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# Regulatory networks, genes and glycerophospholipid biosynthesis pathway in schistosomiasis: A systems biology view for pharmacological intervention

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ABSTRACT

Understanding network topology through embracing the global dynamical regulation of genes in an active state space rather than traditional one-gene–one trait approach facilitates the rational drug development process. Schistosomiasis, a neglected tropical disease, has glycerophospholipids as abundant molecules present on its surface. Lack of effective clinical solutions to treat pathogens encourages us to carry out systems-level studies that could contribute to the development of an effective therapy. Development of a strategy for identifying drug targets by combined genome-scale metabolic network and essentiality analyses through in silico approaches provides tantalizing opportunity to investigate the role of protein/substrate metabolism. A genome-scale metabolic network model reconstruction represents choline–phosphate cytidyltransferase as the rate limiting enzyme and regulates the rate of phosphatidylcholine (PC) biosynthesis. The uptake of choline was regulated by choline concentration, promoting the regulation of phosphocholine synthesis. In *Schistosoma*, the change in developmental stage could result from the availability of choline, hampering its developmental cycle. There are no structural reports for this protein. In order to inhibit the activity of choline–phosphate cytidyltransferase (CCT), it was modeled by homology modeling using 1COZ as the template from *Bacillus subtilis*. The transition-state stabilization and catalytic residues were mapped as 'HXGH' and 'RTEGISTT' motif. CCT catalyzes the formation of CDP-choline from phosphocholine in which nucleotidyltransferase adds CTP to phosphocholine. The presence of phosphocholine permits the parasite to survive in an immunologically hostile environment. This feature endeavors development of an inhibitor specific for cytidyltransferase in *Schistosoma*. Flavonolignans were used to inhibit this activity in which hydnowightin showed the highest affinity as compared to miltefosine.



## 1. Introduction

A prerequisite for understanding any disease is acquiring knowledge of its component interactions between the host–parasite systems. This is possible with the available sequence and omics' data obtained through various high throughput platforms. The volumes of data generated can be analyzed to identify individual component interactions that make up a system, which can be analyzed within a mathematical framework to identify possible targets for new drug discovery (Kitano, 2002).

Under this backdrop our work represents abstracting the complex lipid biosynthetic process in schistosomiasis for novel drug target identification. Schistosomiasis is a tropical disease caused by trematode belonging to the species *Schistosoma*. Control of schistosomiasis is challenging as more than 600 million people in 76 developing countries are at risk and with 200 million people already affected by this deadly disease (Berriman et al., 2009). The intestinal form of the disease is caused by *Schistosoma mansoni*. The availability of genomic data of the intestinal form of the parasite opened new avenues to understand disease novel pathophysiology that can be harnessed to progression of the disease (Useh, 2013). The parasite enters human, a definitive host (snail: *Biomphalaria glabrata*, being the intermediate host) by dissolving the skin barrier and migrates towards the liver and lungs to lay eggs. These eggs are lodged within the blood capillaries which is a major cause of pathology. Praziquantel (Biltricide®) is the effective monotherapy to control schistosomiasis but reports of drug resistance and tolerance have limited its use (Doenhoff et al., 2008). Designing new drug becomes difficult due to the rapid proliferation and changing surface variants of the parasite. One of the important surface components involved in renewal of membrane complex is associated with lipid

*Abbreviations:* PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; GPC, glyceryl phosphorylcholine; CTP, cytidine triphosphate; CCT, choline–phosphate cytidyltransferase; CK, choline kinase; ADP, adenine diphosphate; DAG, diacylglycerol; AAG, alkyl-acylglycerol; IL, interleukin; DC, dendritic cells; Lyso-PC, lysophosphatidylcholine; FBA, flux balance analysis; GEM, genome-scale model; FBA, flux based analysis; MD, Molecular Dynamic; SPC, Simple Point Charge; NVT, Number of moles, Volume, Temperature; NPT, Number of moles, Pressure, Temperature; PME, Particle Mesh Ewald; RMSD, root mean square deviations; RMSF, root mean square fluctuations; LGA, Lamarckian Genetic Algorithm; PE, potential energy.

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metabolism (Ramakrishnan et al., 2012). Impairment reduces these pathogens' ability to proceed in its infectious life cycle and therefore could be a possible source of drug target (Marechal, 2011). Dissection of lipid metabolism using lipidomics in *S. mansoni* can provide cues on the alteration in the lipid pool of parasites that is associated with drug resistance (Lewis et al., 1996). Metabolic profile of *S. mansoni* infected mice liver indicates lower levels of glucose and glycogen but higher levels of choline metabolites, (phosphatidylcholine (PC), glyceryl phosphorylcholine (GPC), and alanine) than in controls suggesting that *Schistosoma* can suppress the metabolic response of the host (Wu et al., 2010). Many evidence suggests that lipids are essential in the life cycle of the parasite, and its regulation mechanisms are largely unknown (Loukas and Maizels, 2000). Glycerophospholipids are one such class of lipids in *Schistosoma* which accounts for PC (25%), phosphatidylserine (PS—15%) and phosphatidylethanolamine (PE—8%). Fatty acids are differentially incorporated into the various phospholipid classes, principally into PC and, to a lesser extent, into PE, lyso-PC, and PS (Furlong and Caulfield, 1989). They show immune modulation properties e.g. PS induces dendritic cells (DC) to polarize IL-4/IL-10 producing T-cells. Whereas lysophosphatidylserine (lyso-PS) specifies DC to induce IL-10 secreting regulatory T cells, thus swaying the immune system away from a protective Th1 immune response (Hewitson et al., 2009a, 2009b). Lysophosphatidylcholine (Lyso-PC) is known to adhere, immobilize and lyse the red blood cell to alter the cell plasma membrane, thereby neutralizing the attacking host cells. It promotes membrane fusion with the host resulting in the acquisition of host membrane components by the parasite. The presence of lyso-PC may alter antigen presentation and immune recognition of the parasite antigens (Golan et al., 1986). These immune modulating lipids confer on the parasite the major adaptation mechanism, allowing survival in an immunologically hostile environment within the host (Schmid-Hempel, 2009). Also PCs are essential in the development of *Schistosoma*, as large amount is needed for a rapid membrane renewal and to renew its outer bilayer surface. The synthesis of PC in *S. mansoni* adults occurs via the Kennedy pathway (de novo synthesis of PC) consisting of three enzymatic steps shown in Fig. 1 (Young and Podesta, 1982). In protozoan parasites like *Trypanosoma brucei*, *Plasmodium falciparum* Kennedy pathways have already been validated as a drug target (Ancelin et al., 2003; Cui and Houweling, 2002; Gibellini and Smith, 2010).

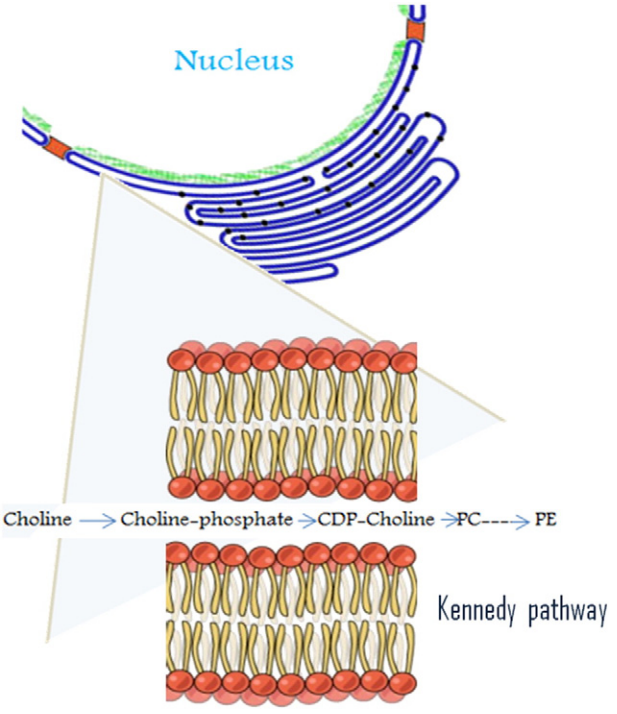


**Fig. 1.** Biosynthesis of phosphatidylcholine.

In order to understand the highly redundant phospholipid pathway and to establish a mechanism to identify newer drug targets a kinetic model was developed in *S. mansoni*. Lipid mining connects the lipid profiles with the metabolic pathways during different stages of disease progression. This may allow deciphering the complexities and regulatory mechanisms involved in maintaining lipid homeostasis in diseased condition. Potential drug targets can also be identified that may be important regulatory hubs in the lipid biosynthesis. Designing small molecule inhibitors against these targets may be valuable 'schistosomicides'. Our current understanding of Kennedy pathway in *Schistosoma* development and pathogenesis could help to develop anti-schistosomal therapies targeting lipid metabolic pathways. We have attempted to delineate lipid metabolism specifically PC biosynthetic pathway in parasite and identify key regulatory enzymes. These were screened against natural compounds like flavonolignan derivatives as potential lead compounds for possible drug design.

## 2. Material and Methods

### 2.1. Metabolic Network Analysis

To address the current challenge of metabolic network biology and understand how organisms adapt to their environment to maintain optimal growth in changing environmental conditions the mathematical model was optimized for PC biomass production. The built model represents a schematic way to unravel the molecular components that underlie cellular processes with the available concentration of metabolites and kinetic data from KEGG database (Kanehisa and Goto, 2000) and Biocyc database (Caspi et al., 2010). The metabolic network was designed using CellDesigner v4.3 (Funahashi et al., 2008; Kitano et al., 2005). The detail work flow is shown in Fig. 2. Reactions in the metabolic network are connected via gene–protein-reaction (GPR) mechanism where the cofactors are considered to be independent of time and thus are not directly considered for the study. The boundary conditions of genes are constant. The reactions in the network are under the control of several kinetic laws like Hill–Hinze, convenience kinetics, and Michaelis–Menten (MM).

Numerical simulation of the built model identifies essential genes/metabolites vital for cell growth under the imposed constraint. The sensitivity of the flux and the topology of the network are studied by summarizing the stoichiometric matrix using a Copasi tool (Hoops et al., 2006). The stoichiometry of the reaction network is described mathematically as a stoichiometric matrix with rows representing reactants and columns corresponding to the reaction deduced by flux balance analysis (FBA) and ExPa (Bell and Palsson, 2005). In ExPa, the topology of the metabolic network is summarized as a stoichiometric coefficient matrix ($S_{ij}$) in which, the rate of change of concentration of a metabolite is the sum of metabolite flow to and from the metabolite represented as $S_{ij} < 0$ when consumed and $S_{ij} > 0$ when produced. An Optim tool box of Matlab version 7.12.0.35 (R2011a) finds the minimum and maximum objective parameters required to get the optimal solution of a linear equation determined by the feasible boundary region (Lee et al., 2005).

### 2.2. Sequence Retrieval, Model Building and Evaluation

The target identified from network analysis was modeled by homology modeling protocol to get an insight into the functional characteristic of the protein. The sequence of choline–phosphate cytidyltransferase (CCT) was retrieved from Uniprot database (Uniprot ID: G4VD67_SCHMA) and blast against RCSB PDB (The Research Collaboratory for Structural Bioinformatics Protein Data Bank) to obtain a template for protein modeling. The PDB ID: 1COZ which is a prototypical glycerol-3-phosphate cytidyltransferase (GCT) from *B. subtilis* was used as a template to model CCT. Fifty models of CCT were generated by modeler v9.10