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Identification of Sodium-Ion–Dependent Neurotransmitter Transporters among Protozoa Parasite Genomes: Structure, Function and Prospects for Drug Discovery

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Abstract Insect-transmitted pathogenic protozoa cause widespread and debilitating diseases in man and his domestic livestock. Malaria, leishmaniasis and trypanosomiasis cause significant morbidity and mortality. A few members of the group, e.g. *Toxoplasma gondii*, are also important in disease of immunocompromised individuals. There are no vaccines against these diseases and most of the available drug treatments are toxic and/or ineffective making drug development a priority. The genomes of many of these protozoan parasites have recently been sequenced, allowing rational design of targeted therapies. Sodium-ion-dependent neurotransmitter transporters play important roles in the physiology of many organisms including protozoan parasites making them ideal candidates as therapeutic targets. In the present study, the analysis of 25 genera of eukaryotic pathogen genomes is described. We show the existence within their genomes of genes encoding putative homologues of sodium-ion-dependent neurotransmitter transporters. Excluding *T. gondii*, we discovered that all protozoan parasites we examined lack genes that encode for sodium-ion-dependent neurotransmitter transporters. Therefore *T. gondii* sodium-ion-dependent neurotransmitter transporter homologues may represent a parasite specific novel target for drug discovery. Furthermore, sequence alignment and evolutionary differences between humans and *T. gondii* may allow pathogen-specific targeting of the transporter homologues identified.
Keywords Sodium-ion-dependent neurotransmitter transporter; Eukaryotic pathogens; *Toxoplasma gondii*; Genomics

Introduction

Despite the recent increase in funding and improved measures introduced for the control of infectious diseases caused by protozoan parasites, many members still exert a heavy toll on human health and those of his livestock (Fletcher et al., 2012). Apicomplexan members of the group cause important diseases such as: Malaria (*Plasmodium* sp), Babesiosis (*Babesia* sp), Cryptosporidiosis (*Cryptosporidium* sp) and Toxoplasmosis (*Toxoplasma gondii*) (Arisue and Hashimoto, 2015). In addition to apicomplexans, diseases caused by trypanosomatid parasites include: Human African Trypanosomiasis (*Trypanosoma* sp), Chagas disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania* sp.) (Docampo and Huang, 2014). Other diseases caused by protozoa parasites include giardiasis (*Giardia intestinalis*), dysentery (*Entamoeba histolytica*) and trichomoniasis (*Trichomonas vaginalis*) (Turkeltaub et al., 2015). For many of these parasites an effective vaccine is lacking for control (Petersen et al., 2011; Castillo et al., 201l), some current drugs of choice for treatment have significant side effects, can be often ineffective and are prone to the emergence of drug resistant strains (Monzote and Siddiq, 2011; Petersen et al., 2011; Castillo et al., 2010). Therefore identifying new targets and developing new drugs against these targets is a priority for effective control. In recent years, many of the genomes of these parasites have been sequenced and have provided a wealth of putative targets (Aaron et al., 2010). This has allowed from their genomes the identification of proteins that can become targets for novel drugs (Garcia, 2011; Fadiel et al., 2009; Prole and Taylor, 2011; Wiser, 2011).

Sodium-ion-dependent neurotransmitter transporters are a class of membrane transport proteins that span

the cellular membranes of neurons (Garcia, 2011). Their primary function is to carry neurotransmitters across these membranes and to direct their further transport to specific intracellular locations. In Sodium-ion-dependent neurotransmitter transporters, neurotransmitters are co-transported with Na+ using the energy stored in transmembrane electrochemical gradients generated by primary ion pumps (Kanner, 1983). Sodium-ion–dependent neurotransmitter transporters transport serotonin, norepinephrine, and dopamine in the presynaptic plasma membrane. They also terminate neuronal signal transmission in the central nervous system through a reuptake mechanism (Nelson, 1998; Torres et al., 2003; Blakely et al., 2005; Iversen, 2006). These systems have been shown to modulate mood, emotion, sleep, and appetite. Besides, they have been implicated in the control of numerous behavioural and physiological functions (Schloss and Williams, 1998). The termination of neurotransmission is achieved by rapid uptake of the released neurotransmitter by specific high-affinity neurotransmitter transporters. Most of these transporters are encoded by a family of genes (Na^+/Cl^- transporters), which has similar membrane topography of 12 transmembrane helices (Nelson, 1998). The presence of the neurotransmitters in animals has now been confirmed for all taxa from Protozoa to Mammals (Rudnick and Clark, 1993). Neurotransmitter transporters have also been identified and studied in *Schistosoma* spp (Ribeiro and Patocka, 2013), however identification of these transporters has not been reported in any protozoan parasite. In bacteria, Singh et al., (2007) identified an antidepressant-binding site in a bacterial homologue of neurotransmitter transporters.

In this report, we show that genes encoding homologues of Sodium-ion-dependent neurotransmitter exist only in *T. gondii* among the many protozoan genomes we examined. We use comparisons of *T. gondii* (protozoa), *Micromonas* sp (green plant), (*Megachile rotundata*) insect, (*Lottia gigantean*) mollusc, and human homologues of sodium-ion-dependent neurotransmitter to identify a conserved region that may be involved in the conduction of ions, or gating. We, suggest that specific targeting of these transporters may be a novel therapeutic strategy in the control of *T. gondii*.

1 Materials and Methods

1.1 Genome Analysis, Sequence Alignments and Topology Analysis

The genomes of the following 25 eukaryotes were searched for Sodium-ion-dependent neurotransmitter transporters; *Acanthamoeba* and *Entamoeba* from AmoebaDB 4.0 (8 May 2014); *Cryptosporidium* from CryptoDB 6.0 (30 January 2014); *Giardia* from GiardiaDB 4.0 (8 May 2014); *Anncaliia, Edhazardia, Encephalitozoon, Enterocytozoon, Hamiltosporidium, Nematocida, Nosema, Vavraia* and *Vittaforma* from MicrosporidiaDB 7.0 (8 May, 2014); *Babesia* and *Theileria* from PiroplasmaDB 5.0 (30 January, 2014); *Plasmodium* from PlasmoDB 11.1 (May 2014); *Eimeria, Gregarina, Neospora* and *Toxoplasma* from ToxoDB 11.0 (8 May 2014); *Trichomonas* from TrichDB (30 January 2014); *Crithidia, Endotrypanum, Leishmania* and *Trypanosoma* from TriTrypDB 8.0 (8 May 2014). All the genome databases are found under EupathDB version 21 (Aurrecoechea et al., 2006). The identified proteins were retrieved and converted to FASTA format using the webserver tool (Dereeper et al., 2010).

All Physical and chemical parameters such as the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydrophobicity (GRAVY) for the proteins were predicted using a webserver tool, ProtParam (http://web.expasy.org/protparam/). The solubility status of the proteins was computed using PROSO (Smialowski et al., 2007). Several procedures ensured that hits were probable Sodium-ion–dependent neurotransmitter transporter homologues. Firstly, the occurrence of multiple putative TMDs was confirmed using OCTOPUS (Viklund and Elofsson, 2008). Secondly, reciprocal BLASTP searches (non-redundant protein database at of the National Center for Biotechnology [NCBI]) were undertaken, using identified parasite hits as bait, and only proteins that gave the original target protein family as hits were analyzed further. Finally *Homo sapiens, Lottia gigantea, Megachile rotundata* and

Micromonas sp. proteins that are highly similar to sodium-ion-dependent neurotransmitter transporter of *T. gondii* were identified and retrieved. Thirdly, conserved domains were identified using the Conserved Domains Database (NCBI). For phylogenetic analysis, multiple sequence alignments were constructed with MUSCLE v3.7 using default parameters. After using GBLOCKS at low stringency to remove regions of low confidence, and removal of gaps, maximum likelihood analysis was undertaken using PhyML v3.0 (WAG substitution model; 4 substitution rate categories; default estimated gamma distribution parameters; default estimated proportions of invariable sites; 100 bootstrapped data sets). Phylogenetic trees are shown using TreeDyn (v198.3). MUSCLE, GBLOCKS, PhyML and TreeDyn are all functions of Phylogeny.fr. Geneious software version 7.1.7 was employed in the final alignment by using cluster algorithm and identification of hydrophilic, hydrophobic, and conservation and typical secondary structural pattern of these sequences by EMBOSS tools for secondary structure prediction (Kearse et al., 2012). The same software was used in identification of similarity and relatedness presented in percent identity matrix (PIM).

2 Results

A total of nine eukaryotic pathogen Sodium-ion–dependent neurotransmitter transporter (TGGT1_208420, TGGT1_264870 and TGGT1_314340 in *T. gondii* GT1; TGME49_208420, TGME49_264870 and TGME49_314340 in *T. gondii* ME49; TGVEG_208420, TGVEG_264870 and TGVEG_314340 in *T. gondii* VEG) genes were identified and retrieved from the Eukaryotic Pathogen Database. All these transporters were identified in *T. gondii* ME49, *T. gondii* GT1 and *T. gondii* VEG strains from ToxoDB genome. TGGT1_208420, TGME49_208420 and TGVEG_208420; TGGT1_264870, TGME49_264870 and TGVEG_264870 and TGGT1_314340, TGME49_314340 and TGVEG_314340 are located on chromosomes 1, 9 and 11 respectively in the *T. gondii* genome. Similar protein sequences with the NCBI accession number NP_001165975.1, ESO97939.1, XP_003705345.1 and XP_002507431.1 from the following organisms; *Homo sapiens, Lottia gigantea, Megachile rotundata* and *Micromonas* sp were also analyzed.

The various physico-chemical properties of the Sodium-ion–dependent neurotransmitter transporter proteins from *T. gondii* strains are presented in Table 1. The net charge of each of the proteins in respect to their corresponding isoelectric point show that all the proteins are positively charged at different alkalinity states. From the extinction coefficient and instability index values we computed, TGGT1_314340, TGME49_314340 and TGVEG_314340 have the highest values and the TGGT1_264870, TGME49_264870 and TGVEG_264870 have the lowest. All the proteins have the same half-life of 30 hours. The aliphatic index obtained show that TGGT1_264870, TGME49_264870 and TGVEG_264870 have the highest values while TGGT1_314340, TGME49_314340 and TGVEG_314340 have the lowest values. The solubility status of the proteins shows that TGGT1_314340, TGME49_314340 and TGVEG_314340 are the only soluble proteins, while the others are insoluble.

Figure 1 shows sequence alignment of the *T gondii* sodium-ion–dependent neurotransmitter transporter proteins compared with that from *H. sapiens*, *L. gigantea*, *M. rotundata* and *Micromonas* sp. For hydrophobicity prediction: Red bars in the aligned sequences represent hydrophobic regions while the sky blue bars represent hydrophilic regions. For secondary structure predictions; blue tubes represent alpha helices, yellow arrows represent beta strand, grey lines represent coils and pink arrows represent turns. For transmembrane; the green represent the regions that are cytoplasmic or extracellular while the dark red is the transmembrane region of the sequence. Black lines in the sequence alignment represent the conserved regions. The transmembrane helices of the proteins are as follows: TGGT1_208420, TGME49_208420 and TGVEG_208420, 15; TGGT1_264870, TGME49_264870 and TGVEG_264870, 14; while TGGT1_314340, TGME49_314340 and TGVEG_314340 have 15, 14 and 12 transmembrane helices respectively. While *H. sapiens*, *L. gigantea*, *M. rotundata* and *Micromonas* sp have 12, 14, 12 and 14 transmembrane helices respectively. None of the identified proteins have signal peptide sequences.

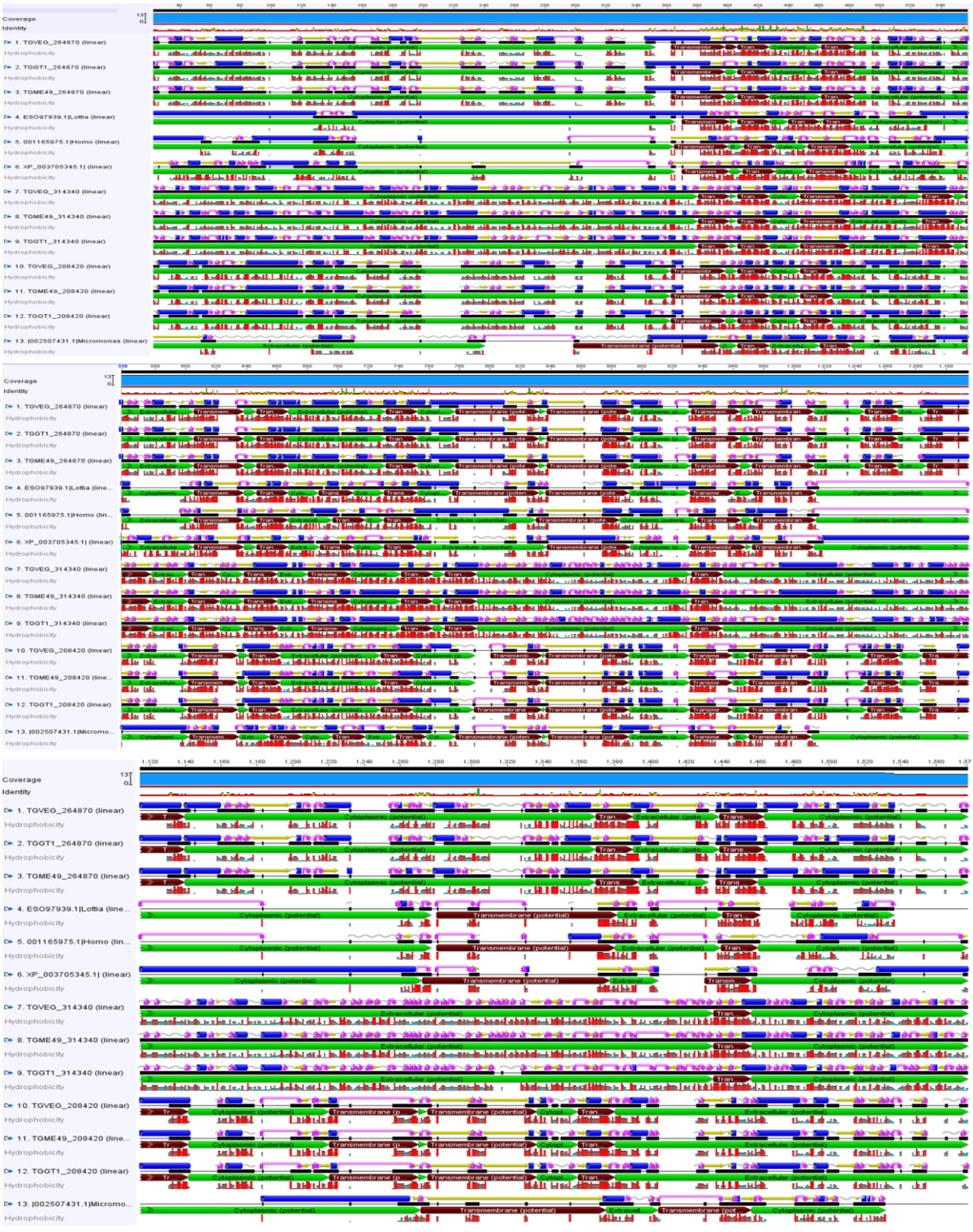

Figure 1 Aligned sequence of neurotransmitter transporter proteins in *T. gondii*. For hydrophobicity prediction: Red bars in the aligned sequences represents hydrophobic regions while the sky blue represent hydrophilic regions. For secondary structure predictions; blue tubes represent alpha helices, yellow arrow represent beta strand, grey line represent coils and while pink arrows represent turns. For transmembrane, the green represented the regions that is cytoplasmic or extracellular while the dark red is transmembrane region of the sequence. While conserved regions in the alignment is represented in black line in each sequence

Table 2 shows percent identity matrix of *T. gondii* Sodium-ion–dependent neurotransmitter transporters. We observed that TGGT1_208420, TGME49_208420 and TGVEG_208420; TGGT1_264870, TGME49_264870 and TGVEG_264870; TGGT1_314340, TGME49_314340 and TGVEG_314340 show a very high percentage of similarity with percent identity of 99.81, 99.32 and 98.53 respectively. Among these three groups, the closest are TGGT1_264870, TGME49_264870 and TGVEG_264870 with about 16.3% identity. The similarities of the other parasite proteins analyzed were very small. The transmembrane topology (Figure 1), phytogenic tree and conserved domain (Figure 2) also support these similarities between the toxoplasma Sodium-ion–dependent neurotransmitter transporters and put these proteins into three groups; with each group containing three members. Results from Table 2 indicate that TGGT1_208420, TGME49_208420 and TGVEG_208420; TGGT1_264870, TGME49_264870 and TGVEG_264870 has SLC5-6-like_sdb superfamily conserved domain representing the solute carrier 6 subfamily, while TGGT1_314340, TGME49_314340 and TGVEG_314340 has the SLC6sbd_NTT5 which represent neurotransmitter transporter 5; solute-binding domain. *L. gigantea*, *M. rotundata* both have SLC5-6-like_sdb superfamily conserved domain. While *H. sapiens* has the SLC6sdb_NET, whcih represents Na(+)- and Cl(-)-dependent Norepinephrine Transporter (NET). While *Micromonas* sp. has SLC6sdb_u2, which represents sodium- and chloride-dependent neurotransmitter transporter family.

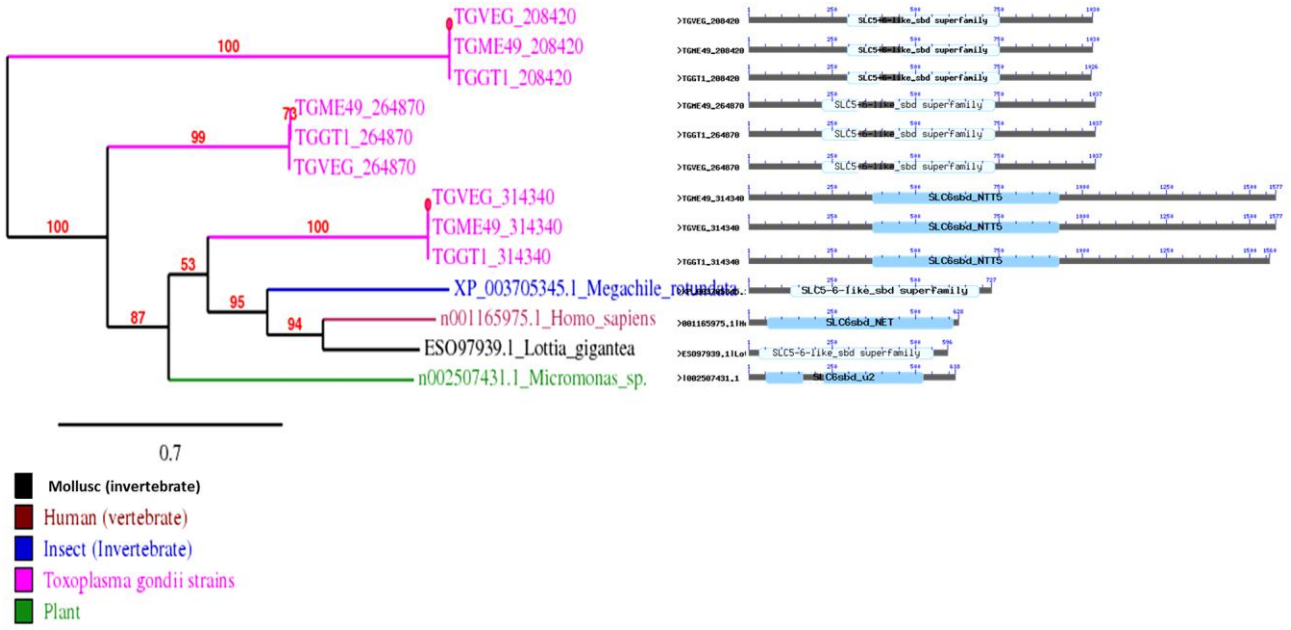

Figure 2 Phylogenetic analysis with corresponding domains. A, phylogram based Maximum Likelihood method. SLC6sbd_NTT5 represent Neurotransmitter transporter 5; solute-binding domain, SLC5-6-like_sdb superfamily represents solute carrier 6 subfamily, SLC6sdb_NET represents Na(+)- and Cl(-)-dependent norepinephrine transporter NET and SLC6sdb_u2 represents sodium- and chloride-dependent neurotransmitter transporter family

3 Discussion

Sodium-ion-dependent neurotransmitter transporters play important roles in the physiology of many organisms including pathogens. The pharmacological potential of these targets may add to the present number of limited protozoan drug targets (Lagrue and Poulin 2010). A paucity of published information on protozoan Sodium neurotransmitter transporters provided a justification to search a host of protozoan parasite genome databases in a bid to identify some of these transporters. Of all protozoan genomes examined, only *T. gondii* strains contain genes encoding for Sodium-ion–dependent neurotransmitter transporters, suggesting that these putative

transporters may not be widespread, which may imply a conserved physiological function for these transporters in Toxoplasma (Gross, 2007; McLean et al., 2011). The absence of these transporters from the other protozoa parasites analyzed may suggest that because these parasites primarily use proton motive force transport, they have seized to require these transporters (Prole and Taylor, 2011). Most of the putative Sodium-ion–dependent neurotransmitter transporters identified in this work are not yet fully annotated in available pathogen databases (http://eupathdb.org/). Experimental studies will be required to confirm the expression and function of these proteins in parasites. Neurotransmitter transporters belong to a class of membrane transport proteins that span the cell membranes. They are attributed to biological processes, cellular component and molecular functions (Camon et al., 2003b; Binns et al., 2009; Gene Ontology Consortium, 2012). They penetrate at least one phospholipid bilayer of membranes indicating that all or part of their peptide sequence is embedded in membranes. Consequently, their position in the membrane help in directing the movement of signals in and out of a cell or between cells and also to catalyse the transfer of solutes across the membrane (Iversen, 2000, Camon et al., 2003a).

Our discovery of these transporters only in *T. gondii* strains, might explain why *T. gondii* can establish a persistent infection in the central nervous system in its hosts, including humans. The identification of these transporters in *T. gondii* may assist in understanding how the parasite manipulates its host's behaviour and cause schizophrenia, since the mechanism(s) responsible for behavioural changes in the host is truly unknown (Lagrue and Poulin 2010; Prandovszky et al., 2011).

The results from the sequence alignment show that these proteins are amphitropic that they exist in two alternative states: a water-soluble and a lipid bilayer-bound (Travaglini-Allocatelli et al., 2009). The Transmembrane helices (TMHs) we predicted here show that neurotransmitter transporters have no equal number of TMH but rather have TMH ranging from 12 to 15. These TMHs come together to form a pore, which transports the neurotransmitters. The 12 TMHs of *T. gondii* Sodium-ion-dependent neurotransmitter; TGGT1_314340, TGME49_314340 and TGVEG_314340 in this report is similar to that obtained by Yamashita and colleagues (2005) who worked on the crystal structure of the bacterial homologue of Na^+/Cl^- dependent neurotransmitter transporters and showed that they posses 12 TMHs. The only non-parasitic organism that shares the same number of TMHs with TGGT1_314340, TGME49_314340 and TGVEG_314340 is *H. sapiens* (Yamashita et al., 2005). Nelson, (1998) suggested that it was likely that the structure of Na^+/Cl^- transporters contains 12 transmembrane helices. However, other neurotransmitter transporter proteins identified in our work have TMHs above 12. Most membrane proteins have being predicted to have 12 TMHs including Na^+/glucose transporter (Hediger et al., 1987), the passive facilitative glucose transporter and the voltage dependent K^+ channels. Genomic and bioinformatic studies of most neurotransporters have shown that they comprise a new super-family of proteins (Worrall and Williams, 1994). The solvent accessibility predicted from the sequences identified from our work show that a larger portion of these proteins are flexible (Karplus and Schulz, 1985; Vihinen et al., 1994) and this is of great practical interest because solvent accessibility gives the measure of the contact surface area and chemical properties of the protein, and this accounts for van der Waals forces and solvation free energy of the protein (Carugo, 2000; Eyal et al., 2004). This may have implications for drug discovery (Eyal et al., 2004).

The neurotransmitter transporter proteins identified in *T. gondii* exhibit high percent identity, as revealed by the percent identity matrix. There is a very high level of identity within the TGGT1_208420 and TGME49_208420, TGGT1_264870 and TGME49_264870, and TGGT1_314340 and TGME49_314340. This level of similarity reflects the highly conserved nature of these proteins, which are often required for basic cellular function, stability or reproduction (Gross, 2007). The highly conserved nature of the gene products of the Na~/Cl transporter family has also been reported (Nelson and Lill, 1994; Uhl and Johnson, 1994). In this work the conservation of protein structures observed in the transporters

identified indicate functional similarity (Gross, 2007). Sequence similarities serve as evidence for structural and functional conservation, as well as of evolutionary relationships between the sequences and hence organisms (Gross, 2007; McLean et al., 2011). Among the most highly conserved sequences are active sites of enzymes and the binding sites of protein receptors, which may be involved in channel conductance or gating (McLean et al., 2011).

The alignment of the conserved regions within each protein as shown by our phylogenic analysis, indicates that proteins with similar functional domain cluster together and may function very similarly. Their channel conductance may therefore be in similar positions (Calin et al., 2007). From the phylograms, the most similar proteins identified shared similar ancestral nodes and are closely related.

TGGT1_314340, TGME49_314340 and TGVEG_314340 have SLC6sbd_NTT5 conserved domain which is a neurotransmitter transporter 5; solute-binding domain. In Humans, the SLC6A16 gene encodes NTT5. NTT5 is expressed in the testis, pancreas, and prostate; its expression is predominantly intracellular, indicative of a vesicular location. However, its substrates are unknown. This subgroup belongs to the solute carrier 6-transporter family (SLC6) (Farmer et al., 2000). TGGT1_264870, TGME49_264870, TGVEG_264870, TGGT1_208420, TGME49_208420 and TGVEG_208420 have SLC5-6-like_sbd superfamily conserved domain, which we also found in *Lottia gigantea*, *Megachile rotundata*. This domain is shared by *T. gondii* and the invertebrates, it is a eukaryotic solute carrier 6 subfamily; solute-binding domain (Rudnick, 2011). SLC6 proteins (also called the sodium- and chloride-dependent neurotransmitter transporter family or Na+/Cl--dependent transporter family) include neurotransmitter transporters (NTTs): these are sodium- and chloride-dependent plasma membrane transporters for the monoamine neurotransmitters serotonin (5-hydroxytryptamine), dopamine, and norepinephrine, and the amino acid neurotransmitters GABA and glycine (Kristensen et al., 2011; Lee et al., 2011). These NTTs are widely expressed in the mammalian brain, and are involved in regulating neurotransmitter signaling and homeostasis, and are the target of a range of therapeutic drugs for the treatment of psychiatric diseases. Bacterial members of the SLC6 family include the LeuT amino acid transporter (Kristensen et al., 2011). The conserved domain in the *H. sapiens* is a Na(+)- and Cl(-)-dependent norepinephrine transporter NET; solute-binding domain. NET (also called NAT1, NET1), is a transmembrane transporter that transports the neurotransmitter norepinephrine from synaptic spaces into presynaptic neurons (Guptaroy et al., 2011). The SLC6A2 gene encodes human NET; NET is expressed in brain, peripheral nervous system, adrenal gland, and placenta (Kim et al., 2010). NET may play a role in diseases or disorders including depression, orthostatic intolerance, anorexia nervosa, cardiovascular diseases, alcoholism, and attention-deficit hyperactivity disorder (Kristensen et al., 2011; Kohli et al., 2011).

In conclusion in most organisms, some neurotransmitter transporters have been implicated as important sites for drug action. However the structural basis of this transporter in eukaryotic pathogens is not completely known, neither the pore-forming region nor the active sites defined. Therefore, the conservation, hydrophobic, hydrophilic, solvent accessibility and secondary structure predicted in this work can help in understanding the mechanism by which these transporters function in *T. gondii* a representative eukaryotic protozoa. We suggest further studies that will prove the drug target potentiality of these transporters. The evolutionary position, history and relationship of these proteins can also help in identifying transporters with similar structure and function in other organisms.

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Table 1 Physical and chemical parameters of Neurotransmitter transporters proteins in different T. *gondii* strains

		TGME49_208420	TGME49_264870	TGME49_314340	TGGT1_208420	TGGT1_264870	TGGT1_314340	TGVEG_208420	TGVEG_264870	TGVEG_314340
1.	Number of amino acids	1030	1037	1577	1026	1037	1560	1030	1037	1577
2.	Molecular weight	113409.5	109080.0	165858.8	113014.1	109004.9	164321.1	113409.5	109033.9	165858.8
3.	Theoretical pI	8.87	8.55	5.93	8.83	8.39	5.90	8.87	8.31	5.93
4.	Total number of negatively charged residues (Asp + Glu)	71	66	149	71	67	149	71	68	149
5.	Total number of positively charged residues (Arg + Lys)	86	75	134	85	74	134	86	74	134
6.	Formula	$C_{5182}H_{7954}N_{1366}O_{1397}S_{52}$	$C_{4956}H_{7702}N_{1310}O_{1385}S_{40}$	$C_{7273}H_{11440}N_{2042}O_{2274}S_{62}$	$C_{5167}H_{7921}N_{1361}O_{1390}S_{52}$	$C_{4957}H_{7697}N_{1303}O_{1386}S_{40}$	$C_{7203}H_{11328}N_{2022}O_{2253}S_{63}$	$C_{5182}H_{7954}N_{1366}O_{1397}S_{52}$	$C_{4959}H_{7700}N_{1302}O_{1387}S_{40}$	$C_{7273}H_{11440}N_{2042}O_{2274}S_{62}$
7.	Total number of atoms	15951	15393	23091	15891	15383	22869	15951	15388	23091
8.	Ext. coefficient	157565	127780	151850	157565	126290	151850	157565	126290	151850
9.	Estimated half-life (hrs)	30	30	30	30	30		30	30	30
10.	Instability index	42.33	55.49	69.36	42.32	55.48	68.00	42.33	55.61	69.36
11.	Aliphatic index	92.72	95.09	75.12	92.70	95.00	75.12	92.72	95.18	75.12
12.	Grand average of hydropathicity (GRAVY)	0.267	0.401	-0.124	0.274	0.410	-0.121	0.267	0.412	-0.124
13.	solubility	insoluble; 0.363	insoluble; 0.392	soluble; 0.608	insoluble; 0.369	insoluble; 0.409	soluble; 0.619	insoluble; 0.363	insoluble; 0.416	soluble; 0.608

Table 2 Percent identity matrix analysis of *T. gondii* strains, green plant, insect, mollusc, and human homologues of sodium- ion–dependent neurotransmitter transporters

	TGVEG_264870	TGGT1_264870	TGME49_264870	ESO97939.1|Lottia	001165975.1|Homo	XP_003705345.1|	TGVEG_314340	TGME49_314340	TGGT1_314340	TGVEG_208420	TGME49_208420	TGGT1_208420	002507431.1|Micromonas
TGVEG_264870		99.5	98.8	14.4	16.3	16.6	16.6	16.6	16.5	16.5	16.5	16.5	16
TGGT1_264870	99.5		99.3	14.6	16.3	16.5	16.6	16.6	16.5	16.4	16.4	16.4	16
TGME49_264870	98.8	99.3		14.5	16.3	16.4	16.7	16.7	16.6	16.5	16.5	16.5	15.9
ESO97939.1|Lottia	14.4	14.6	14.5		43.1	26.8	6.7	6.7	6.7	8.5	8.5	8.4	25.1
001165975.1|Homo	16.3	16.3	16.3	43.1		27.2	7.7	7.7	7.7	9.9	9.9	10	23.9
XP_003705345.1|	16.6	16.5	16.4	26.8	27.2		8.4	8.4	8.5	10.5	10.5	10.7	19.9
TGVEG_314340	16.6	16.6	16.7	6.7	7.7	8.4		100	97.4	10.8	10.8	10.8	6.3
TGME49_314340	16.6	16.6	16.7	6.7	7.7	8.4	100		97.4	10.8	10.8	10.8	6.3
TGGT1_314340	16.5	16.5	16.6	6.7	7.7	8.5	97.4	97.4		11	11	11	6.3
TGVEG_208420	16.5	16.4	16.5	8.5	9.9	10.5	10.8	10.8	11		100	98.8	8.9
TGME49_208420	16.5	16.4	16.5	8.5	9.9	10.5	10.8	10.8	11	100		98.8	8.9
TGGT1_208420	16.5	16.4	16.5	8.4	10	10.7	10.8	10.8	11	98.8	98.8		9
002507431.1|Micromonas	16	16	15.9	25.1	23.9	19.9	6.3	6.3	6.3	8.9	8.9	9	