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RESEARCH NOTE

In Silico Screening of Schistosome Membrane Proteins as Candidate Diagnostic Antigens for Asian Schistosomiasis

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Asian schistosomiasis is primarily caused by *Schistosoma japonicum*. Given the relatively low prevalence of the disease, efforts are now focused on its elimination. However, the low sensitivity of the current diagnostic techniques underestimates the actual prevalence of schistosomiasis in areas with low endemicity. Therefore, this study aims to identify candidate diagnostic antigens that are potentially useful for the development of a reliable serological diagnosis of Asian schistosomiasis using the genomic databases of *S. haematobium* and *S. japonicum*. On the SchistoDB database, this study first employed a preliminary genomic filtration on 12,021 *S. haematobium* genes – including the presence of transmembrane domain, signal peptides, isoelectric point, and molecular weight yielding 113 genes. Further gene ontology (GO) enrichment analysis resulted in 22 candidate *S. haematobium* genes. These *S. haematobium* genes were then compared with the *S. japonicum* genome in the WormBase ParaSite, identifying 36 *S. japonicum* homologous proteins. The amino acid sequences of the 36 proteins were aligned using ClustalW to identify identical proteins, resulting in 21 distinct *S. japonicum* proteins considered as the final set of candidate diagnostic antigens. Therefore, these 21 candidate diagnostic antigens can be useful in developing a reliable serological diagnosis of Asian schistosomiasis, which may be accomplished through *in vitro* validation, protein characterization, and serological evaluation.

Schistosoma japonicum is the main species causing intestinal schistosomiasis in Asia. In the Philippines, schistosomiasis is known to be endemic in 28 out of the 81 provinces of the Philippines and spatially distributed among 12 regions (Leonardo *et al*. 2015). Due to the continuous mass drug administration, some of these endemic areas are now nearing elimination having a low prevalence in humans (Inobaya *et al.* 2018). The low sensitivity of microscopy used in the diagnosis of schistosomiasis in these endemic areas underestimates the actual prevalence of schistosomiasis in areas with low endemicity. Thus, the use of serological techniques is then recommended in these areas. However, currently available serological tests use crude antigens, which may result in cross-reactivity with other helminthiases, low sensitivity, and inability to distinguish past from active infections (Carvalho *et al*. 2017). Recent advances in serological assays have addressed these diagnostic disadvantages, such as the use of schistosome-derived recombinant proteins (Angeles *et al*. 2011; Hinz *et al*. 2017). Several recombinant proteins have been already evaluated for their diagnostic potential in Asian schistosomiasis and showed promising sensitivity and specificity for both humans (Angeles *et al.* 2011) and animals (Angeles *et al*. 2012, 2019). However, discrepancies in the diagnostic sensitivities were seen in the performance of the recombinant antigens in detecting schistosome infection in different endemic geographical

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areas (Angeles *et al*. 2020). Therefore, there is still a need to analyze other candidate proteins for their diagnostic use in schistosomiasis.

Due to their direct exposure to the host's immune response, schistosome tegument and membrane proteins have become ideal targets n for the development of an accurate serodiagnostic test. Twenty-eight (28) percent of the proteins analyzed using *S. mansoni* tegumental sub-proteome were reported to lack sequence similarity to any other protein in any species other than schistosomes (van Balkom *et al*. 2005), suggesting that tegument proteins may be highly antigenic. This study aims to identify candidate diagnostic proteins from the tegument of *S. japonicum* in the available genomic database using bioinformatic tools.

SchistoDB, an online resource for genomic data for the *Schistosoma* parasite (Zerlotini *et al.* 2012), was used for the screening of schistosome genes. This database provides data-mining capabilities of both comparative and functional genomic data which grant researchers to apply a technique known as genomic filtering, allowing the identification of certain gene products based on a combined set of criteria under the same principles of antigen mining (Zerlotini *et al*. 2009, 2013). However, the current version of SchistoDB only features the *S. haematobium* genome data, which may limit the study from missing out on possible useful antigens from *S. japonicum*. The genome sequence used for *S. haematobium* has accession no. PRJNA78265 (Stroehlein *et al.* 2019). Genomic filtration of *S. haematobium* genes was done by intersecting the following criteria available in the database: [1] presence of transmembrane domains, [2] predicted signal peptides, [3] isoelectric points of 5.5–8, and [4] molecular weights of 10–50 kDa. This resulted in identifying 113 *S. haematobium* genes. These genes were then narrowed down by using GO enrichment analysis for selecting genes that can be found as an integral component of the schistosome's membrane, as dictated by the extracellular or plasmatic membrane localization criterion. GO enrichment further screened the genes into 22 *S. haematobium* genes.

To ensure that the chosen genes have a low similarity in the human genome, nucleotide BLAST (BLASTN) analysis was performed on the National Center for Biotechnology and Information database. All genes showed no significant similarity with the human genome. Then, BLASTP was performed in the WormBase ParaSite using the amino acid sequences of the selected *S. haematobium* genes to identify homologous proteins in *S. japonicum*. Two available *S. japonicum* genomes were used in the analysis with accession nos. PRJEA34885 (*Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium 2009) and PRJNA520774 (Luo *et al*. 2019). For each amino acid sequence of *S. haematobium* analyzed, the resulting *S. japonicum* homologs with the highest percent identity (% ID) were included in the final list of candidate antigens for *S. japonicum*. BLASTP was then able to identify 36 *S. japonicum* homologous proteins in the WormBase ParaSite.

The identified *S. japonicum* candidate diagnostic antigens were grouped according to their protein family. Multiple sequence alignment using ClustalW was then done for *S. japonicum* proteins found to be homologous to the same *S. haematobium* amino acid sequence to confirm whether these proteins are the same. Sequence alignment was also done for the proteins belonging to the same protein family to confirm that each one is distinct from one another. Sequence alignment analysis was able to differentiate 21 *S. japonicum* proteins, listed in Table 1, which could be further analyzed *in vitro* for their diagnostic potential.

Table 1. The 21 *S. japonicum* candidate diagnostic antigens and their genomic locations, product prescriptions (protein families), and *S. haematobium* homologs.

***S. haematobium* gene ID**	**Homologous *S. japonicum* gene ID**	**Genomic location**	**Product description (protein family)**	**Known characteristics**
MS3_01370	*EWB00_001015.1*	Scaffold_140 1245152–1245349 (–)	Tetraspanin	Tetraspanins Sj-tsp-1, Sj-tsp-8, Sj-tsp-14, and Sj-tsp-26 have shown to be potential protective antigens (Wu *et al.* 2011).
	EWB00_001015.2			
MS3_01730	*EWB00_006997.1*	Scaffold_408 620610–620786 (–)		
	EWB00_006997.2			
MS3_02232	*Sjp_0016930*	SJC_S000081 413160–421441 (–)		
	EWB00_003303	Scaffold_205 1283447–1291610 (–)		
MS3_09789	*Sjp_0083380*	SJC_S001704 115007–115340 (+)		
MS3_05289	*EWB00_006390*	Scaffold_396 1375477–1377427 (–)	Tetraspanin (putative tetraspanin similar to uroplakin 1)	

Table 1. Cont.

S. haematobium gene ID	Homologous *S. japonicum* gene ID	Genomic location	Product description (protein family)	Known characteristics
MS3_09198	*EWB00_004892.1* *EWB00_004892.2*	Scaffold_321 95209–95545 (–)	23 kDa (2.3 x 10^4 g/mol) integral membrane protein, tetraspanin	Sj23 was reported to have an immunomodulatory function, which includes the ability to suppress host responses to other parasite antigens and to polarize the host immune system to produce non-protective IgG2a antibodies (Jiang *et al.* 2010).
MS3_07058	*EWB00_005148.1* *EWB00_005148.2*	Scaffold_341 667903–673289 (–)	Sodium/potassium/calcium exchanger 4 (NCKX4 or SLC24A4)	NCKX4 is a potassium-dependent Na+/Ca2+ exchanger that transports four ions of sodium in exchange for one ion of calcium and one ion of potassium (Stephan *et al.* 2012).
MS3_00606	*Sjp_0029070* *EWB00_010540*	SJC_S000184 20106–20234 (+) Scaffold_814 14209–14337 (–)	Solute carrier family 35 member (SLC35F1/F2/F6)	Solute carrier family 35 (SLC35F1/F2/F6) protein is a known nucleotide sugar transporter (Hadley *et al.* 2019).
MS3_02758	*EWB00_007622.4*	Scaffold_425 936105–936164 (–)	Solute carrier family 2, facilitated glucose transporter member 3; sugar/inositol transporter; major facilitator superfamily (SLC2A3 or GLUT3)	GLUT3, also known as the neuronal GLUT, is more specifically associated with the transport of glucose, mannose, galactose, xylose, 2-deoxyglucose, fucose, and dehydroascorbate in the brain (Simpson *et al.* 2008).
MS3_11437	*Sjp_0028910* *EWB00_003676*	SJC_S000182 86294–86779 (–) Scaffold_222 127600–128085 (–)	Solute carrier family 43 members 3; major facilitator superfamily (SLC43A3)	
MS3_00861	*Sjp_0053290*	SJC_S000528 22363–22467 (+)	Major facilitator superfamily; sugar transporter-like	
MS3_06093	*Sjp_0013750*	SJC_S008058 14461–15027 (–)	Major facilitator superfamily	
	EWB00_005320.1 *EWB00_005320.2*	Scaffold_357 416110 Scaffold_357 406758–416061 (–)	Protein spinster 1; major facilitator superfamily	Protein spinster 1 homolog of the MFS has varying functions such as transporting sphingolipids and involvement in endosome and lysosome storage (*S. japonicum* Genome Sequencing and Functional Analysis Consortium 2009).
MS3_05373	*EWB00_005349*	Scaffold_359 406416–411758 (+)	Zinc transporter 6; cation efflux transmembrane domain superfamily (ZnT-6)	ZnT-6 is a cation efflux protein with increased tolerance towards zinc metal ions, mediating its transport across cell membranes, and was shown to be expressed predominantly in female adult stages (Schulte *et al.* 2011).

Table 1. Cont.

S. haematobium gene ID	Homologous *S. japonicum* gene ID	Genomic location	Product description (protein family)	Known characteristics
MS3_10729	*Sjp_0127360*	SJC_S021615 477–2102 (+)	Cytochrome c oxidase subunit I (COX1), copper binding site	COX1 is a proton-pumping heme-copper oxidase located in the inner membrane of the mitochondrion and is a multi-pass membrane protein that specifically functions to catalyze the reduction of oxygen to water (Gao *et al.* 2012).
	Sjp_0126290	SJC_S020357 466–1147 (–)	NADH: ubiquinone oxidoreductase, subunit 1/ F420H2 oxidoreductase subunit H; cytochrome c oxidase subunit I	NADH: ubiquinone oxidoreductase, subunit 1/ F420H2 oxidoreductase subunit H is part of the assembly required for catalysis and electron transfer process from NADH to the respiratory chain wherein the immediate electron acceptor for the enzyme is ubiquinone (Walker 1992).
	Sjp_0124510	SJC_S017625 920–985 (–)		
MS3_10879	*EWB00_006105*	Scaffold_391 547139–547198 (–)	Gamma-secretase subunit Aph-1	This enzyme is a multi-pass membrane protein and an enzyme that mainly functions as an endoprotease complex that catalyzes the intramembrane cleavage of integral proteins including Notch receptors like lin-12 or glp-1 (Zhang *et al.* 2014).
	Sjp_0114800	SJC_S008418 9451–9510 (+)		
MS3_01878	*Sjp_0025050*	SJC_S000146 545088–545453 (+)	G protein-coupled receptor, rhodopsin-like, 7TM	GPCRs are known to be important in innate immunity through immunomodulation responses of the worm, *C. elegans* (Venkatesh and Singh 2021).
	EWB00_006475	Scaffold_398 301986 –302351 (–)		
MS3_08313	*Sjp_0082170*	SJC_S001624 67226–68101 (–)		
MS3_09781	*EWB00_005519.4*	Scaffold_363 755112–769953 (–)	B-cell receptor-associated protein 29/31 (BAP29/31)	Its main functions are quality control and sorting, and the regulation of intracellular protein transport between the endoplasmic reticulum and the Golgi complex (Annaert *et al.* 1997)
	EWB00_005519.2			
	EWB00_005519.5			
MS3_06630	*Sjp_0133010*	SJC_S026712 370–6175 (+)	Neural proliferation differentiation control-1 (NPDC-1)	In *C. elegans*, the C-terminal of one of its proteins called CAB-1 is an ortholog of NPDC-1, which is said to be involved in chemical synaptic transmission, positive regulation of anterior or posterior axon guidance, and ventral cord development (Iwasaki and Toyonaga 2000).
MS3_07576	*Sjp_0030970*	SJC_S000198 91535–91597 (+)	Concanavalin A-like lectin/glucanase, EGF-like calcium-binding domain	Lectins/glucanases are a diverse group of proteins containing concanavalin A-like (con A) domains, which play a role in cell recognition and adhesion (Gough *et al.* 2001).
MS3_03458	*Sjp_0061850*	SJC_S000716 49385–49468 (–)	Hypothetical protein	

The 21 candidate diagnostic antigens derived from the antigen mining of the *S. haematobium* and *S. japonicum* genomes include tetraspanin, transmembrane transporters, cytochrome c oxidase subunit I, gamma-secretase subunit APH-1, rhodopsin-like G protein-coupled receptor, B-cell receptor-associated protein 29/31 (BAP29/31), neural proliferation differentiation control-1 (NPDC-1), and concanavalin A-like lectin/glucanase with EGF-like calcium-binding domain. Most of these identified proteins are not yet fully characterized in any schistosome species except for tetraspanin (Jiang *et al*. 2010) and zinc-transporter (Schulte *et al*. 2011).

With the search for candidate antigens accomplished by the proteins derived from the antigen mining of the *S. haematobium* and *S. japonicum* genomes, further research and analyses may be done through *in vitro* means. Priority candidate antigens could be identified through their constitutive expression in the intra-mammalian stages of *S. japonicum*.

To conclude, this *in silico* study has identified 21 candidate diagnostic antigens, which may be used in the development of a highly sensitive and specific immunodiagnostic test for the detection of *S. japonicum*. This could contribute to improved surveillance of Asian schistosomiasis in areas of low endemicity having individuals with low intensity of infection.

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