears with the exception of sialin.

7. Medical Relevance

No drugs targeting SLC17 family members are currently used to treat human diseases. However, given their functions (Figure 3), the potential for pharmacological targeting is great and the implications diverse.

The association of polymorphisms in *SLC17A1* /NPT1 and *SLC17A3*/NPT4 and their functional characterization as urate transporters suggest that manipulations that increase their expression or activity might be useful for the treatment of hyperuricemia and gout. Alternatively, their role in secretion of drugs such as antibiotics and anti-inflammatory drugs suggests that inhibiting their activity might enhance the bioavailability of these drugs.

Impairment of *SLC17A5*/sialin function is associated with a lysosomal storage disorder and inhibitors of sialin are unlikely to be of benefit as pharmaceutical agents. However, data suggesting that sialin may be mislocalized in Salla disease and in one mutation associated with ISSD, indicates that if the mutated proteins are still functional, drugs that increase their trafficking to the lysosome may be of interest. Similarly compounds that increase expression of mutant proteins that have reduced, but not absent activity might also be of clinical utility.

The vesicular glutamate transporters (SLC17A6-8) are ideal targets for attenuating glutamateric neurotransmission. Reducing their activity should reduce storage of glutamate in synaptic vesicles and its subsequent release. This could be useful for the treatment of neurodegenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's disease and Huntington's disease in which chronic excessive glutamate signaling (excitotoxicity) has been implicated, as well as for treatment of epilepsy and pain. Several compounds have been shown to block synaptic vesicle glutamate uptake, but efficacy may be limited by delivery to the central nervous system and synaptic vesicles. Among the most promising compounds are Rose Bengal and its derivatives, which appear to be membrane permeant (Thompson et al., 2005; Pietrancosta et al., 2010; Ahmed et al., 2011).

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Figure 1. Phylogenetic tree with human isoforms of the SLC17 family

Computer-based phylogenetic analysis of the identified members of the SLC17 family of proteins illustrates four distinct branches. There is a high degree of similarity among the VGLUT sequences (74–82% identity) and sialin and VNUT are more closely related to the VGLUTs (38% identity and 57% similarity over 489 amino acids for VGLUT1 (SLC17A7) and sialin (SLC17A5) and 27% identity and 47% similarity over 436 amino acids for VGLUT1 and VNUT (SLC17A9)) than to the type I phosphate transporters (37% identity and 54% similarity between over 467 amino acids for NPT1 (SLC17A1) and sialin and 24% identity and 43% similarity over 397 amino acids for VNUT and NPT1= 170/397 (43%)). The tree was generated using the AllAll program of the Computational Biochemistry Research Group, Swiss Federal Institute of Technology, Zurich, Switzerland (<http://www.cbrg.ethz.ch/services/AllAll>) using protein translations of the mRNA sequences listed under the following accession numbers: NM_005074.3, NM_003052.4, NM_001098486.1, NM_006632.3, NM_005495.2, NM_012434.4, NM_020346.2, NM_020309.3, NM_139319.2, NM_001145288.1, and NM_022082.3.

Figure 2. Conserved residues in the SLC17 family of proteins

A) PredictProtein-derived topology model for human sialin sequence with manual adjustments. The circled residues are conserved among all nine SLC17 proteins across vertebrate species in which the proteins have been identified. B) Inward (cytoplasmic)-facing and outward (luminal/extracellular)-facing models of sialin based on structures of the bacterial MFS transporters GlpT and FucP, respectively. Structures are sequentially color coded from blue at the N-terminus to red at the C-terminus. Sequence-structure alignments were provided by Lei Shi (Weill Cornell Medical College) and derived with PROMALS3D. Images were generated in MacPymol2 (PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC).

Figure 3. Physiology of the SLC17 transporters

A) The type I phosphate transporters NPT1 and NPT3 have been implicated in secretion of organic ions including urate and drugs in the renal tubules for secretion. B) Sialin mediates the H⁺ coupled efflux of sialic acid from the acidic lysosome. The degradation of oligosaccharides and glycoproteins leads to the generation of free sialic acid (SA) in lysosomes. The free sialic acid released into the cytoplasm for further metabolism or incorporation into newly synthesized glycoproteins and glycolipids in the golgi. C) The VGLUTs and VNUT both concentrate neurotransmitters (glutamate for VGLUTs and purines including ATP for VNUT) into neurosecretory vesicles for regulated release. This activity requires the electrochemical gradient generated by a H⁺-ATPase on the secretory vesicles. Both glutamate and ATP are present in all cells, but their release through exocytosis requires active transport into secretory vesicles by these transporters. The VGLUTs and VNUT are thus the defining molecular markers for glutamatergic and purinergic neurons. D) Proposed transport mechanisms for SLC17 transporters. The best characterized transport mechanism among in the SLC17 family is symport of acidic sugars and H⁺ by sialin. Electrogenic uniport of substrate with allosteric modulation by Cl⁻ has been proposed for NPT1, the VGLUTs and VNUT. Other transport mechanisms that have been proposed include exchange of glutamate and H⁺ for the VGLUTs, an inorganic anion channel activity for NPT1 and VGLUT1, and Na⁺ - PO₄³⁻ cotransport for NPT1, VGLUT1, and VGLUT2.

Human gene name	Protein	Aliases	Predominant substrates	Transport type /Coupling ions	Tissue distribution (selected)	Predominant subcellular distribution	Link to disease	Human gene locus	Sequence accession ID
SLC17A1	NPT1	NaPi1	organic anions; Phosphate; chloride	electrogenic, Cl ⁻ -dependent; C/Na ⁺ ; channel	kidney, liver	plasma membrane	gout	6p22.2	NM_005074.3
SLC17A2	NPT3		unknown	unknown	heart, muscle, brain, placenta, lung, liver kidney, pancreas	unknown	none	6p22.2	NM_003052.4
SLC17A3	NPT4		Organic anions	electrogenic	liver, kidney, small intestine, testis	Plasma membrane	gout	6p22.2	NM_001098486.1 NM_006632.3
SLC17A4	Na ⁺ /PO ₄ ⁻ cotransporter homologue		unknown	unknown	small intestine, colon, liver, pancreas	unknown	none	6p22.2	NM_005495.2
SLC17A5	sialin	AST VEAT	Sialic acid, other acidic sugars	C/ H ⁺	ubiquitous	lysosome	Sialic acid storage disease (Salla disease)	6q13	NM_012434.4
SLC17A6	VGLUT2	DNPI	glutamate	electrogenic, Cl ⁻ -dependent	brain (neurons only), endocrine	synaptic vesicle	none	11p14.3	NM_020346.2
SLC17A7	VGLUT1	BNPI	glutamate	electrogenic, Cl ⁻ -dependent	brain (neurons only), endocrine	synaptic vesicle	none	19q13.33	NM_020309.3
SLC17A8	VGLUT3		glutamate	electrogenic, Cl ⁻ -dependent	brain (neurons and glia), liver, kidney	synaptic vesicle	Autosomal Dominant non-syndromic deafness (DFNA25)	12q23.1	NM_139319.2 NM_001145288.1
SLC17A9	VNUT		Purine nucleotides	electrogenic, Cl ⁻ -dependent	brain (neurons), adrenal, thyroid	synaptic vesicle	none	20q13.33	NM_022082.3