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Phylogenetic Analysis of the SLC22 Transporter Family

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Christopher Zhu

Committee in charge:

Professor Sanjay Nigam, Chair

Professor Milton Saier

Professor Scott Rifkin

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Co-Chair

Chair

University of California, San Diego

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ABSTRACT OF THE THESIS

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Abstract

Organic anion transporters (OATs), organic cation transporters (OCTs) and organic carnitine and zwitterion transporters (OCTNs), which belong to the solute carrier 22 family (SLC22);TC# 2.A.1.19, transport a wide variety of metabolites, environmental toxins and drugs. Evolutionary analysis of these so called “drug” transporters may shed light on the endogenous role of SLC22 transporters which is only beginning to be explored. We took advantage of the sequencing of multiple genomes, and here, present a phylogenetic analysis of 31 known SLC22 members. Our studies indicate that they evolved from a common ancestor over 450 million years ago before the separation of bony fish and land vertebrates. Several putative SLC22 orthologs exist in worms, sea urchins, flies, and ciona while several other OAT, OCT, and OCTN family members first appear in mammals based on phylogenetic tree topologies. There is a particularly large expansion of SLC22 members in mammals, suggesting a physiological and/or toxicological role for these SLC22 transporters in the successful mammalian radiation into new environments. Moreover, phylogenetic analysis indicates that OATs (e.g. SLC22A6 also known as OAT1 or NKT) and OCTs (SLC22A1 or OCT1) represent two major subclades with the OAT subclade being by the largest; nevertheless there are clear OCTN (e.g. OCTN1 or SLC22A4, OCTN2 or

SLC22A5), OAT-like (e.g. OAT10 or SLC22A13, SLC22A14 or OCTL2), OCTN-like subclades, as well as a distantly related group which we have named the OAT-related group containing SLC22A17, SLC22A18, SLC22A23, and SLC22A31. The uniqueness of the OAT-related subgroup raises the possibility of distinct functions for this set of transporters. Motif analysis suggests the first extracellular region is highly conserved within subclades but not between subclades and may be particularly important for subclade-specific function.

Introduction

The solute carrier 22 protein family (SLC22 family) is a group of membrane transporter proteins containing at least 31 known members confirmed in either mouse or human. The transport capabilities of some of these proteins is varied and is shared by the more sequence-related members of this family which are involved in the transport of toxins, drugs and endogenous metabolites such as prostaglandins, urate, and carnitine across the cell membrane [1,2,3,4,5,40]. Members of this family have been classified in the IUBMB-approved Transporter Classification (TC) system within the Major Facillitator Superfamily (MFS) (TC# 2.A.1) as organic anion transporters (OAT) and organic cation transporters (OCT) which denote the respective substrate affinities, although some substrates are shared by both [43]. In the last few years, there has been much progress in understanding mammalian SLC22 proteins through site-specific mutagenesis of some transporters and construction of 3D models of SLC22A1 (OCT1), SLC22A2 (OCT2), and SLC22A6 (OAT1) [6,7,8,9].

The evolutionary basics of the SLC22 genes were described mostly prior to the complete genome sequencing of many relevant organisms; hence, the lineage of the SLC22 family remains unclear [2,5,10,11]. For example, when did the SLC22 proteins diverge into the various members in primates and rodents, and which are the ancestral members in each group? To best observe the evolution of the SLC22 transporters, specific organismal classes were chosen to represent the evolution of vertebrates and of the SLC22 family in parallel. Thus, we have analyzed SLC22 protein sequences from vertebrates including lamprey, shark, bony fish, amphibians, birds, monotremes, marsupials, mice, old/new world monkeys, and humans; invertebrate organisms such as fly (*D. melanogaster)*, worm (*C. elegans*), ciona (*C. intestinalis)*, and sea urchin (*S. purpuratus)* were also included. Phylogenetic analyses indicate the origin of some SLC22 transporters that have clear orthologs in both humans and rodents which arose before the separation of bony fish and land vertebrates [12]. Seven distant subclades were identified, including a relatively uncharacterized distant subclade consisting of SLC22A17, A18, A23, and A31. Fourteen sequence motifs were analyzed in the context of the six subclades. There also

appears to be an expansion of SLC22 members along with the evolution of mammals via gene duplication and divergence, further implying the importance of the SLC22 family in the development of mammals. These analyses may prove useful for future studies to clarify the true physiological functions of the various subclades of the SLC22 transporters as well as certain “orphan” members of the family [1,44,27]

Materials and Methods

Phylogenetic Calculations

NCBI BLASTp [13] was used to collect the majority of the sequences with the exception of several shark, lamprey, and sea urchin sequences for which a BLASTp was used in their respective databases (http://esharkgenome.imcb.a-star.edu.sg/)(http://jlampreygenome.imcb.a-star.edu.sg/) (http://www.spbase.org/SpBase/).

The phylograms were calculated using the neighbor-joining algorithm (ClustalX 2.1) and the Multiple Alignment using Fast Fourier Transform (MAFFT) [15,16]. Branch lengths are proportional to sequence divergence i.e. the longer the branch length, the more two sequences have diverged from one another. An example of this can be seen in the comparisons between SLC22A1 orthologs of primates (human and chimpanzee) and rodents (rat and mouse) [Figure 1.2]. Most of the branch lengths are proportional to the putative evolutionary distance between these species. Accession numbers are shown in Table 2.1. An online tool, Interactive Tree of Life (http://itol.embl.de/) was used to display the phylograms generated from ClustalX 2.1. Additionally, bootstraps were calculated using ClustalX 2.1 at 1000 trials per node with a random number generator seed at 111. Higher bootstrap values indicate higher confidence in the representation of the data alignment.

Motif Analysis

Motif comparisons were performed on the 31 members included in this analysis using the Multiple Expectation-maximum for Motif Elicitation (MEME) suite (http://alternate.meme-suite.org/tools/meme), the details of which have been described elsewhere [41]. In an unbiased approach to detect any motifs found within our set of 112 SLC22 sequences, a threshold of 16 motifs at a range of 6 to 20 amino acid length was set with the normal discovery mode. This detection method yielded a set of evolutionarily conserved motifs across all members, SLC22A1 through SLC22A31, used in the analysis. A separate analysis using clade-specific SLC22 members were then used at a threshold of 16 motifs at a range of 6 to 20 amino acid length in

order to refine the motif detection to the specific subclade. The same set of several human homologs were then chosen from both analyses to compare clade-specific and evolutionarily conserved motifs to further refine which motifs were truly clade-specific. In the 2D representation of these sequences, the Web-based Hydropathy, Amphipathicity and Topology (WHAT) suite was used to predict transmembrane locations which uses HMMTOP as the transmembrane prediction engine. Motif locations and TMD locations were input into TOPO2, a web-based software, to draw the 2D representation (http://www.sacs.ucsf.edu/cgi-bin/open-topo2.py). This was repeated for OAT, OCT, OAT-like, OCTN, OCT-related, and OAT-related subclades.

Results

Table 1 presents a summary of our overall findings with respect to membership in the six subclades. From the right, specific members and their gene locations in humans and mice are listed along with their specific members in numerical order. Seen farther left, these specific members are organized according to their major and minor clades identified in Figure 1, the general overview. The OAT major subclade is colored orange with its minor clades in various shades of orange. The OCT major subclade is colored green with its minor clades in various shades of green.

The list of accession numbers for the various databases used to generate Figure 1 are listed in Table 2.1. Common names for each accession number are listed in Table 2.2. The tables present the presence and absence of members across the each member, which are listed numerically. Ancestry of each member is also described by the title columns which are ordered according to known evolutionary histories; the listed organisms on the farther left have split from the evolution of humans farther back in time than those listed on the right [12,45]. As presented here, the invertebrates are most likely related to OCT-related subclade found in humans.

Identification of orthologs and paralogs of 31 SLC22 Transporters

Evolutionary analysis of the SLC22 genes depends on the tracing of their orthologs in multicellular organisms. Two genes are orthologs if they have evolved via speciation events i.e. mouse Slc22a2 versus human SLC22A2. Orthologs can be identified by their phylogenetic topology. For example, the lamprey sequences are identified as orthologs of the OAT subclade, OCT subclade, and the OAT-like subclade [Figure 1]. Paralogs develop via gene duplication and sequence divergence in the same species i.e. human SLC22A1 vs human SLC22A2 [17]. In humans there are 23 homologs, 1 of which appears unique to primates (SLC22A25). In rodents there are 25 homologs, 5 of which appear unique to rodents (Slc22a26-Slc22a30) [30]. We have also found 17 homologs in marsupials, 8 homologs in monotremes, 10 homologs in birds, 6 homologs in amphibians (frog), 13 homologs in bony fish, 10 homologs in shark, and possibly 3 homologs in lamprey [Table 2.1]. Partial sequences that were found were not included in this

phylogenetic analysis which are color-coded in blue in Table 2.1. Distant SLC22 orthologs have been previously reported in fly, worm and other species, however it is difficult to determine which subclade they belong to (OAT, OCT, OCTN etc…)[2].

Major Clades and Subclades Identified

In our analysis, we have identified several subclades within our major clades of OAT and OCT. Figure 1 describes the general overview of aligned proteins from all members used in this analysis (SLC22A1 – SLC22A31). The supplementary figures (Figures 1.1 and 1.2) provide greater detail on the evolutionary spread of the SLC22 family members within each subclade. To differentiate the grouping patterns within a phylogenetic tree, the term clade was chosen to describe the rooted estimation of the phylogenies for the SLC22 amino acid sequences. For genetic grouping, the term cluster was chosen to describe the chromosomal locations for each clade if the members were located next to each other on a chromosome.

The General Overview (Figure 1)

Figure 1 was generated using the results from NCBI's BLASTp from 162 known SLC22 family members A1 through A31 [13][Table 2.1]. Several vertebrate classes were chosen to represent time points in which specific SLC22 transporters seemed to have developed. MAFFT and neighbor-joining alignments are in agreement that there appear to be two major branches which then split into various minor branches. The larger branch of the two is the Organic Anion Transporter (OAT) major clade consisting of three minor clades which include the OAT subclade, OAT-like subclade, and a relatively uncharacterized OAT-related subclade. The OAT subclade is by far the best taxonomically represented subclade, especially within mammals, which include SLC22A6-SLC2A8 (OAT1, OAT2, OAT3). Within this subclade, there are also species-specific members such as SLC22A21 (Octn3) for the mouse and SLC22A25 (UST6) for the human [30] [Figure 1.1]. The OAT-like subclade consists of members SLC22A13 and SLC22A14 (OAT10 and OCTL2) [27][Figure 1.1]. Many of these proteins have been shown, by in vitro transport assays and to some extent in vivo knockouts, to transport organic cations and anions, although zwitterionic substrates have also been identified for example SLC22A8 OAT3 [36]. The OAT-

related subclade consists of members SLC22A17, A18, A23, and A31 [Figure 1.1]. The evolutionary distance suggests that this subclade is structurally and functionally unique, although still related enough by sequence to be grouped with the prototypical OAT subclade. Although not well studied, current literature suggests they may have different substrates and different expression patterns than the OATs and OCTs [42].

A second distinct branch has been identified as the Organic Cation Transporter clade (OCT) major clade. The OCTs further branch into three more closely related subclades: the classical organic cation transporters (OCTs) subclade (SLC22A1-A3) [Figure 1.2], OCTN and OCT-related subclades (SLC22A4-A5 and SLC22A15-16) [Figure 1.2]. As presented, SLC22A1-A3 genes are found in many different vertebrates including humans, birds, and bony fish. Although the exact functions of the orthologs may vary from organism to organism, SLC22A1-A3 are known to transport many cationic drugs and metabolites while SLC22A4-5 (OCTN1-2) and SLC22A15-16 (FLIPT1 and FLIPT2 originally identified by Eraly et al. 2002) are generally considered zwitterionic transporters with carnitine as a probable physiological substrate [27].

Because there were several branches with exceedingly low bootstrap values, specifically regarding the more primitive species, a separate alignment was generated (data not shown) to confirm that the putative SLC22 members for the lower species belonged to the major OAT or OCT clade. The resulting alignment confirmed that these lower species align within the OCT clade.

Major Clades and subclades

In order to view the OAT and OCT major clades without mutual alignment influences of each other, the OAT and OCT major clades were split into subfigures. Also, additional sequences were included within the subclades (e.g. OAT-like, OCTN, etc…) to further distinguish the evolutionary details.

SLC22A6-A30 (OAT) subclade (Figure 1.1)

Figure 1.1 uses 175 sequences from NCBI. The OAT subclade contains 12 known members including, SLC22A6, SLC22A7, SLC22A8, SLC22A9, SLC22A10, SLC22A11, SLC22A12, SLC22A20, SLC22A22, SLC22A24, SLC22A25, and SLC22A26 - SLC22A30. The prototypical OAT, OAT1 (SLC22A6), was identified as NKT [28]. As presented here, SLC22A6 or OAT1 is found in nearly all classes of vertebrate organisms included in this analysis with the exception of birds and monotremes. In the Japanese lamprey genome database, a protein match was found and included into this phylogeny (jlampreygenome accession JL188). Interestingly, this sequence is most likely equally related to SLC22A6 as a known relative, SLC22A20 [Figure1.1]. Though a shark Slc22a6 was not found, the presence of the shark Slc22a20 relative, along with the evidence of fish SLC22A6 orthologs in zebrafish and salmon, indicate ancestry in this subclade. SLC22A8, another close relative of SLC22A6, may have first appeared in marsupials. SLC22A7 appears to be equally ancestral to SLC22A6 and is represented in nearly all classes of vertebrate organisms used in this analysis including cartilaginous fish, bony fish, birds, rodents, marsupials, and placental animals. These analyses places the development of the ancestral OAT3 gene after the development of the OAT1 and OAT2 genes; however, additional analysis will be required to determine whether OAT1 or OAT2 is most ancestral. The several slc22a6 proteins belonging to various fish appear to align best with SLC22A20, OAT6 in mice. This may suggest that OAT1 (SLC22A6) and OAT6 (SLC22A20) evolved in parallel with each other or that these fish sequences are more similar to the mammalian SLC22A20 than previously expected. Sequence identity analysis shows that zebrafish SLC22A6 is 49% identical to both mouse Slc22A6 and Slc22A20.

Interestingly, there appears to be a mammalian expansion of SLC22 members consisting of the members SLC22A9, SLC22A10, SLC22A11, SLC22A12, SLC22A22, SLC22A24, SLC22A25, and SLC22A26- SLC22A30. BLASTp searches suggest SLC22A25 to be unique to primates and SLC22A26-A30 to be unique to rodents. This expansion of SLC22 members appears unique to mammals and only within the OAT subclade.

SLC22A13-A14 (OAT-like) subclade (Figure 1.1)

SLC22A13 (OAT10) is a urate transporter expressed in the kidney and to some extent, the brain [29]. Our data show ancestry that in this subclade extends to lamprey (accession JL188). SLC22A13 has also been found in zebrafish, chicken, platypus, and all mammals used in this analysis. SLC22A14 appears to be a more recent development with its first appearance in opossum. This subclade appears to be a close relative of the larger OAT subclade.

SLC22A17-A31 (OAT-related) subclade (Figure 1.1)

The OAT-related subclade is in a separate branch in our phylogeny. This subclade is a somewhat closer relative of the traditional organic anion transporters (OATs and OAT-like) than the organic cation transporters (OCTs) based on the branching schemes calculated [Figure 1]. SLC22 members A17, A18, A23, and A31 appear to group together. Although these four group together in the general overview [Figure 1], there appears to be a subdivision within this group between A18 and A17, A23, and A31. SLC22A17 is shown to exist in organisms up to opossum and there is possibly a related sequence in lamprey (jlampreygenome accession JL10482). SLC22A23 and SLC22A31 were found to exist in all mammals and up to the bony fishes such as pufferfish and coelacanth though these fish sequences may have functionally diverged substantially. Interestingly, *Caenorhabditis elegans* (NCBI accession AAF73198.1) sequence was found (identified as a putative OAT sequence by Eraly et al. 2004 based on the homology to fly like transporters) to align within this subclade though it is unclear which member of this subclade is most similar to it in multiple alignments. SLC22A18 appears fairly diverged compared to the rest of the OAT-related subclade. However, bootstrap support is high enough to conclude these four SLC22 members are the most distantly related subclade of the SLC22 family.

Because of the sequence divergence seen in this subclade relative to the other SLC22 members, members of other drug transporter families were investigated for homology. A separate alignment was done including members of The Drug H^+ Antiporter-1 family (DHA1, TC# 2.A.1.2) which was found to have high homology to SLC22A18. The top hits with the TCBLAST scores of 100 or over were included into a multiple global alignment of SLC22 family members A1-A31 (data not shown). Our findings indicate SLC22A18 to group with DHA1 sequences of the SLC22

family. This implies the SLC22A18 to be more distantly related to the SLC22 family than previously expected and probably more related to the DHA1 family.

SLC22A1-A3 (OCT) subclade (Figure 1.2)

135 sequences were used to generate Figure 1.2. The OCT subclade consists of members SLC22A1, SLC22A2, and SLC22A3. A BLASTp search of the NCBI database has revealed the OCT subclade (SLC22A1, SLC22A2, and SLC22A3) to be found in a wide range of organisms. From the Japanese lamprey genome database, an uncharacterized protein with close homology with the OCT subclade was found, implying ancestry in this family to at least the evolution of lampreys (jlampreygenome accession JL2643) and sharks [Figure 1.2]. As is apparent from the available sequences, SLC22A1 (OCT1) is only present in mammals while the SLC22A2 (OCT2) and SLC22A3 (OCT3) ancestry extends to lampreys, 360 million years ago [37].

SLC22A4, A5, A15, A16 and A21 (OCTN and OCT-related) subclade (Figure 1.2)

Alignments indicate SLC22A4 and SLC22A5 to be within the Major OCT clade of Figure 1. OCTN identification and functions have been previously described as carnitine transporters in mice and humans [22,23,24]. Both SLC22A4 (OCTN1) and SLC22A5 (OCTN2) are revealed to be present in marsupials (opossum), monotremes (platypus), avians (chicken), bony fish (zebrafish and salmon), ciona, and sea urchin implying ancestry in this group to be before the separation of bony fish and land animals over 450 million years ago [12,25,26]. Slc22A21 (OCTN3) is found to exist only in mice and is closely related to existing rodent Slc22A5.

SLC22A15 and SLC22A16, previously described as FLIPT1 and FLIPT2, are related to the OCT and OCTN subclades by sequence [10]. SLC22A16 (FLIPT2) is OCT-related which has been characterized and may be involved in the development of spermatozoa in humans [27]. The OCT-related subclades, SLC22A15 and SLC22A16, do not group very well with the OCT or OCTN subclades nor do they group very well relative to each other [Figure 1]. The lineage of the human variants SLC22A15 and SLC22A16 therefore appeared to have diverged substantially

from the better known OCT and OCTN subclades. Additional work will be required to further distinguish when these members diverged from the rest of the OCT and OCTN subclades or if these are a separate category of SLC22 members.

Ancestral subclade predictions

The OCT, OAT, and OAT-like subclades are similar in phylogenetic topology, and each include one lamprey homolog. The OAT-related (SLC22A17, A18, A23, A31) subclade appears to have both a lamprey homolog as well as a putative worm SLC22 member. This suggests that the OAT-related subclade may predate the OAT, OCT, and OAT-like subclades. However, there were no fly or sea urchin homologs found in either NCBI or their genome-specific databases that grouped with the OAT-related subclade. The OCT-related subclades (FLIPT) appears to diverge substantially from the other subclades of the OCT major clade. However, NCBI contains several sea urchin sequences that appear to belong to the OCT-related subclade with one sea urchin sequence (NCBI accession XP_003730263.1) annotated as SLC22A15 (FLIPT1).

Invertebrates within the phylogeny

Putative worm, fly, sea urchin, and ciona SLC22 sequences are included in this phylogenic analysis. The topology of the branches indicates that the invertebrate sequences align in OCTN or OCT-related subclade. However, the bootstrap support for the exact subclade they belong to is low. Based on the available data, Figure 1 indicates the Major OCT clade predates the Major OAT clade. With the exception of a single *C. elegans* sequence in the OAT major cluster, only the Major OCT clade contains the invertebrate organisms such as fly, worm, and sea urchin. Despite the ambiguity among the invertebrate sequences, there were several sequences that appear to have high enough bootstrap values to group with one of the existing subclades. Ciona sequences appear in the OCTN, SLC22A15, and SLC22A16 subclades (NCBI accession numbers XP_002132109.1, XP_009860851.1, XP_002124941.1). A single sea urchin sequence was found to belong in the SLC22A15 subclade (NCIB accession number XP_003730263.1). These findings indicate much of the OCT major clade originate either during the time of the split between the OCT major clade and the OAT major clade or prior to this separation.

Of the fly and worm sequences identified by Dr. Satish Eraly, the 4 worm and 9 fly sequences were input into the global phylogeny. The phylogeny yielded from this alignment showed similar alignments as did in his original work; these invertebrate 'homologs' are equally distantly related to the OCT and OCTN subclades. Interestingly, there is no evidence for these sequences in the OAT major clade.

Subclade specific Motifs

Prototypical SLC22 members used as reference sequences for motifs are as follows: OAT subclade includes SLC22A6, SLC22A8, and SLC22A12 (hOAT1 - NP_695008.1, hOAT3 - NP_004245.2, hURAT1 - NP_653186.2) with 87 sequences. The OAT-like subclade includes SLC22A14 (hOCT-like2 - NP_004794.2) with 22 sequences. The OCT subclade includes SLC22A1, SLC22A2, and SLC22A3 (hOCT1 - NP_003048.1, hOCT2 - NP_003049.2, hOCT3 - NP_068812.1) with 51 sequences. The OCTN subclade includes SLC22A4 and SLC22A5 (hOCTN1 - NP_003050.2, hOCTN2 - NP_003051.1) 38 sequences. The OCT-related subclade includes SLC22A15 and SLC22A16 (hFLIPT1 - NP_060890.2 and hFLIPT2 - NP_149116.2) with 35 sequences. The subclade motifs were compared to a set of 16 motifs of 112 sequences including SLC22 members A1-A31. All motifs found had significant E-values of less than 0.05. Table 5.1 contains 16 motif sequences from each of the members above.

The members of the OAT subclade contain a unique region within the first extracellular region beginning on L67 in SLC22A6 (hOAT1). Homologous sections in the other two members, SLC22A8 (hOAT3) and SLC22A12 (hURAT1) also show this substantial 20 amino acid length motif. Other significant motifs include an H130-M142, L253-F259, and a cytoplasmic region L283-R298 on hOAT1 and homologous sections in hOAT3 and hURAT1.

The OAT-like members SLC22A13 and SLC22A14 contain motif topologies similar to the OAT subclade members. Within the first cytoplasmic region, there exists a clade-specific 18 amino acid motif in both SLC22A13 (P73-P90) and SLC22A14 (P121-P137). Both members also contain homologous motifs T214-F233 (SLC22A13) and Q292-V311 (SLC22A14) which is predicted to be cytoplasmic.

Within the OCT clade, an interesting finding emerges when comparing the first cytoplasmic region among the three OCT members: SLC22A1, SLC22A2, and SLC22A3 (OCT1, OCT2, and OCT3). SLC22A1 and SLC22A2 share a homologous motif (G87-T106 in OCT1) in the first cytoplasmic region which excludes SLC22A3. This suggests SLC22A1 and SLC22A2 to be sister genes while SLC22A3 to be a sister gene of an ancestral SLC22A1/A2.

The OCTN subclade shows a similar extracellular motif location beginning on V77-G96 on both SLC22A4 and SLC22A5, OCTN1 and OCTN2 respectively. Surprisingly, there is a lack of a motif within the cytoplasmic region beginning at the 'ESPAR' region found in many other SLC22 transporters in this family.

Multiple alignments show the OCT-related subclades (FLIPT) are the most distant of the OCT major clade members. SLC22A15 (FLIPT1) does not share several common domains that are expected in the SLC22 transporter family the in first extracellular loop. This difference in extracellular domain could be a significant factor in its characterization as an SLC22 transporter and could imply differences in function relative to the other SLC22 family members. SLC22A16 (FLIPT2) contains the common motifs found in the first extracellular region as well as a member defining motif N92-R106. Similar to the OCTN subclade, the OCT-related subclade lacks a significant motif found the better known SLC22 family members such as SLC22A1 and SLC22A2 in the 'ESPAR' intracellular domain.

Mapped Mutagenesis and SNP (Single Nucleotide Polymorphisms)

Mutagenesis and SNP data were gathered and mapped onto their respective 2D topologies of OAT1, OAT3, URAT1, OCT1, OCT2, and OCTN1/2. OCTN1 and OCTN2 data were mapped onto the OCTN2 2D model because of their relatively high identity and similarity between them and because the majority of the data available were of OCTN2 origin. Three areas of interest emerged from the mapping: the first large extracellular loop, the central region near or at the large intracellular loop, and the 9^{th} and 10^{th} transmembrane domains. Interestingly, many of the mapped residues that were found in first large extracellular loop reside in the clade specific motif we have identified in this study, colored in red. The other residues that appear crucial for

transport reside in TMD 9 and 10 which have been confirmed in various uptake experiments. Another interesting observation is that SNPs associated with disease, in the case of URAT1 and OCTN2, fall near or within the central intracellular loop of these proteins, at the signature 'ESPXR' region of the SLC22 transporter family.

OAT-related subclade

The OAT-related subclade, consisting of the remaining SLC22 members A17, A18, A23, and A31, contains 10 of 16 motifs detected in the A1-A31 analysis. Unlike the 5 other subclades identified, the OAT-related subclade does not contain an obvious "prototypical" member that can generalized for the other members. In the motif detection within all 31 members, the human SLC22A17 sequence only contains 9 out of the 16 common motifs detected; human SLC22A18 sequence contains 3 out of the 16 motifs common detected; human SLC22A23 contains 9 out of the 16 common motifs detected; human SLC22A31 contains 10 of the 16 common motifs detected. Within the clade-specific MEME motif detection, the SLC22A17 sequence contains the most motifs in common with the subclade at 16 out of 16 subclade motifs detected followed by SLC22A31 (15/16), SLC22A23 (14/16), and SLC22A18 (2/16). The relatively few common motifs found within SLC22A18 suggest it is the most distantly related member of the SLC22 family.

Gene Locations

Pairing and clustering of the SLC22 family members in human and rodent genomes have been previously described [2,30]. Using the UCSC genome browser and previously mapped gene locations for the SLC22 genes, gene clusters in human were identified. The majority of the OAT subclade exists on chromosome 11. The OAT-like subclade, SLC22A13 and SLC22A14, exists on chromosome 3. The OCT subclade, SLC22A1, A2, and A3, cluster on chromosome 6. The OCTN subclade cluster on chromosome 5. However, there are several human SLC22 members within these subclades that do not follow the genomic pattern. SLC22A7 (OAT) and SLC22A23 (OAT-related) are found on chromosome 6, which the OCT cluster is also on, though not part of the same phylogenetic clade. SLC22A15 (hFLIP1) appears on chromosome 1 which does not have any other known SLC22 member. SLC22A17 and SLC22A31, both part of the OAT-related

subclade, also exist on separate chromosomes without other known SLC22 members nearby on chromosome 14 and chromosome 16 respectively. SLC22A18 (OAT-related) exists on chromosome 11 on which the known OAT cluster exists 60 million base pairs away.

Discussion

Our phylogenetic analysis of 31 SLC22 transporters in mammalian as well as non-mammalian sequences indicates the existence of 6 subclades, including an evolutionary distant and functionally unclear subgroup consisting of SLC22A17, A18, A23, and A31. Using the available genome databases along with the current NCBI database, we were also able to identify putative ancestral clades within our subclades.

Expansion of SLC22 proteins in mammals

An interesting finding that emerges from the phylogenetic analysis is that there was an expansion of the OAT, OCT, and OCTN clades in mammals through gene duplication and divergence from which SLC22A1, SLC22A8, SLC22A9, SLC22A10, SLC22A11, SLC22A12, SLC22A14, SLC22A18, SLC22A19, SLC22A21, SLC22A22, SLC22A24-SLC22A30 have evolved [30]. This suggests a role for these SLC22 genes in the physiology of mammals and possibly in their successful radiation in the last 60 million years. The majority of the observed expansion appears to be organic anion transporters (OATs).

Organic cation transporters (OCTs) and organic carnitine/zwitterion transporters (OCTN) expansion is limited to SLC22A1-5 and SLC22A15-A16. OCT1 (SLC22A1) appears to be mammalian-exclusive, and the first sequence identified by us appears in opossum [Figure 1.2]. Current literature on the functionality of each subclade OCT, OCTN, and OCT-related include transport of drugs, metabolites and of toxins for OCTs and OCTNs to possible roles in the maturation of spermatozoa in humans for the OCT-related genes [10, 27].

The OAT-related cluster contains members SLC22A17, A18, A23, and A31. SLC22A17 and SLC22A23 do not share many of the common OCT substrates associated with the SLC22 family such as 1-methyl-4phenyl-pyridinium (MPP+) and carnitine despite showing expression in the choroid plexus and liver, similar to many of the known SLC22 members [42]. There is also evidence SLC22A18 regulation may play a role in the development of non-small cell lung cancer. This may imply novel function for SLC22A17 and the associated proteins in this cluster [18, 19].

Non-mammalian SLC22 proteins

Fish SLC22 genes have diverged substantially from their mammalian orthologs, which seems reasonable from an evolutionary standpoint because fish and mammals have different physiological requirements are likely to encounter different chemicals for membrane transport. OCT2 has duplicated genes in salmon and tetraodon, which is consistent with genome duplication in teleosts [31,32,33]. Alignments within our analysis, for example, show zebrafish slc22a6 grouped with known mouse and human SLC22A20 orthologs, which suggests some ambiguity as to which subclade certain fish orthologs belong to.

An interesting finding was that BLAST searches did not find certain SLC22 members in frogs, platypuses or opossums. Their absence could be due to loss of the gene or to incomplete sequencing of the genome. Of note is that the platypus is missing several genes that would be expected to be present. For example, platypus lacks an ortholog of SLC22A2 (OCT2), which does not seem consistent with the presence of SLC22A2 (OCT2) in birds and fish [Figure 1.2]. However, platypus contains an ortholog of SLC22A3 (OCT3), which may perform some of the functions of SLC22A2.

Lamprey and shark sequences were identified which have close homology with known SLC22 subclades. These subclades include the OCT (SLC22A1-A3), the OAT (SLC22 members A6-A30), the OAT-like subclades (SLC22A13-A14), and the OAT-related subclade. Based from our analyses, the OAT cluster appears the most ubiquitous throughout all organisms. SLC22A6 (OAT1) is one of the more well known within this cluster and is expressed almost exclusively in the kidney [26]. Furthermore, transport capabilities of OAT1 and others within this cluster (OAT2 - OAT10) have been documented and is found to have affinities for hundreds of endogenous and exogenous compounds that are mostly, though not exclusively, organic anions of considerable physiological as well as toxicological and pharmaceutical significance [34,27,35].

Ancestry of the SLC22 proteins have been identified here to possibly extend as far as the worm; however, bootstrap support for some of the lower branches remains low [Figure 1]. It appears OCTs predate OATs in evolutionary time because of the invertebrate SLC22-like

transporters (see OCT-related subclade, Figure 1.2) that exist in ciona, worms, flies, and sea urchins. These findings imply a basic function for the SLC22 transporters that have not been fully characterized and should be investigated for further clarification regarding the true physiological function. The most distantly related of the SLC22 family, the OAT-related subclade, is most likely a unique subset of the SLC22 family. However, current sequencing data show it has existed as long as many of the better known SLC22 members such as SLC22A1 (OCT1) and SLC22A6 (OAT1), and it is possibly even older.

Motifs found within the SLC22 family

The motif patterns found within our subclades place a particular focus on the first extracellular domain with several TMDs uniquely present in some cases. This is particularly clearly seen when comparing 2D topologies of SLC22A1, SLC22A6, and SLC22A12, all of which show subclade unique domains in this region as well as the flanking common motifs. The evolutionarily conserved flanking regions may hold the structurally important features of the SLC22 family while the subclade-specific motif regions allow for the transporter's flexibility in handling a wider range of substrates. SNP studies in SLC22A6 (OAT1), SLC22A8 (OAT3), and SLC22A12 (URAT1) have shown evidence of stabilizing selection in this extracellular region, which implies the sequence and structure of this region may be important for its function [38]. Mutagenesis studies of SLC22A6 and SLC22A8 finds cysteine residues on TMD 10 are related to the sensitivities to Hg^{2+}, a region that was detected in these analyses to be a significant motif within the OAT subclade [39].

Our motif predictions along with mutagenesis and SNP data suggests there are 3 conserved areas of these proteins that are responsible for function, the first extracellular domain, the central large intracellular domain, and the adjacent transmembrane domains 9 and 10. The substrate kinetics associated with the reported residues suggests these three domains work in conjunction with one another to allow substrate specificity as no single residue was found to be responsible for the transport. Additional work will be required to identify the roles of each of these

three domains identified through our analysis [Figures 3.1-3.3]. Additional information per residue can be found in Tables 6.1-6.3.

Genomic Locations

Genomic locations and the relative similarities of these genes strongly suggest duplication events which have produced the SLC22 subclades we have found [30,33]. Our motif and current substrate data also suggest initial functional similarities between the duplicated sister genes that have diverged over time into the various subclades present. Some SLC22 family members do not cluster on the same locations as does the OAT, OCT, and OCTN subclades. Thus, there may be other factors at play here that describe the prevalence of the SLC22 family members across different chromosomes. Nevertheless, the close homology of these sequences and the similarity in substrates for current members of the same clade indicate there to have been ancestrally similar functions between the SLC22 members; for example in the OAT subclade, all homologs with substrate data that has currently classified as organic anion transporters, also cluster together on chromosome 11 with the exception of SLC22A7 (OAT2).

In summary, the historical phylogeny of the SLC22 family members has been described here. We present the evidence for this family's beginnings to extend beyond the evolution of worms and flies. As described in the Remote Sensing and Signaling hypothesis, drug transporters are likely to play important homeostatic functions in present day organisms [1,27,36]. Mammals, in particular, have taken advantage of the innate functions of the organic anion transporters (OATs) and have developed a mammalian exclusive SLC22 family member set. Additional work on the OAT-related members, though currently not functionally well characterized, may hold important clues as to how these members have diverged in function while maintaining sequence similarity. Motif analysis suggests that the first extracellular region is important for function in each particular subclade as it is highly conserved across members of a subclade (OAT/OCT/OCTN etc…) while not conserved across members of different subclades. The conserved regions across all SLC22 members appear to flank the subclade-specific regions which include extracellular, cytoplasmic, and transmembrane regions.

Figures

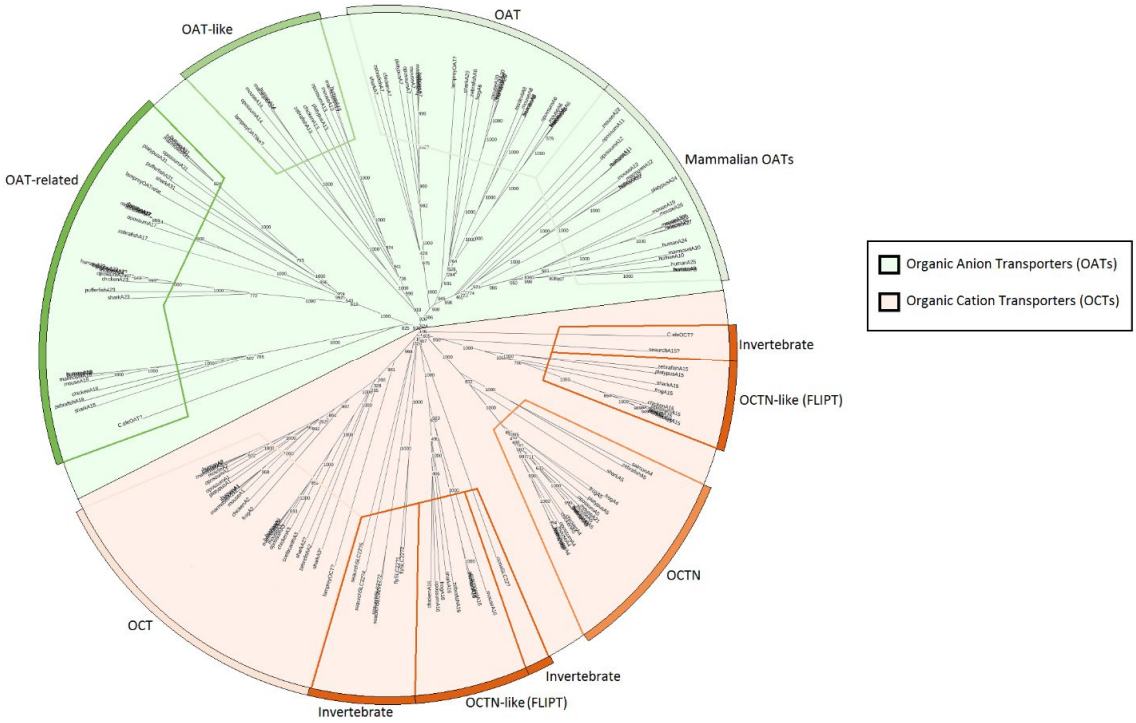


Figure 1: The General Overview. This is an unrooted phylogeny of the SLC22 transporter family. It uses 161 sequences various organisms from each of the 31 SLC22 members. Accession numbers are listed in Table 2.1. In green is the OAT major clade. In outlined varying shades of green are the subclades OAT, OAT-like, and OAT-related. In orange is the OCT major clade. In outlined varying shades of orange are the subclades, OCT, OCTN, and OCT-related. Bootstrap values are shown at each node of this phylogeny

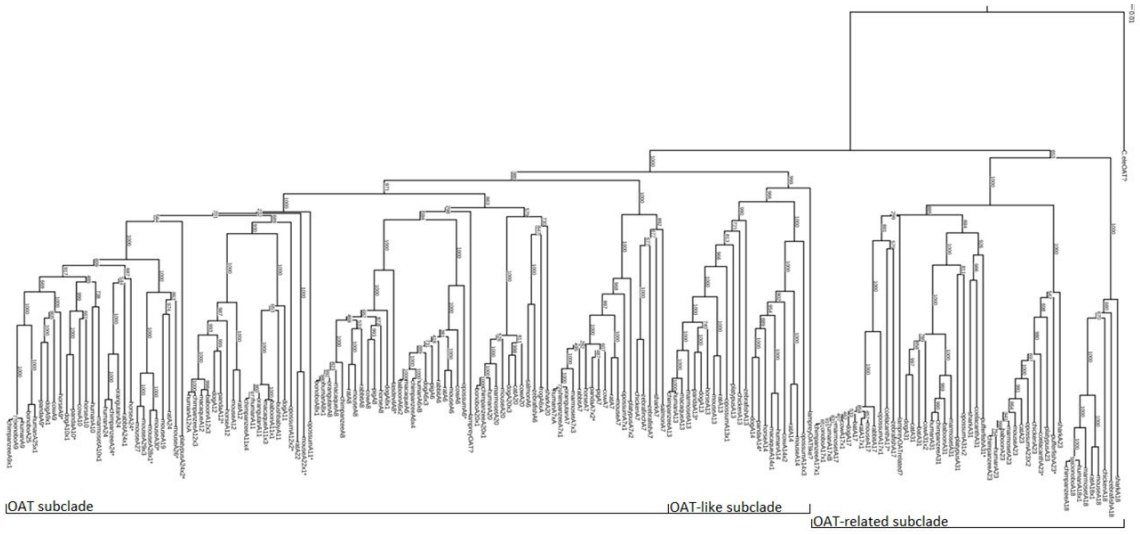


Figure 1.1: The major OAT clade. 175 sequences were used to generate this figure. The branching scheme outlined here show the three subclades we have identified and is rooted on the most ancient sequence, *C. elegans*. There are three subclades identified here, the OAT subclade, the OAT-like subclade, and the OAT-related subclade. Bootstrap values in this phylogeny describe the confidence in each node. Table 3.1 lists the accession numbers used to generate this phylogeny.

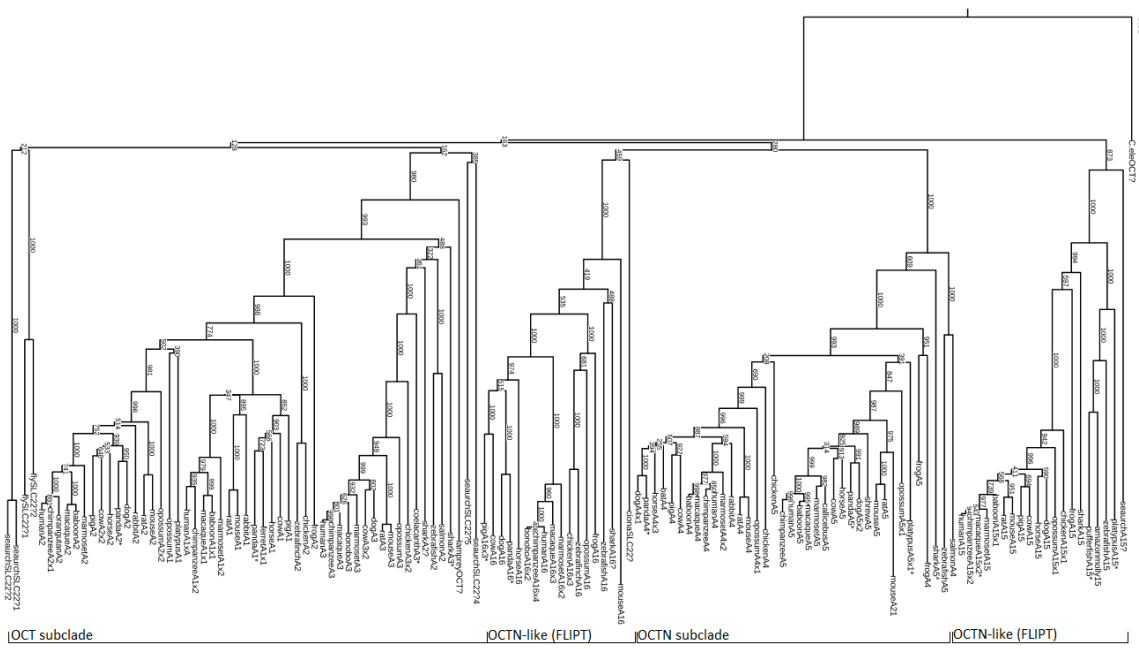
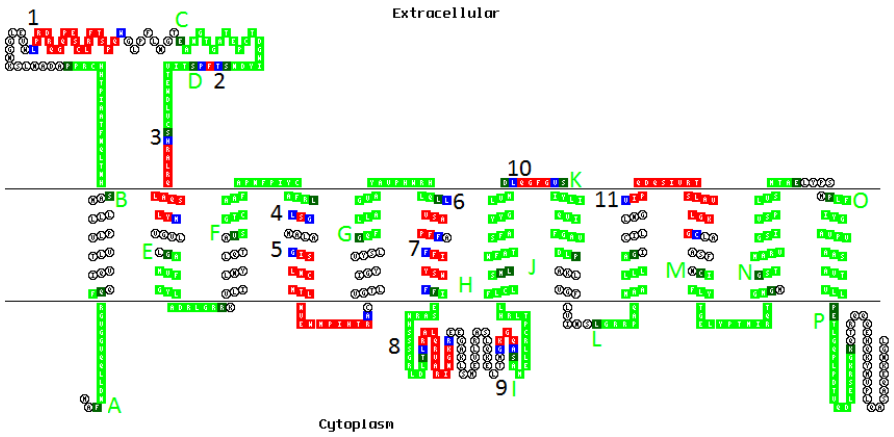


Figure 1.2: The major OCT clade. This figure was generated from 133 sequences from various organisms. There are three subclades identified here: the OCT subclade, the OCTN subclade, and the OCT-related subclade. The phylogeny is rooted to the most ancient sequence, *C. elegans*. Invertebrate sequences are difficult to assign to subclades due to their low bootstrap values within this phylogeny as well as relatively low homology to the other members of the major OCT clade.

Figure 2: Family conserved motifs versus Clade conserved motifs. This is a figure that overlays the 16 evolutionarily conserved family motifs on 16 evolutionarily conserved subclade motifs. Conserved family motifs are assigned letters A-P in the order of appearance in human OAT1 (human SLC22A6). Subclade motifs are either whole or partial and are not numbered. The first box shows the predicted large extracellular loop for each sequence. The second box shows the predicted intracellular loop for each sequence. Only human orthologs were chosen in this analysis.



OAT clade specific motifs

Motif #	Region	% present	MEME #	E-value	Sequence
1	67-86	1.00	4	1.0e-1078	LPRDRQGQPESCLRFTSPQW
2	115-117	1.00	1	3.2e-1323	TFP
3	130-142	1.00	3	1.1e-1079	HRALRQLAQSLYM
4	194-196	1.00	13	1.2e-878	LSG
5	201-220	0.99	16	1.6e-636	GISLNCMTLNVEWMPIHTRA
6	253-259	1.00	12	7.3e-977	LVSAPFF
7	261-267	0.97	11	9.6e-751	FFIYSWF
8	285-298	0.95	7	4.6e-894	LRALQRVARINGKR
9	320-324	0.95	14	2.1e-638	GKGQA
10	360-365	0.92	8	7.6e-806	LQGFGV
11	414-433	0.92	15	5.0e-719	VIPQDQSIVRTSLAVLGKGC

Common Motifs

Motif letter	%present	MEME#	E-value	SLC22A6 Sequence
A	0.78	9	4.2e-493	FNDLLQQVGGVGRFQ
B	0.66	8	1.3e-597	SHNTLQNFTAAIPTHHCRPP
C	0.74	7	1.4e-593	EANGTGATEPCTDGWIYDNS
D	0.88	5	6.6e-637	STIVTEWDLVCS
E	0.96	3	1.0e-717	GAMVFGYLADRLGRR
F	0.98	14	2.3e-471	VSGTCAAFAPNFPIYCAFRL
G	0.95	6	6.7e-830	GQFLLAGVAYAVPHWRHLQL
H	0.92	2	7.9e-867	FIESARWHSSSGRLDLT
I	0.95	12	2.0e-560	SAMELLRCPTLRHLFLCLSM
J	0.71	11	5.9e-421	LWFATSFAYYGLVMD
K	0.70	10	3.7e-427	SIYLIQVIFGAVDLP
L	0.95	16	2.8e-331	LGRRPAQMAALLLAG
M	0.90	4	2.2e-941	CIFLYTGELYPTMIRQTGMG
N	0.63	13	6.2e-446	GSTMARVGSIVSPLVSMTAE
O	0.88	15	7.1e-515	PLFIYGAVPVAASAVTVLLP
P	0.87	1	2.9e-663	ETLGQPLPDTVQDLESRKGK

Figure 2.1: Human SLC22A6 2D topology with family and subclade motifs. Human OAT1 (SLC22A6) was chosen to map family and subclade conserved motifs. Listed in the tables are the subclade motifs in red with the regions that they were found, the length, the E-score that was reported for the motif, and the sequence.

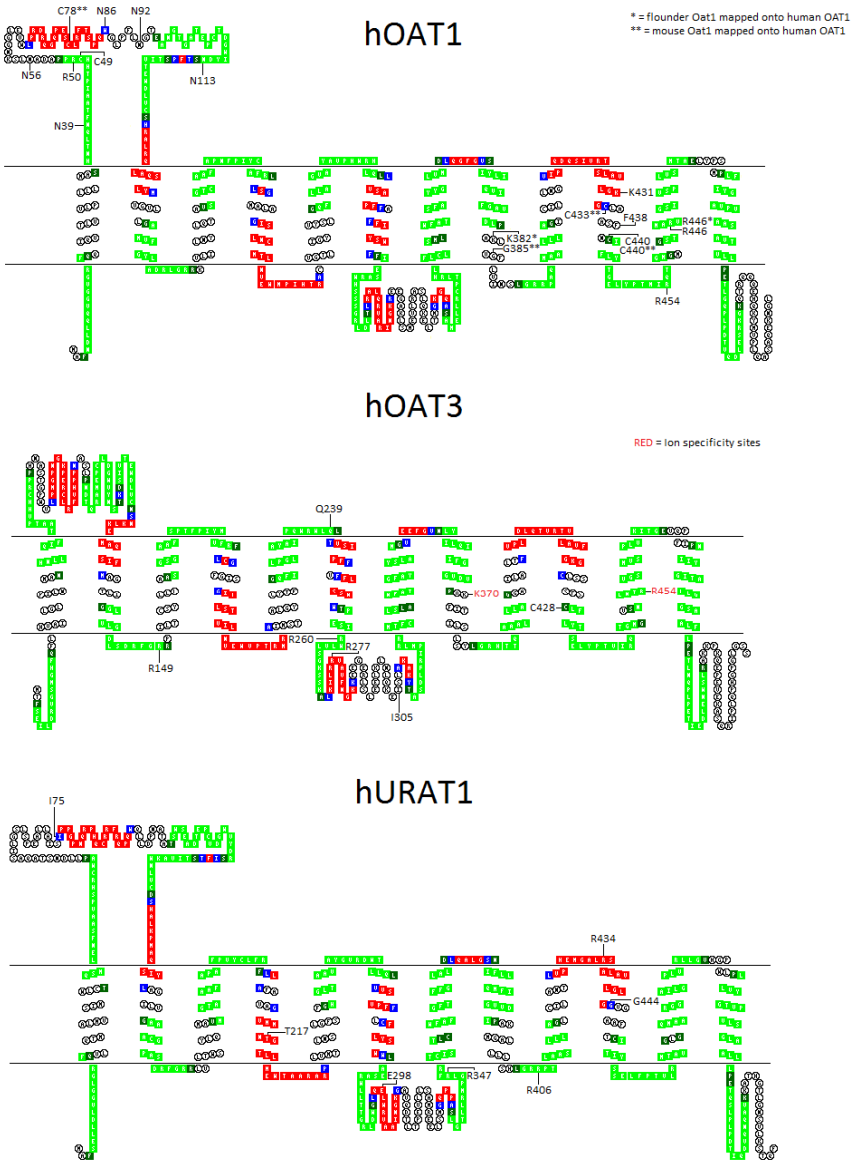


Figure 3.1: Mapped OAT residues. These series of figures describes mutagenesis and SNP data collected for OAT1, OAT3, and URAT1. Human variants were chosen to display these residues, although there other model organism variant data which are starred.

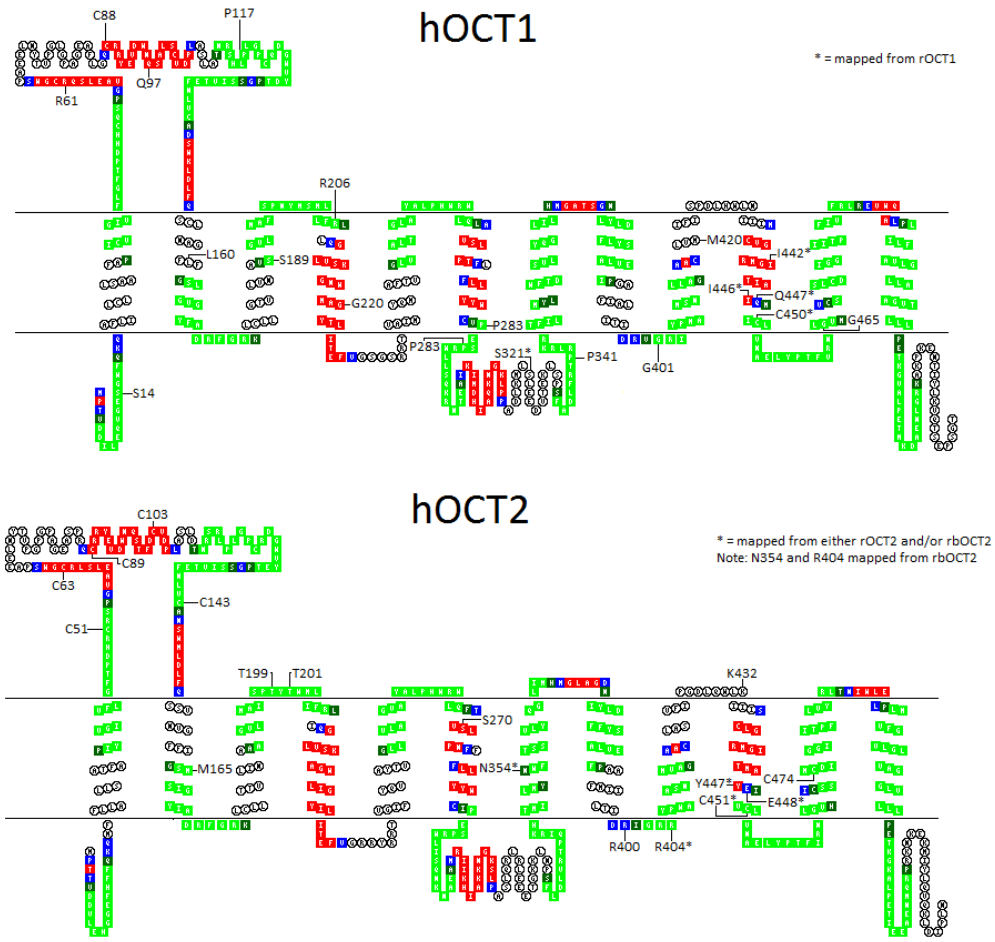


Figure 3.2: Mapped OCT residues. These series of figures describes mutagenesis and SNP data collected for OCT1 and OCT2. Human variants were chosen to display these residues, although there other model organism variant data which are starred.

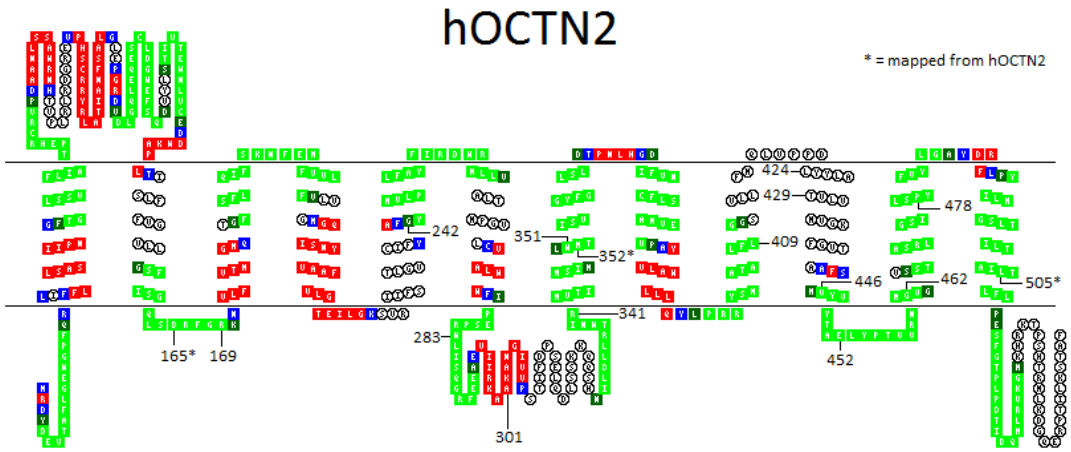


Figure 3.3: Mapped OCTN residues. These series of figures describes mutagenesis and SNP data collected for OCTN1 and OCTN2. hOCTN2 was chosen to represent the residues found because the majority of the available data was of hOCTN2 origin. OCTN1 residues ere also mapped on this sequence as homology between OCTN1 and OCTN2 are relatively high which are starred

Tables

Table 1: Summary Table

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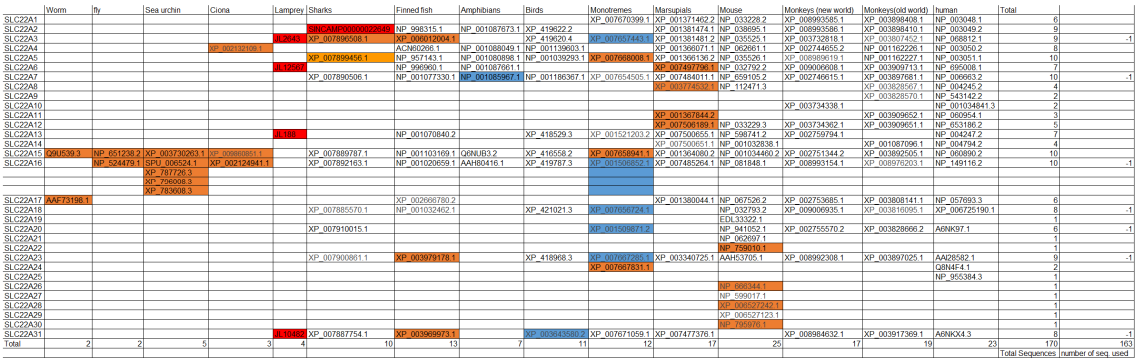
Major Clade	Minor Clade	first split?	which ancestral?	member (common name)	exact location (human)	location abv.	chromosome(human)	member (common name)	exact location (mouse)	location abv.	chromosome(mouse)
OAT	OAT	lamrey	Unclear. Lamprey is an outgroup of A6,A8, and A20	SLC22A6 (hOAT1)	chr11:62,744,069-62,752,495	11q12.3	11	slc22a6 (mOAT1)	chr19:8,617,996-8,628,299	19 A; 19 5.44 cM	19
OAT	OAT			SLC22A7 (hOAT2)	chr6:43,265,998-43,273,276	6p21.1	6	slc22a7 (mOAT2)	chr17:46,432,185-46,438,477	17; 17 C	17
OAT	OAT			SLC22A8 (hOAT3)	chr11:62,760,296-62,783,317	11q11	11	slc22a8 (mOAT3)	chr19:8,591,254-8,611,835	19; 19 A	19
OAT	OAT			SLC22A9 (hOAT7)	chr11:63,137,261-63,177,712	11q13.1	11				
OAT	OAT			SLC22A10 (hOAT5)	chr11:63,057,430-63,079,246	11q12.3	11				
OAT	OAT			SLC22A11 (hOAT4)	chr11:64,323,098-64,338,999	11q13.1	11				
OAT	OAT			SLC22A12 (hURAT1)	chr11:64,358,282-64,369,825	11q13.1	11	slc22a12 (mURAT1)	chr19:6,535,856-6,543,070	19; 19 A	19
OAT	OAT							slc22a19 (Oat5)	chr19:7,673,076-7,711,310	19; 19 A	19
OAT	OAT			SLC22A20 (hOAT6)	chr11:64,981,311-64,993,511	11q13.1	11	slc22a20 (mOAT6)	chr19:5,970,234-5,986,143	19; 19 A	19
								slc22a21 (Octn3)	chr11:53,951,256-53,979,857	11; 11 B1.3	11
OAT	OAT							slc22a22 (Oatpg)	chr15:57,243,771-57,477,625	15; 15 D1	15
OAT	OAT			SLC22A24	chr11:62,847,412-62,911,693	11q12.3	11				
OAT	OAT			SLC22A25 (UST6)	chr11:62,931,296-62,997,124	11q12.3	11				
OAT	OAT							slc22a26 (mOAT6)	chr19:7,781,981-7,802,667	19; 19 A	19
OAT	OAT							slc22a27	chr19:7,864,389 -7,866,027	19; 19 A	19
OAT	OAT							slc22a28	chr19:8,062,209-8,131,982	19; 19 A	19
OAT	OAT							slc22a29	chr19:8,160,168-8,218,839	19; 19 A	19
	OAT							slc22a30	chr19:8,343,301-8,405,105	19; 19 A	19
OAT	OAT-like	lamprey	Oldest seems to be SLC22A13 because of shark and fish	SLC22A13 (hOAT10)	chr3:38,307,298-38,319,806	3p21.3	3	slc22a13 (mOAT10))	chr9:119,192,978-119,209,105	9; 9 F3	9
OAT	OAT-like			SLC22A14 (hOCTL2)	chr3:38,347,445-38,359,859	3p21.3	3	slc22a14 (mOCTL2)	chr9:119,169,456-119,190,393	9; 9 F3	9
OAT	OAT-related	C. elegans	Oldest seems to be SLC22A31 because of lamprey homology	SLC22A17 (hBOCT1)	chr14:23,815,520-23,822,123	14q11.2	14	slc22a17 (mBOCT1)	chr14:54,906,727-54,913,132	14; 14 C3	14
OAT	OAT-related			SLC22A18 (hORCTL2)	chr11:2,920,951-2,946,476	11p15.5	11	slc22a18 (mORCTL2)	chr7:143,473,755-143,499,332	7 F5; 7 88.22 cM	7
OAT	OAT-related			SLC22A23	chr6:3,269,207-3,456,793	6p25.2	6	slc22a23	chr13:34,179,158-34,345,182	13; 13 A3.3	13
OAT	OAT-related			SLC22A31	chr16:89,262,169-89,266,529	16q24.3	16				
OCT	OCT	lamprey	Oldest seem to be OCT3 because of lamprey homology	SLC22A1 (hOCT1)	chr6:160,542,863-160,579,750	6q25.3	6	slc22a1 (mOCT1)	chr17:12,648,875-12,675,838	17 A1; 17 8.63 cM	17
OCT	OCT			SLC22A2 (hOCT2)	chr6:160,637,794-160,675,963	6q25.3	6	slc22a2 (mOCT2)	chr17:12,584,189-12,628,488	17 A1; 17 8.61 cM	17
OCT	OCT			SLC22A3 (hOCT3)	chr6:160,769,405-160,873,611	6q25.3	6	slc22a3 (mOCT3)	chr17:12,419,574-12,507,704	17 A1; 17 8.52 cM	17
OCT	OCTN	shark	Oldest seems to be SLC22A4 because of bird homology	SLC22A4 (hOCTN1)	chr5:131,630,145-131,679,899	5q31.1	5	slc22a4 (mOCTN1)	chr11:53,983,126-54,028,090	11 B1.3; 11 32.07 cM	11
OCT	OCTN			SLC22A5 (hOCTN2)	chr5:131,705,401-131,731,306	5q23.3	5	slc22a5 (mOCTN2)	chr11:53,864,542-53,891,703	11 B1.3; 11 32.02 cM	11
OCT	OCTN-like	C. elegans	Unclear. However, sea urchin is confirmed to have SLC22A15	SLC22A15 (hFLIPT1)	chr1:116,519,119-116,612,675	1p13.1	1	slc22a15 (mFLIPT1)	chr3:101,855,771-101,924,453	3; 3 F2.2	3
OCT	OCTN-like			SLC22A16 (hFLIPT2)	chr6:110,745,892-110,797,844	6q22.1; 6q21-q22.1	6	slc22a16 (mFLIPT2)	chr10:40,570,362-40,604,132	10; 10 B1	10

Table 1: Summary Table. This table summarizes our findings. Listed from right to left are the Major clades, minor clades, most ancestral organism that the sequence was found in, the most ancestral member of the specific subclade, the existing human SLC22 members, genomic locations in humans, the existing mouse SLC2 members, and genomic locations in mice. The various shades of green are part of the major OAT clade. The various shades of orange are part of the major OCT clade.

Table: 2.1 Accession numbers for Figure 1.



	Worm	fly	Sea urchin	Ciona	Lamprey	Sharks	Finned fish	Amphibians	Birds	Monotremes	Marsupials	Mouse	Monkeys (new world)	Monkeys(old world)	human	Total	
SLC22A1										XP_007670399.1	XP_001371462.2	NP_033228.2	XP_008993585.1	XP_003898408.1	NP_003048.1	6	
SLC22A2						SINCAMP000000228549	NP_998315.1	NP_001087673.1	XP_419622.2		XP_001381474.1	NP_038695.1	XP_008993586.1	XP_003898410.1	NP_003049.2	9	
SLC22A3					IL2643	XP_007896508.1	XP_006012004.1		XP_419620.4	XP_007657443.1	XP_001381481.2	NP_035525.1	XP_003732818.1	XP_003807452.1	NP_068812.1	9	-1
SLC22A4				XP_002123109.1			ACN60266.1	NP_001088049.1	NP_001139603.1		XP_001366071.1	NP_062661.1	XP_002746655.2	NP_001162226.1	NP_003050.2	8	
SLC22A5						XP_007899456.1	NP_957143.1	NP_001080898.1	NP_001039293.1	XP_007668008.1	XP_001366136.2	NP_035526.1	XP_008989919.1	NP_001162227.1	NP_003051.1	10	
SLC22A6					IL12567		NP_996960.1	NP_001087661.1			XP_007497796.1	NP_032792.2	XP_009006808.1	XP_003909713.1	NP_695008.1	7	
SLC22A7						XP_007890506.1	NP_001077330.1	NP_001085967.1	NP_001186367.1	XP_007654505.1	XP_007484011.1	NP_659105.2	XP_002746615.1	XP_003897681.1	NP_006963.2	10	-1
SLC22A8											XP_001374532.2	NP_112471.3		XP_003828567.1	NP_004245.2	4	
SLC22A9														XP_003828570.1	NP_543142.2	2	
SLC22A10													XP_003734338.1		NP_001034841.3	2	
SLC22A11											XP_001367844.2			XP_003909652.1	NP_060954.1	3	
SLC22A12											XP_007506189.1	NP_033229.3	XP_003734362.1	XP_003909651.1	NP_653186.2	5	
SLC22A13					IL188		NP_001070840.2		XP_418529.3	XP_001521203.2	XP_007506655.1	NP_598741.2	XP_002759794.1		NP_004247.2	7	
SLC22A14											XP_007506511.1	NP_001032838.1		XP_001087096.1	NP_004784.2	4	
SLC22A15	Q9UJS9.3	NP_651238.2	XP_003730263.1	XP_009680851.1		XP_007889787.1	NP_001103169.1	Q8NUB3.2	XP_416558.2	XP_007658941.1	XP_001364080.2	NP_001034460.2	XP_002751344.2	XP_003892505.1	NP_060890.2	10	
SLC22A16		NP_524479.1	SPU_006524.1	XP_002124941.1		XP_007892163.1	NP_001020659.1	AAH80416.1	XP_419787.3	XP_001506852.1	XP_007485264.1	NP_081848.1	XP_008993154.1	XP_008976203.1	NP_149116.2	10	-1
			XP_787726.3														
			XP_796608.3														
			XP_783608.3														
SLC22A17	AAF73198.1						XP_002668780.2				XP_001380044.1	NP_067526.2	XP_002753685.1	XP_003808141.1	NP_057693.3	6	
SLC22A18						XP_007885570.1	NP_001032462.1		XP_421021.3	XP_007656724.1		NP_032793.2	XP_009006935.1	XP_003816095.1	XP_006725190.1	8	-1
SLC22A19												EDL33322.1				1	
SLC22A20						XP_007910015.1				XP_001509671.2		NP_941052.1	XP_002755570.2	XP_003828666.2	A6NK97.1	6	-1
SLC22A21												NP_062697.1				1	
SLC22A22												NP_759010.1				1	
SLC22A23						XP_007900861.1	XP_003979178.1		XP_418968.3	XP_001507201.1	XP_003340725.1	AAH53705.1	XP_008992308.1	XP_003897025.1	AAI28582.1	9	-1
SLC22A24										XP_007667831.1					G8N4F4.1	2	
SLC22A25															NP_955384.3	1	
SLC22A26												NP_599034.1				1	
SLC22A27												NP_599017.1				1	
SLC22A28												XP_006527242.1				1	
SLC22A29												XP_006527123.1				1	
SLC22A30												XP_003970114.1				1	
SLC22A31					IL16482	XP_007887754.1	XP_003969973.1		XP_003943580.2	XP_007671059.1	XP_007477376.1		XP_008984632.1	XP_003917369.1	A6NKX4.3	8	-1
Total	2	2	5	3	4	10	13	7	11	12	17	25	17	19	23	170	163
																Total Sequences	number of seq. used

Table: 2.1 Accession numbers for Figure 1. This is a list of accession numbers relating to the used to generate Figure 1. In orange are accession numbers annotated as “SLC22-like” In blue are partial sequences and were not included in the phylogeny. In red are sequences not found in GenBank. The totals of each column and row that were used in the phylogeny are also displayed.

Table 2.2: Common names for Table 2.1.

	Worm	fly	Sea urchin	Ciona	Lamprey	Sharks	Finned fish	Amphibians	Birds	Monotremes	Marsupials	Rodents	Monkeys (new world)	Monkeys(old world)	Recent primates	Total	
SLC22A1										platypus	opossum	mouse	marmoset	baboon	human	6	
SLC22A2						ghost shark	zebrafish	frog	chicken		opossum	mouse	marmoset	baboon	human	9	
SLC22A3					Lamprey	ghost shark	coelacanth		chicken	platypus	opossum	mouse	marmoset	bonobo	human	9	-1
SLC22A4				Ciona			salmon	frog	chicken		opossum	mouse	marmoset	baboon	human	8	
SLC22A5						ghost shark	zebrafish	frog	chicken	platypus	opossum	mouse	marmoset	baboon	human	10	
SLC22A6					Lamprey		zebrafish	frog			opossum	mouse	marmoset	baboon	human	7	
SLC22A7						ghost shark	zebrafish	frog	chicken	platypus	opossum	mouse	marmoset	baboon	human	10	-1
SLC22A8											tasdevil	mouse		bonobo	human	4	
SLC22A9														bonobo	human	2	
SLC22A10													marmoset		human	2	
SLC22A11											opossum			baboon	human	3	
SLC22A12											opossum	mouse	marmoset	baboon	human	5	
SLC22A13					Lamprey		zebrafish		chicken	platypus	opossum	mouse	marmoset		human	7	
SLC22A14											opossum	mouse		macaque	human	4	
SLC22A15	C. elegans	M. drasophila 1	sea urchinA15	Ciona		ghost shark	zebrafish	frog	chicken	platypus	opossum	mouse	marmoset	baboon	human	10	
SLC22A16		M. drasophila 2	sea urchin 1	Ciona		ghost shark	zebrafish	frog	chicken	platypus	opossum	mouse	marmoset	bonobo	human	10	-1
			sea urchin 2														
			sea urchin 3														
			sea urchin 4														
SLC22A17	C. elegans						zebrafish				opossum	mouse	marmoset	bonobo	human	6	
SLC22A18						ghost shark	zebrafish		chicken	platypus		mouse	marmoset	bonobo	human	8	-1
SLC22A19												mouse				1	
SLC22A20						ghost shark				platypus		mouse	marmoset	bonobo	human	6	-1
SLC22A21												mouse				1	
SLC22A22												mouse				1	
SLC22A23						ghost shark	pufferfish		chicken	platypus	opossum	mouse	marmoset	baboon	human	9	-1
SLC22A24										platypus					human	2	
SLC22A25															human	1	
SLC22A26												mouse				1	
SLC22A27												mouse				1	
SLC22A28												mouse				1	
SLC22A29												mouse				1	
SLC22A30												mouse				1	
SLC22A31					Lamprey	ghost shark	pufferfish		chicken	platypus	opossum		marmoset	baboon	human	8	-1
Total	2	2	5	3	4	10	13	7	11	12	17	25	17	19	23	170	163
																Total Sequences	number of seq. used

Table 2.2: Common names for Table 2.1. This is a list of common names of each accession number used in Table 2.1. In orange are accession numbers annotated as “SLC22-like” In blue are partial sequences and were not included in the phylogeny. In red are sequences not found in GenBank. The totals of each column and row that were used in the phylogeny are also displayed.

Table 3.1 Accession numbers for Figure 1.1.

	Worm	Lamprey	Sharks	Finned fish	Amphibians	Birds	Monotremes	Marsupials	Other placental mammals	Monkeys (new world)	Monkeys(old world)	human	
SLC22A6		JL12567		NP_001133616.1	NP_001087661.			XP_007497796.1	NP_001001143.1		XP_003909713.1	NP_695008.1	15
				NP_996960.1					XP_005631653.1		XP_001160252.1		
									NP_032792.2		NP_001252596.1		
									NP_001001261.1				
									NP_001075596.1				
									NP_058920.1				
SLC22A7			XP_007890506.1	ACI33410.1		NP_001186367.1		XP_007484011.1	NP_001094517.1	XP_002746615.1	XP_003897681.1	NP_006663.2	18
				NP_001077330.1		XP_002195618.1			XP_001918258.2		XP_001137440.1		
									NP_659105.2		NP_001127633.1		
									XP_002914519.1				
									NP_001038082.1				
									NP_001076111.1				
									NP_445989.2				
SLC22A8								XP_003774532.1	NP_001193175.1		XP_003828567.1	NP_004245.2	13
									XP_005631651.1		XP_508510.4		
									XP_001495264.1		NP_001181622.1		
									NP_112471.3		NP_001125961.1		
									NP_999620.1				
									NP_001075590.1				
									NP_112622.1				
SLC22A9									NP_001039471.1		XP_003828570.1	NP_543142.2	7
									XP_533255.2		XP_001160644.1		
									XP_001495463.3				
									XP_002925397.1				
SLC22A10									XP_002699367.1	XP_003734338.1	XP_508511.4	NP_001034841.3	7
									XP_533256.2				
									P_001503029.2				
									XP_002925418.1				
SLC22A11								XP_001367844.2	XP_854924.2		XP_003909652.1	NP_060954.1	7
											ACH92547.1		
											XP_001084980.1		
											NP_001127182.1		
SLC22A12								XP_007506189.1	NP_001271402.1		XP_003909651.1	NP_653186.2	10
									XP_001489890.3		XP_001165302.1		
									NP_033229.3		NP_001258575.1		
									XP_002916755.1				
									NP_001030115.1				
SLC22A13		JL188		NP_001070840.2		XP_418529.3	XP_001521203.2	XP_007500655.1	XP_542706.2	XP_002759794.1	XP_526175.2	NP_004247.2	14
									XP_001488889.1		XP_001087330.1		
									NP_598741.2				
									XP_002914692.1				
									NP_001119757.1				
SLC22A14								XP_007500651	XP_601004.3		XP_001087096.1	NP_004794.2	9
									XP_005634309.1				
									XP_005601107.1				

									NP_001032838.1				
									XP_002914690.1				
									NP_001101663.1				
SLC22A19									EDL33322.1				1
SLC22A20			XP_007910015.1						XP_003993638.1	XP_002755570.2	XP_003828666.2	A6NK97.1	9
									XP_002699386.1		XP_001169267.2		
									XP_854865.2				
									NP_941052.1				
SLC22A22									NP_759010.1				2
									NP_001013969.1				
SLC22A24							XP_007667831.1		XP_008505067.1		XP_009421581.1	Q8N4F4.1	7
									XP_008272574.1		NP_001125361.1		
									NP_620263.1				
SLC22A25											XP_003828572.1	NP_955384.3	2
SLC22A26									NP_666344.1				1
SLC22A27									NP_599017.1				1
SLC22A28									XP_006527242.1				1
SLC22A29									XP_006527123.1				1
SLC22A30									NP_795976.1				1
SLC22A17	AAF73198.1			XP_005994492.1				XP_001380044.1	XP_006776882.1	XP_002753685.1	XP_003808141.1	NP_057693.3	14
				XP_002666780.2					NP_001124227.1		XP_001162356.1		
									XP_005623963.1				
									NP_067526.2				
									XP_008267601.1				
									NP_803156.2				
SLC22A18			XP_007885570	NP_001032462.1		XP_421021.3			NP_032793.2	XP_009006935.1	XP_003816095.1	XP_006725190.1	9
									NP_001004260.1		XP_009457982.1		
SLC22A23			XP_007900861	XP_006004499.1		XP_418968.3	XP_007667285	XP_003340725.1	XP_006931564.1	XP_008992308.1	XP_003897025.1	AAI28582.1	12
				XP_003979178.1					AAH53705.1		XP_009448686.1		
SLC22A31		JL1048	XP_007887754.1	XP_003969973.1			XP_007671059.1	XP_007477376.1	XP_005862737.1	XP_008984632.1	XP_003917369.1	A6NKX4.3	14
				XP_006001096.1					XP_004001564.1		XP_009429710.1		
									XP_005218549.1				
									XP_005620617.1				
	1	3	5	12	1	5	4	10	74	8	36	16	175

Table 3.1 Accession numbers for Figure 1.1. Listed are accession numbers used to generate Figure 1.1. In orange are the “SLC22-like” annotated sequences. The sequences per clade are listed and a grand total is also listed at the bottom right.

Table 3.2: Common names for Table 3.1.

	Worm	Lamprey	Sharks	Finned fish	Amphibians	Birds	Monotremes	Marsupial	Other placental	Monkeys (new world)	Monkeys(old world)	human	
SLC22A6		Lamprey		salmon	frog			opossum	cow		baboon	human	15
				zebrafish					dog		chimpanzee		
									mouse		macaque		
									pig				
									rabbit				
									rat				
SLC22A7			ghost shark	salmon		chicken		opossum	cow	marmoset	baboon	human	18
				zebrafish		zebrafinc			horse		chimpanzee		
									mouse		orangutan		
									panda				
									pig				
									rabbit				
									rat				
SLC22A8								tasdevil	cow		bonobo	human	13
									dog		chimpanzee		
									horse		macaque		
									mouse		orangutan		
									pig				
									rabbit				
									rat				
SLC22A9									cow		bonobo	human	7
									dog		chimpanzee		
									horse				
									panda				
SLC22A10									cow	marmoset	chimpanzee	human	7
									dog				
									horse				
									panda				
SLC22A11								opossum	dog		baboon	human	7
											bushbaby		
											macaque		
											orangutan		
SLC22A12								opossum	dog		baboon	human	10
									horse		chimpanzee		
									mouse		macaque		
									panda				
									rat				
SLC22A13		Lamprey		zebrafish		chicken	platypus	opossum	dog	marmoset	chimpanzee	human	14
									horse		macaque		
									mouse				
									panda				
									rat				
SLC22A14								opossum	cow		macaque	human	9
									dog				
									horse				
									mouse				
									panda				
									rat				
SLC22A19									mouse				1
SLC22A20			ghost shark						cat	marmoset	bonobo	human	9
									cow		chimpanzee		
									dog				
									mouse				
SLC22A22									mouse				2
									rat				
SLC22A24							platypus		horse		chimpanzee	human	7
									rabbit		orangutan		
									rat				
SLC22A25											bonobo	human	2
SLC22A26									mouse				1
SLC22A27									mouse				1
SLC22A28									mouse				1
SLC22A29									mouse				1
SLC22A30									mouse				1
SLC22A17	C. elegans			coelacanth				opossum	bat	marmoset	bonobo	human	14
				zebrafish					cow		chimpanzee		
									dog				
									mouse				
									rabbit				
									rat				
SLC22A18			ghost shark	zebrafish		chicken			mouse	marmoset	bonobo	human	9
									rat		chimpanzee		
SLC22A23			ghost shark	coelacanth		chicken	platypus	opossum	cat	marmoset	baboon	human	12
				pufferfish					mouse		chimpanzee		
SLC22A31		Lamprey	ghost shark	pufferfish			platypus	opossum	bat	marmoset	baboon	human	14
				coelacanth					cat		chimpanzee		
									cow				
									dog				
	1	3	5	12	1	5	4	10	74	8	36	16	175

Table 3.2: Common names for Table 3.1. Listed are common names for table 3.1. In orange are the “SLC22-like” annotated sequences. The sequences per clade are listed and a grand total is also listed at the bottom right.

Table 4.1: Accession numbers for Figure 1.2.

	Invertebrates	Lampre	Sharks	Finned fish	Amphibians	Birds	Monotremes	Marsupials	Other placental mammals	Monkeys (new world)	Monkeys(old world)	human	
	NP_524479.1												
	NP_651238.2												
	Q9U539.3												
	XP_002124941.1												
	XP_003453.1												
	XP_787726.3												
	XP_796008.3												
	XP_783608.3												
SLC22A1 (OCT)							XP_007670399.	XP_001371462.	NP_001094568.1	XP_008993585.1	XP_003898408.1	NP_003048.	15
									XP_004770900.1		XP_001151564.2		
									XP_001491464.4		XP_005551559.1		
									NP_033228.2				
									XP_002925382.1				
									NP_999154.1				
									NP_001075491.1				
									NP_036829.1				
SLC22A2 (OCT)		JL2643	SINCAMP00000022649	NP_998315.1	NP_001087673.	XP_419622.2		XP_001381474.	XP_599284.3	XP_008993586.1	XP_003898410.1	NP_003049.	22
				ACN11115.1		XP_002189094.2			NP_001273890.1		XP_003311617.1		
									XP_001500595.2		XP_005551558.1		
									NP_038695.1		NP_001126767.1		
									XP_002925381.1				
									NP_999067.1				
									NP_001075584.1				
									Q9R0W2.1				
SLC22A3 (OCT)			XP_007896508.1	XP_006012004.1		XP_419620.4		XP_001381481.	XP_002690418.1	XP_003732818.1	XP_003807452.1	NP_068812.	13
									XP_533467.3		XP_001152133.1		
									NP_035525.1		XP_005551557.1		
									NP_062103.1				
SLC22A15 (OCTlike)	XP_003730263.1		XP_007889787.1	XP_007568088.1	Q6NUB3.2	XP_416558.2	XP_007658941.1	XP_001364080.2	NP_001180022.1	XP_002751344.2	XP_003892505.1	NP_060890.2	20
				XP_003963824.1					XP_005630710.1		XP_001148217.1		
				NP_001103169.1					XP_001496384.2		XP_001112207.1		
									NP_001034460.2				
									XP_001927939.2				
									XP_001112207.1				
SLC22A4				ACN60266.1	NP_001088049.	NP_001139603.		XP_001366071.	ACC68852.1	XP_002744655.2	NP_001162226.1	NP_003050.	18
									NP_001193918.1		XP_001163062.1		
									XP_005626571.1		XP_005557778.1		
									XP_005599500.1				
									NP_062661.1				
									XP_002912999.1				
									NP_001139224.1				
									NP_001164817.1				
									NP_071606.1				
SLC22A5			XP_007899456.1	NP_957143.1	NP_001080898.1	NP_001039293.	XP_007668008.	XP_001366136.	NP_001039967.1	XP_008989619.1	NP_001162227.1	NP_003051.	19
									XP_860734.1	ACB21281.1	XP_003310855.1		

									XP_001917996.1		XP_002804555.1		
									NP_035526.1				
									XP_002912941.1				
									NP_062142.1				
									XP_004610019.1				
SLC22A16			XP_007892163.1	NP_001020659.	AAH80416.1	XP_419787.3		XP_007485264.	NP_081848.1	XP_008993154.1	XP_008976203.1	NP_149116.2	17
						XP_002192085.			XP_002927601.1		XP_518688.2		
									XP_532263.2		XP_001088078.1		
									NP_001069792.2				
									XP_005597022.1				
									XP_005652520.1				
SLC22A21									NP_062697.1				1
	9	1	5	9	5	8	3	7	49	8	22	7	133

Table 4.1: Accession numbers for Figure 1.2. Listed are accession numbers used to generate Figure 1.2. In orange are the “SLC22-like” annotated sequences. The sequences per clade are listed and a grand total is also listed at the bottom right. In orange are annotated “SLC22-like” sequences.

Table 4.2 Common names for Table 4.1.

	Invertebrates	Lamprey	Sharks	Finned fish	Amphibians	Birds	Monotremes	Marsupials	Other placental mammals	Monkeys (new world)	Monkeys(old world)	human	
	fly												
	fly												
	C. elegans												
	ciona												
	sea urchin												
	sea urchin												
	sea urchin												
	sea urchin												
SLC22A1 (OCT)							platypus	opossum	cow	marmoset	baboon	human	16
									ferret		chimpanzee		
									horse		macaque		
									mouse				
									panda				
									pig				
									rabbit				
			2						rat				
SLC22A2 (OCT)		Lamprey	ghost shark	zebrafish	frog	chicken		opossum	cow	marmoset	baboon	human	22
				salmon		zebrafinch			dog		chimpanzee		
									horse		macaque		
									mouse		orangutan		
									panda				
									pig				
									rabbit				
									rat				
SLC22A3 (OCT)			ghost shark	coelacanth		chicken		opossum	cow	marmoset	bonobo	human	13
									dog		chimpanzee		
									mouse		macaque		
									rat				
SLC22A15 (OCTlike)	sea urchin		ghost shark	amazon molly	frog	chicken	platypus	opossum	cow	marmoset	baboon	human	19
				pufferfish					dog		chimpanzee		
				zebrafish					horse		macaque		
									mouse				
									pig				
									rat				
SLC22A4				salmon	frog	chicken		opossum	bat	marmoset	baboon	human	18
									cow		chimpanzee		

									dog		macaque		
									horse				
									mouse				
									panda				
									pig				
									rabbit				
									rat				
SLC22A5			ghost shark	zebrafish	frog	chicken	platypus	opossum	cow	marmoset	baboon	human	19
									dog	callicebus	chimpanzee		
									horse		macaque		
									mouse				
									panda				
									rat				
									shrew				
SLC22A16			ghost shark	zebrafsh	frog	chicken		opossum	mouse	marmoset	bonobo	human	17
						zebrafinch			panda		chimpanzee		
									dog		macaque		
									cow				
									horse				
									pig				
SLC22A21									mouse				1
	9	1	6	9	5	8	3	7	49	8	22	7	134

Table 4.2 Common names for Table 3.1. Listed are accession numbers used to generate Figure 4.1. In orange are the “SLC22-like” annotated sequences. The sequences per clade are listed and a grand total is also listed at the bottom right. In orange are annotated “SLC22-like” sequences.

Table 5.1: Conserved motif sequences.

Motif Letter	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Motif Number	Motif 9	Motif 8	Motif 7	Motif 5	Motif 3	Motif 14	Motif 6	Motif 2	Motif 12	Motif 11	Motif 10	Motif 16	Motif 4	Motif 13	Motif 15	Motif 1
SLC22A15	VEEAFQAVGEMGIYQ			TSIASEWFLIAN	GVISFGQLSDRFGRK	LFAIANGFSPSYEFFAVTRF	GIAQYALLGYFIRSWRTLAI	IPESPRWLYSQGRLSEA	SFLDLFRYRVLLGHTLILMF	IWFVCSLVYYGLTLS	SIYANLALSGLIEIP	FGRKRTLSAFLCLGG	IVYIYTSELYPTVIRNVGLG	CSMFSRVGGIIAPFIPSLKY	PFIVFGATGLTSGLLSLLLP	ETLNSPLLETFSDLQVYSYR
SLC22A16	FEGIYDHVGHFGRFQ	IHYLASVFMGVTPHHVCRPP	EYTGSKKEFPCVDGYIYDQN	STAVTQWNLVCD	GSVTFGYFSDRLGRR	LFGIAAAFAVDYYTFMAARF	GTLLVALTGYLVRTWWLYQM	LPETPPWLLSEGRYEEA		IWFTGSLGFYSFSLN	NEYLNLFLLGVVEIP	VGRRTVLAYSLFCSA	LIYLYTAELYPTIVRSLAVG	GSMVCRLASILAPFSVDLSS	PQLFVGTMALLSGVLTLKLP	ETLGKRLATTWEEAAKLESE
SLC22A17				TNAIGQWDLVCD	GYLFLGYPADRFGRR	PCGVGGAAAGSSTGVMALRF	GHFLFLGLALVSKDWRFLQR	FLESARWLIVKRQIEEA	SFASLLNYRNIWKNLLILGF			FGRRGILLLSMTLTG	LSTLLAAEVIPTTVRGRGLG		QHVVLAACALLCILSIMLLP	ETKRKLLPEVLRDGELCRRP
SLC22A18					GGPVFGRFADQRGAR	VVGLAMAWMSSVFHFCLLVP			AIASLLRLPDVPRIFLVKVA							
SLC22A23				QNVVSKWDLVCD	GYLITGCIADWVGRR	LTVALSVNVTMFSTLRFFEG	GQFLMPGLAALCRDWQVLQA	FPESLRWLMATQQFESA	AVGSLSVFFCAEITPTVIRC			LGRRGGLLLFMILTA		AVGSLSVFFCAEITPTVIRC		ESRDQNLPENISNGEHYTRQ
SLC22A31				AAAGPTWNLVCG	GCVILGAGCDRFGRR	GLGASEALAASFPTLLVLRL	GTLLLPGLAALVQDWRLLQG	FPESPCWLLATGQVARA	SPLGLLRTRVTWRNGLILGF			CGRRPVLLLGTMVTG	LSSLFAAEVFPTVIRGAGLG		QQVVFASLAVLALLCVLLLP	ESRSRGLPQSLQDADRLRRS
SLC22A6	FNDLLQQVGGVGRFQ	SHNTLQNFTAAIPTHHCRPP	EANGTGATEPCTDGWIYDNS	STIVTEWDLVCS	GAMVFGYLADRLGRR	VSGTCAAFAPNFPIYCAFRL	GQFLLAGVAYAVPHWRHLQL	FIESARWHSSSGRLDLT	SAMELLRCPTLRHLFLCLSM	LWFATSFAYYGLVMD	SIYLIQVIFGAVDLP	LGRRPAQMAALLLAG	CIFLYTGELYPTMIRQTGMG	GSTMARVGSIVSPLVSMTAE	PLFIYGAVPVAASAVTVLLP	ETLGQPLPDTVQDLESRKGK
SLC22A8	FSEILDRVGSMGHFQ	NHNLLQIFTAATPVHHCRPP	PNDTQRAMEPCLDGWVYNST	DSIVTEWDLVCN	GGLVLGDLSDRFGRR	ASGSGAAFSPTFPIYMVFRF	GQFILPGLAYAIPQWRWLQL	TPESIRWLVLSGKSSKA	TASDLFRIPMLRRMTFCLSL	AWFATGFAYYSLAMG	NLYILQIIFGGVDVP	LGRHTTQAAALLLAG	CLFLYTSELYPTVIRQTGMG	SNLWTRVGSMVSPLVKITGE	PNIIYGITALLGGSAALFLP	ETLNQPLPETIEDLENWSLR
SLC22A12	FSELLDLVGGLGRFQ	TQSMLENFSAAVPSHRCWAP	TSWSEADTEPCVDGWVYDRS	STIVAKWNLVCD	GAAACGPASDRFGRR	VMGTAAAFAPAFPVYCLFRF	GHGLTAAVAYGVRDWTLLQL	LAESARWLLTTGRLDWG	SLGTLLRMPGLRFRTCISTL	CWFAFGFTFFGLALD	NIFLLQMFIGVVDIP	LGRRPTLAASLLLAG	CITIYSSELFPTVLRMTAVG	GQMAARGGAILGPLVRLLGV	PLLVYGTVPVLSGLAALLLP	ETQSLPLPDTIQDVQNQAVK
SLC22A13	FVQVLAEIGDFGRFQ	FYFFAHVFMVLDEPHHCAVA	LSHRFNETQPCDMGWEYPEN	PSLKNEFNLVCD	GTLMFGPLCDRIGRK	LIGLATAFVPSFELYMALRF	GQMVLAGLAYGFRNWRLLQI	LPESARWLLTRGRMDEA	NALDLFRHPQLRKVTLIIFC	VWFVDSLGYYGLSLQ	DVYLTQLIFGAVEVP	FGRKWSQLGTLVLGG	ISYVYSAELFPTILRQTGMG	VGIFSRIGGILTPLVILLGE	PMLIYGSLPIVAGLLCTLLP	ETHGQGLKDTLQDLELGPHP
SLC22A14	FANLLDAVGEFGTFQ		IQFGLNDTDTCQDGWIYPDA	RSLINEFDLVCG	GSLIFRLITDKMGRY	IFGFGTAFMNSFHLYLFFRF	GAVLLTGIAYSLPHWQLLFL	LPESPRWLMMKGKVKEA	SVLDFCKNRQLCKVTLVMSC	VWFTVSYTYFTLSLR	SVHFRHVVPSIMEVP	IGRKWSLAVTLLQAI	VFFLYTAELLPTVLRATGLG			ETRDQPLSESLNHSSQIRNK
SLC22A1	VDDILEQVGESGWFQ	PICVGIVFLGFTPDHHCQSP	TNRSHLPLGPCQDGWVYDTP	SSIVTEFNLVCA	GSLGVGYFADRFGRK	VSGVLMAFSPNYMSMLLFRL	GLVALTGLAYALPHWRWLQL	VPESPRWLLSQKRNTEA	SFADLFRTPRLRKRTFILMY	LWFTDSVLYQGLILH	NLYLDFLYSALVEIP	VGRIYPMAMSNLLAG	MICLVNAELYPTFVRNLGVM	CSSLCDIGGIITPFIVFRLR	PLILFAVLGLLAAGVTLLLP	ETKGVALPETMKDAENLGRK
SLC22A2	VDDVLEHGGEFHFFQ	PIYVGIVFLGFTPDHRCRSP	TNRSRLPLGPCRDGWVYETP	SSIVTEFNLVCA	GSMSIGYIADRFGRK	AAGVLMAISPTYTWMLIFRL	GLLVLAGVAYALPHWRWLQF	IPESPRWLISQNKNAEA	SFLDLVRTPQIRKHTMILMY	NWFTSSVLYQGLIMH	NIYLDFFYSALVEFP	IGRRYPWAASNMVAG	IVCLVNAELYPTFIRNLGVH	CSSMCDIGGIITPFLVYRLT	PLMVFGVLGLVAGGLVLLLP	ETKGKALPETIEEAENMQRP
SLC22A3	FDEALQRVGEFGRFQ		FPNRSAPLVPCRGGWRYAQA	STIVSEFDLVCV	GAFTLGYAADRYGRI	VTGVVVAFAPNFPVFVIFRF	GIIILPGIAYFIPNWQGIQL	VPESPRWLITRKKGDKA	SFLDLVRTPQMRKCTLILMF	AWFTSAVVYQGLVMR	NLYIDFFISGVVELP	LGRRLPFAASNIVAG	IVYLVNSELYPTTLRNFGVS	CSGLCDFGGIIAPFLLFRLA	PLIIFGILASICGGLVMLLP	ETKGIALPETVDDVEKLGSP
SLC22A4	YDEVIAFLGEWGPFQ	FNGMSVVFLAGTPEHRCRVP	VDLGQLEQESCLDGWEFSQD	STVVTEWNLVCE	GSFVSGQLSDRFGRK	VLFATMAVQTGFSFLQIFSI	GYMLLPLFAYFIRDWRMLLL	IPESPRWLISQRRFREA	FILDLFRTRNIAIMTIMSLL	LWMLTSVGYFALSLD	DAYLNCFLSALIEIP	LPRRYIIAAVLFWGG	MLYVFTAELYPTLVRNMAVG	TSTASRVGSIIAPYFVYLGA	PYIVMGSLTVLIGILTLFFP	ESLGMTLPETLEQMQKVKWF
SLC22A5	YDEVTAFLGEWGPFQ	FTGLSSVFLIATPEHRCRVP	VDLGQLEQESCLDGWEFSQD	STIVTEWNLVCE	GSFISGQLSDRFGRK	GFSFLQIFSKNFEMFVVLFV	GYMVLPLFAYFIRDWRMLLV	IPESPRWLISQGRFEEA	NILDLLRTWNIRMVTIMSIM	LWMTISVGYFGLSLD	DIFVNCFLSAMVEVP	LPRRYSMATALFLGG	MVYVYTAELYPTVVRNMGVG	SSTASRLGSILSPYFVYLGA	PYILMGSLTILTAILTLFLP	ESFGTPLPDTIDQMLRVKGM

Table 5.1: Conserved motif sequences. Listed are family conserved motif sequences found in SLC22A15, SLC22A16, SLC22A17, SLC22A23, SLC22A31, SLC22A6, SLC22A8, SLC22A12, SLC22A13, SLC22A14, SLC22A1, SLC22A2, SLC22A3, SLC22A4, and SLC22A5. At the top are the associated letters for each common motif as well as their respective MEME number. In red are the missing motifs.

Table 5.2: Clade unique motif sequences.

SLC22A15 Region	107-119	140-143	171-171	178-197	206-209	262-262	293-293	332-334	350-351	361-362	378-380	382-401	413-416	437-437	533-547		
SLC22A15 Sequence	RSYKVSAASSFFF	KVYL	L	GMSLVAFVLLNECVGTAYWA	FFAV	E	G	LGG	SY	KW	LAC	IVMFLPEKKDTGVFAVVNSH	AAFN	T	EEEFYDADEETQMIK		
SLC22A16 Region	29-35	92-106	150-162	183-186	214-214	221-240	249-252	305-305	344-364	383-385	401-402	410-411	427-429	456-459	480-480		
SLC22A16 Sequence	FQNISCG	NGEIWELSRCSRNKR	RKWLAMLIQPLFM	VVLW	F	GYLVVGFVYVMEFIGMKSRT	FFAV	Q	HNLSYLFYNWSITKRTLTVWL	LGG	AY	DK	LAC	AAFG	S		
SLC22A17 Region	96-104	129-139	160-163	166-185	198-198	269-288	314-324	334-361	387-406	431-445	449-456	500-519					
SLC22A17 Sequence	LGWQVILEQ	GIVLLTLGLVG	LLGF	AGVDLGVYLMRLELCDPTQR	G	MLGEEAQEALQDLENTCPLP	TNFIAHAIRHC	PSDFYLCSLLASGTAALACVFLGVTVDR	DYLNEAAITTFSVLGLFSSQ	LIMALGALGGLSGPA	HMGHGAFL	RQPPPTRCDHVPLLATPNPA					
SLC22A18 Region	148-167	203-204	220-222														
SLC22A18 Sequence	SALGRSSVILLTYVLAATEL	LL	AAL														
SLC22A23 Region	224-233	241-242	258-260	292-292	295-314	327-327	437-447	462-476	488-490	506-507	530-549	570-588	592-611	615-618			
SLC22A23 Sequence	DNAWKVHIAK	IF	PVL	F	AGIILTLYALRIELCPPGKR	A	NSLTGYGIHHC	LENFYADYYTTASIA	RFL	AS	DSVKDKFSIAFSIVGMFASH	GGLGLVLASAGFGMLTAPI	HNQKGYFLHHIIFACCTLIC	ILLL			
SLC22A23 Region	125-133	141-142	158-160	189-192	195-214	227-227	347-357	366-380	392-393	409-411	419-438	463-477	481-488	530-549			
SLC22A31 Sequence	DGWKVPLEQ	LL	AVF	LHGG	AGALLALYLARLELCDPPHR	V	SSLVGGGIRAS	VPTFYLPYFLEAGLE	DC	LAS	QYLPGWTVLFLSVLGLLASR	LVLGAGFLGQAAGPL	HGRQGFFL	LLRGRPRQDHLPLLPPSNSY			
SLC22A6 Region	67-86	115-117	130-142	194-196	201-220	253-259	261-267	285-298	320-324	360-365	414-433						
SLC22A6 Sequence	LPRDRQGQPESCLRFTSPQW	TFP	HRALRQLAQSLYM	LSG	GISLNCMTLNVEWMPIHTRA	LVSAPFF	FFIYSWF	LRALQRVARINGKR	GKGQA	LQGFGV	VIPQDQSIVRTSLAVLGKGC						
SLC22A8 Region	62-81	105-105	118-130	182-184	189-208	241-247	249-255	273-286	308-312	348-353	402-421						
SLC22A8 Sequence	LPMGPNGKPERCLRFVHPPN	K	SNKLKEMAQSIFM	LCG	GITLSTVILNVEWVPTRMRA	TVSIPFF	FFLSSWW	LKILRRVAVFNGKK	AKAKY	VEEFGV	FVPLDLQTVRTVLAVFGKGC						
SLC22A12 Region	77-96	126-128	141-153	205-207	212-231	264-270	272-278	296-309	331-335	371-376	425-444						
SLC22A12 Sequence	IPPGPNQRPHQCRRFRQPQW	IFT	SHALKPMAQSIYL	LLA	GVMMNTGTLLMEWTAARARP	VVSVPFF	CFLYSWW	LQELWRVAAINGKG	GQPPA	LQALGS	LVPHEMGALRSALAVLGLGG						
SLC22A13 Region	1-3	19-20	27-33	54-67	73-90	119-119	133-150	197-201	214-233	269-270	288-288	292-311	358-360	376-377	385-386	402-404	455-455
SLC22A13 Sequence	MAQ	IQ	VLNFLSP	WVKNHTFNLSAAEQ	PLDTAGHPEPCLMFRPPP	R	RKHLKDTTQSVFMAGLLV	AVATA	TEWVGPSWRTQAVVLAQCNF	WA	I	QKAASVNRRKLSPELMNQLV	FGL	AR	QR	LMC	L
SLC22A14 Region	53-68	75-94	96-105	126-128	146-158	174-184	205-210	228-243	264-278	296-301	315-334	371-376	418-424	432-450			
SLC22A14 Sequence	ANLLDAVGEFGTFQQR	IPSIMSAFFMFADHFVFTAQ	PYCNTSWILA	GSF	QFGLNDTDTCQDG	DLVCGMETKKD	TDKMGR	TAFMNSFHLYLFFRFG	VGEHRAHAIILGHCF	WQLLFL	WILPESPRWLMMKGKVKEAK	FCKNRQ	IMEVPAR	QQIGRKWSLAVTLLQAIIW			
SLC22A1 Region	1-3	19-20	54-65	87-106	131-131	144-152	209-228	267-273	275-281	299-314	368-373	398-399	415-417	435-447	468-468	489-494	
SLC22A1 Sequence	MPT	KQ	GVAELSQRCGWS	QCRRYEVDWNQSALSCVDPL	G	DSWKLDLFQ	QGLVSKGNWMAGYTLITEFV	AVSLPTF	FLLYYWC	IKIMDHIAQKNGKLPP	MGATSG	DR	AAC	MCVGRMGITIAIQ	V	EVWQAL	
SLC22A2 Region	2-4	20-21	55-66	88-107	132-132	145-153	210-229	268-274	276-282	300-315	369-374	399-400	416-418	436-448	469-469	490-495	
SLC22A2 Sequence	PTT	KQ	GVAELSLRCGWS	QCRRYEVDWNQSTFDCVDPL	G	NSWMLDLFQ	QGLVSKAGWLIGYILITEFV	TVSLPNF	FLLYYWC	MRIIKHIAKKNGKSLP	MGLAGD	DR	AAC	SCLGRMGITMAYE	I	NIWLEL	
SLC22A3 Region	1-3	19-20	47-66	137-137	150-158	215-234	273-279	281-287	305-320	372-377	402-403	419-421	439-451	472-472	493-498		
SLC22A3 Sequence	MPS	RR	DHYWCRGPSAAALAERCGWS	H	NAWMLDLTQ	QGVFGKGTWMTCYVIVTEIV	AITLPSF	FLLYYWV	LQILRRIAKCNGKYLS	LGIIGG	ER	VAC	ATLGRLGITMAFE	L	AVWLEL		
SLC22A4 Region	1-3	19-20	22-33	54-65	77-96	99-102	138-145	171-171	205-224	239-241	270-276	294-309	363-368	384-394	440-442	484-488	
SLC22A4 Sequence	MRD	RL	FFLLSASIIPNG	DAANLSSAWRNN	VPHSCSRYRLATIANFSALG	PGRD	DNWKVPLT	N	MGQISNYVVAFILGTEILGK	FAV	CVPLWWF	EDIIQKAAKMNNIAVP	APNLHG	AYITAWLLLRT	AFS	YNRML	
SLC22A5 Region	1-3	19-20	22-33	54-65	77-96	99-102	138-145	171-180	205-224	239-241	270-276	294-309	365-370	386-396	442-444	486-490	
SLC22A5 Sequence	MRD	RL	FFLLSASIIPNG	DAANLSSAWRNH	VPHSCRRYRLATIANFSALG	PGRD	DDWKAPLT	NVLFVTMGMQ	MGQISNYVAAFVLGTEILGK	YAF	CVALWWF	EVIIRKAAKANGIVVP	TPNLHG	AYVLAWLLLQY	AFS	YDRFL	

Table 5.2: Clade unique motif sequences. Listed are subclade conserved motif sequences found in SLC22A15, SLC22A16, SLC22A17, SLC22A23, SLC22A31, SLC22A6, SLC22A8, SLC22A12, SLC22A13, SLC22A14, SLC22A1, SLC22A2, SLC22A3, SLC22A4, and SLC22A5. Here, the region that the motifs are found in and the sequences are listed.

Table 6.1: Residue Information – OATs.

hOAT1 table

Residue	Position	Mutation	Effect	Reference	Notes
N	39	Q	Abrogates PAH uptake	Tanaka et al. 2004a	
C	49	A	Reduced sensitivity to PCMBS	Tanaka et al. 2004b	
R	50	H	Little effect on transport	Fujita et al. 2005	
P	104	L	Little effect on transport	Fujita et al. 2005	
C	122	A	Little effect on transport	Tanaka et al. 2004b	
C	172	A	Little effect on transport	Tanaka et al. 2004b	
C	183	A	Little effect on transport	Tanaka et al. 2004b	
C	200	A	Little effect on transport	Tanaka et al. 2004b	
I	226	T	Little effect on transport	Fujita et al. 2005	
A	256	V	Little effect on transport	Fujita et al. 2005	
R	293	W	Little effect on transport	Fujita et al. 2005	
K	431	A	Reduced PAH transport	Perry et al. 2006	
F	438	A	Reduced PAH transport	Perry et al. 2006	
C	440	A	Reduced HgCL2 inhibition	Astorga et al. 2011	
R	454	Q	Non-functional to PAH, OchA, methotrexate	Fujita et al. 2005	
R	466	K	Reduced interaction with chloride	Rizwan et al. 2007	
K	394*	A	Loss of interaction with dicarboxylates but not PAH	Rizwan et al. 2007	*Mutant in flOAT1
R	478*	D	Loss of interaction with dicarboxylates but not PAH	Rizwan et al. 2007	*Mutant in flOAT1
C	78*	A	Little effect on transport	Tanaka et al. 2004b	*Mutant in mOAT1
C	99*	A	Little effect on transport	Tanaka et al. 2004b	*Mutant in mOAT1
C	334*	A	Reduced PAH transport	Tanaka et al. 2004b	*Mutant in mOAT1
C	326**	A	Little effect on transport	Tanaka et al. 2004b	**Mutant in mOAT1, quad mutant
C	379**	A	Reduced PAH transport	Tanaka et al. 2004b	**Mutant in mOAT1, quad mutant
C	427**	A	Reduced PAH transport	Tanaka et al. 2004b	**Mutant in mOAT1, quad mutant
C	434**	A	Reduced PAH transport	Tanaka et al. 2004b	**Mutant in mOAT1, quad mutant
N	92***	Q	Required for transporter activity	Tanaka et al. 2004a	***quadruple mutant
N	56***	Q	Required for transporter activity	Tanaka et al. 2004a	***quadruple mutant
N	86***	Q	Required for transporter activity	Tanaka et al. 2004a	***quadruple mutant
N	113***	Q	Required for transporter activity	Tanaka et al. 2004a	***quadruple mutant

hOAT3 table

Residue	Position	Mutation	Effect	Reference	Notes
R	149	S	Non-functional to ES, OchA, methotrexate	Erdman et. Al 2005	
Q	239	STOP	Non-functional to ES, OchA, methotrexate	Erdman et. Al 2005	
I	260	R	Reduced ES and Cimetidine uptake	Erdman et. Al 2005	
R	277	Y	Reduced ES and Cimetidine uptake	Erdman et. Al 2005	
I	305	F	Reduced ES and Increased Cimetidine uptake	Erdman et. Al 2005	
A	310	V	No effect on ES or Cimetidine uptake	Erdman et. Al 2005	
A	399	S	No effect on ES or Cimetidine uptake	Erdman et. Al 2005	
C	428	A	Limited thiol reactivity	Astorga et. Al 2011	
V	448	I	No effect on ES or Cimetidine uptake	Erdman et. Al 2005	
K	370	A	Increased affinity for MPP+*	Feng et al. 2001	*double mutant K370A/R454D
R	454	D	Increased affinity for MPP+*	Feng et al. 2001	*double mutant K370A/R454D
K	370	A	Increased affinity for MPP+*	Rizwan et al. 2007	*double mutant K370A/R454D
R	454	D,N	Increased affinity for MPP+*	Rizwan et al. 2007	*double mutant K370A/R454D

hURAT1 table

Residue	Position	Mutation	Effect	Reference	Notes
I	75	T	Reduced uric acid uptake	Tasic et al. 2011	
T	217	M	Reduced uric acid uptake	Enomoto et al. 2002	
E	298	D	Reduced uric acid uptake	Enomoto et al. 2002	
R	347	S	Reduced uric acid uptake	Tasic et al. 2011	
V	388	M	No effect on uric acid uptake	Tasic et al. 2011	
R	406	C	Reduced uric acid uptake	Dinour et al. 2011	
R	434	C	Abrogated uric acid uptake	Tasic et al. 2011	
R	434	H	Reduced uric acid uptake	Tasic et al. 2011	
G	444	R	Reduced uric acid uptake	Dinour et al. 2011	

Table 6.1: Residue Information – OATs. Listed here are specific residue information on the 2D models in Figure 3.1. In green are residues with positive results.

Table 6.2 Residue Information – OCTs.

hOCT1 table

Residue	Position	Mutation	Effect	Reference	Notes
S	14	F	Increased MPP uptake	Shu et al. 2003	
S	14	F	Increased metformin uptake	Shu et al. 2007	
R	61	C	Reduced MPP+ uptake	Shu et al. 2003	
R	61	C	Reduced metformin uptake	Shu et al. 2007	
R	61	C	Reduced metformin uptake	Kerb et al. 2002	
L	85	F	No effect on MPP+ uptake	Shu et al. 2003	
C	88	R	Reduced metformin uptake	Kerb et al. 2002	
Q	97	K	Reduced metformin uptake	Chen L et al. 2010	
P	117	L	Reduced metformin uptake	Chen L et al. 2010	
L	160	F	Slight Reduced MPP+ uptake	Shu et al. 2003	
L	160	F	No effect on metformin uptake	Shu et al. 2007	
F	160	L	No effect on MPP uptake	Sakata et al. 2004	
S	189	L	No effect on MPP+ uptake	Shu et al. 2003	
S	189	L	Reduced metformin uptake	Shu et al. 2007	
R	206	C	Reduced metformin uptake	Chen L et al. 2010	
G	220	V	Reduced MPP+ uptake	Shu et al. 2003	
G	220	V	Reduced metformin uptake	Shu et al. 2007	
P	283	L	Abrogated TEA and MPP+ uptake	Sakata et al. 2004	
P	287	G	Abrogated TEA and MPP+ uptake	Sakata et al. 2004	
C	322	A	Critical for choline binding	Sturm et al. 2007	Mutant in rOCT1
P	341	L	Reduced MPP+ uptake	Shu et al. 2003	
P	341	L	No effect on metformin uptake	Shu et al. 2007	
P	341	L	Reduced TEA uptake	Sakata et al. 2004	
R	342	H	No effect on MPP+ uptake	Shu et al. 2003	
R	342	H	No effect on metformin uptake	Shu et al. 2007	
G	401	S	Reduced MPP+ uptake	Shu et al. 2003	
G	401	S	Reduced metformin uptake	Shu et al. 2007	
G	401	S	Reduced MPP+ uptake	Kerb et al. 2002	
M	408	V	No effect on MPP+ uptake	Shu et al. 2003	
V	408	M	No effect on metformin uptake	Shu et al. 2007	
M	420	DEL	No effect on MPP+ uptake	Shu et al. 2003	
M	420	DEL	Reduced metformin uptake	Shu et al. 2007	
M	440	I	No effect on MPP+ uptake	Shu et al. 2003	
A	443	I	Critical for substrate binding	Gorboulev et al. 2005	Mutant in rOCT1
L	447	Y	Critical for substrate binding	Gorboulev et al. 2005	Mutant in rOCT1
Q	448	E	Critical for substrate binding	Gorboulev et al. 2005	Mutant in rOCT1
C	451	A	Critical for choline binding	Sturm et al. 2007	Mutant in rOCT1
V	461	I	No effect on MPP+ uptake	Shu et al. 2003	
G	465	R	Reduced MPP+ uptake	Shu et al. 2003	
G	465	R	Reduced metformin uptake	Shu et al. 2007	
D	475	E	Increased TEA, NMN and choline uptake	Gorboulev et al. 1999	Mutant in rOCT1
R	488	M	No effect on MPP+ uptake	Shu et al. 2003	
R	488	M	No effect on metformin uptake	Shu et al. 2007	

hOCT2 table

Residue	Position	Mutation	Effect	Reference	Notes
C	51	A	Reduced MPP+ uptake	Pelis et al. 2012	
C	63	A	Reduced MPP+ uptake	Pelis et al. 2012	
C	89	A	Reduced MPP+ uptake	Pelis et al. 2012	
C	103	A	Reduced MPP+ uptake	Pelis et al. 2012	
C	143	A	Reduced MPP+ uptake	Pelis et al. 2012	
M	165	I	Altered drug* interactions	Leabman et al. 2002	*Metformin, Phenformin, Procainamide, Quinidine
T	199	I	Reduced metformin uptake	Song et al. 2008	
T	201	M	Reduced metformin uptake	Song et al. 2008	
A	270	S	Enhanced metformin transport	Chen Y et al. 2009	
A	270	S	Altered drug* interactions	Leabman et al. 2002	*Metformin, Phenformin, Procainamide, Quinidine
A	270	S	Increased picoplatin uptake	More et al. 2010	
R	400	C	Altered drug* interactions	Leabman et al. 2002	*Metformin, Phenformin, Procainamide, Quinidine
K	432	Q	Altered drug* interactions	Leabman et al. 2002	*Metformin, Phenformin, Procainamide, Quinidine
Y	447	L	Increased corticosterone IC50	Gorboulev et al. 2005	Mutant in rOCT2
E	447	K,R	Abrogated PAH and TEA transport	Zhang et aL. 2005	Mutant in rbOCT2
E	447	L	Reduced TEA transport, retaining MTMA transport	Zhang et aL. 2005	Mutant in rbOCT2
Q	448	E	Increased corticosterone IC50	Gorboulev et al. 2005	Mutant in rOCT2
C	451	A	Critical for choline binding	Sturm et al. 2007	Mutant in rOCT2
C	474	A	Responsible for TEA binding	Pelis et al. 2012	
N	353*	L	No effect on cimetidine and MTMA	Zhang et aL. 2005	Mutant in rbOCT2,*double mutant decreased affinity
R	403*	I	No effect on cimetidine and MTMA	Zhang et aL. 2005	Mutant in rbOCT2,*double mutant decreased affinity

Table 6.2 Residue Information – OCTs. Listed here are specific residue information on the 2D models in Figure 3.2. In green are residues with positive results.

Table 6.3 Residue Information – OCTNs.

hOCTN2 table

Residue	Position	Mutation	Effect	Reference	Notes
R	169	W	Abrogates carnitine transport	Wang et al. 2000b	
G	242	V	Abrogates carnitine transport	Wang et al. 2000b	
W	283	R	Reduces carnitine transport	Mayatepek et al. 2000	
A	301	D	Abrogates carnitine transport	Wang et al. 2000b	
R	341	A	Reduces carnitine transport	Amat di San Filippo et al. 2003	
W	351	R	Reduces carnitine transport	Wang et al. 2000b	
L	409	W	Reduces carnitine transport	Amat di San Filippo et al. 2003	
T	429	I	Reduces carnitine transport	Amat di San Filippo et al. 2003	
V	446	F	Reduces carnitine transport	Mayatepek et al. 2000	
E	452	K	Reduces carnitine transport	Wang et al. 2000a	
E	452	Q,D,A,K	Reduces carnitine transport	Yuhuan et al. 2000	
P	478	L	Abrogates carnitine transport	Tang et al. 1999	
D	165*	G	Reduced ergothioneine transport	Urban et al. 2007	*mapped from hOCTN1
L	505*	F	Increases affinity for TEA	Peltekova et al. 2004	*mapped from hOCTN1
L	505*	F	Lowered acetylcholine efflux	Pochini et al. 2012	*mapped from hOCTN1

Table 6.3 Residue Information – OCTNs. Listed here are specific residue information on the 2D models in Figure 3.3. In green are residues with positive results

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