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Using Protein-Protein Interaction Network to Prioritize Drug Targets for Malaria

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ABSTRACT

Malaria is caused by the parasites of genus Plasmodium. Among five parasite species known to cause malaria in humans, *P. falciparum* and *P. vivax* are responsible for the majority of malaria cases. Malaria turns out to be a severe global health threat, affecting around 225 million people every year. Emergence of multidrug resistance continues to be a major problem in the treatment of malaria. To encounter this problem, it is essential to identify promising therapeutic drug targets to develop new drugs. A systems level analysis of malarial drug targets within the proteome of *P. falciparum* has the potential to provide insights into the dynamic interactions between them. In this study, therapeutic drug targets of malaria were retrieved from target DB. High confidence interactions of the drug targets were obtained using STRING. This was followed by the construction of a protein protein interaction network of *P. falciparum* drug targets and their interacting partners. Cluster analysis of this network revealed important proteins that are essential for survival of the pathogen. Such proteins were not included in targetDB. Proteins such as ring infected erythrocyte surface antigen, Erythrocyte binding antigen-175 among others were identified as potential drug target candidates and should be given priority for future drug development projects.

KEYWORDS: *Plasmodium falciparum*, therapeutic drug target, drug resistance, pathogenesis, systems biology.

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INTRODUCTION

Malaria remains a major global health concern in endemic countries. Clinical symptoms of malaria are high fever, headache, shaking chills and vomiting. The parasites responsible for causing malaria are from genus *Plasmodium*. Five species such as *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* cause the disease in humans. Among these, *P. falciparum* and *P. vivax* are known to be the most widespread and devastating species. Malaria is one of the major vector-borne diseases in India. Around 80.5% of Indian population live in malaria risk areas. Every year, there are 1.5-2 million cases of malaria are reported. These cases are divided evenly between *P. falciparum* and *P. vivax*[1].

One of the most important challenges in fighting malaria is the emergence of drug resistance. Chloroquine has been the drug of choice in India and other parts of the world. However, the development of chloroquine-resistance against *P. falciparum* has made it unsuitable for treating malaria[2]. Another drug sulphadoxine used in combination with pyrimethamine is effective against *P. falciparum* however some studies have reported resistance against this treatment in Northeastern parts of India. Quinine is although effective in treating malaria, but it produces several side effects with oral and parenteral use. Other concerns associated with antimalaria drugs include patient compliance, side effects, safety in pregnant women, infants and children. These issues call for search for more potent and less toxic therapeutics to combat malaria. Thus, there is an urgent need to revisit the molecular mechanism underlying the disease and the drug targets used for designing antimalarials.

In recent years, numerous studies have reported the success of systems level approaches to understand the behaviour of a complex biological system. Such approaches have been applied in different aspects such as defining the protein protein interaction network, predicting the host-pathogen interactions, revealing drug resistance pathways[3,4,5]. In this paper, we have attempted to unveil the most important proteins that are critical for survival of the pathogen using protein protein interaction network. Such proteins should be the top priority therapeutic drug targets for consideration during development of antimalarial drugs.

METHODOLOGY

Therapeutic drug targets specific for malaria were retrieved from TargetDb[6]. Annotations of these target proteins were also obtained. Interacting partners of these drug targets were predicted from STRING database[7]. *Plasmodium falciparum* was selected as organism. STRING database includes both physical and functional interactions derived from various sources such as co-

expression analysis, co-existence, detection of shared signals across genomes, gene neighbourhood, text-mining of scientific literature, interaction between organisms based on orthology. A combined score is assigned to each pair of proteins based on confidence scores obtained by benchmarking the performance of the predictions against a common reference set of true interactions. A higher score interaction indicates that the interaction is supported by various types of evidence. To include only high confidence predictions, the interactions having combined score more than 0.8 were selected. A protein protein interaction network of Malaria drug targets and their interacting partners was constructed using Cytoscape[8]. Network analyzer plugin was used to compute degree, clustering coefficient, number of self-loops and various other parameters for every node in the network. The shortest paths between all pairs of nodes were determined. A subset of densely connected network and overlapping regions within the protein protein interaction network were selected using ClusterOne plugin provided alongwith Cytoscape. The plugin searches for various groups with high cohesiveness based on the minimum density, minimum size, parameters and edge weights. The minimum size of the cluster was set as 10.

RESULTS AND DISCUSSION

Firstly, therapeutic drug targets for malaria were obtained from TargetDb. Total 32 unique drug targets were retrieved from the database. List of these proteins is given in Table 1. Interacting partners of all the drug targets were predicted from STRING database. Only high confidence interactions with the combined score of more than 0.8 were considered. These interactions were compiled in a single file. The file was used to prepare an input file for Cytoscape. Interactions of therapeutic drug targets with other proteins of *P. falciparum*, discerned from the STRING database, were used to construct a protein protein interaction network with the help of Cytoscape. Such network would provide the insights into the spread of malarial drug targets within the protein-protein interactome of *P. falciparum*. The network captures various types of interactions provided in the STRING database which includes direct as well as indirect associations. Thus, this network represents a comprehensive view of highly evident associations of drug targets within the *P. falciparum* proteome.

Table No. 1 : "List of malaria drug targets obtained from targetDB"

S. No.	Drug Target	S. No.	Drug Target
1	Fructose-1,6-Bisphosphate Aldolase	17	Pfg27
2	D-UTPase	18	Triosephosphate Isomerase
3	Hydrolase	19	Phosphoglycerate Kinase
4	Dihydrooretate Dihydrogenase	20	Proplasmepsin Ii
5	Putative Ribulose 5-Phosphate3-Epimerase	21	Plasmepsin Ii
6	Thioredoxin	22	Plasmesin Iv
7	Purine Nucleoside Phosphorylase	23	Merozoite Surface Protein 1 (Msp-1)
8	Kinesin	24	Peptide Deformylase
9	Peptite Deformylase	25	Enoyl-Acyl-Carrier-Protein Reductase
10	Lactate Dehydrogenase	26	Dihydrofolate Reductase-Thymidylate Synthase
11	Cyclophilin	27	Ferredoxin
12	Polymorphic Antigen Spam-H1	28	Apical Membrane Antigen 1
13	Adenylosuccinate Synthatase	29	Acyl-Coa Binding Protein
14	Glutathione Reductase	30	Rab6
15	Glutathione S-Transferase	31	Malarial Purine Phosphoribosyltransferase
16	Pfpk5 (Protein Kinase)	32	Purine Nucleotide Phosphorylase

The protein protein interaction network is represented as undirected graph that consists of a set of nodes and set of edges. Each node represents a protein and an edge represents an interaction between a pair of nodes. The interaction network thus obtained contains 206 nodes (proteins) and 1215 edges (interactions). The network is shown in fig. 1.

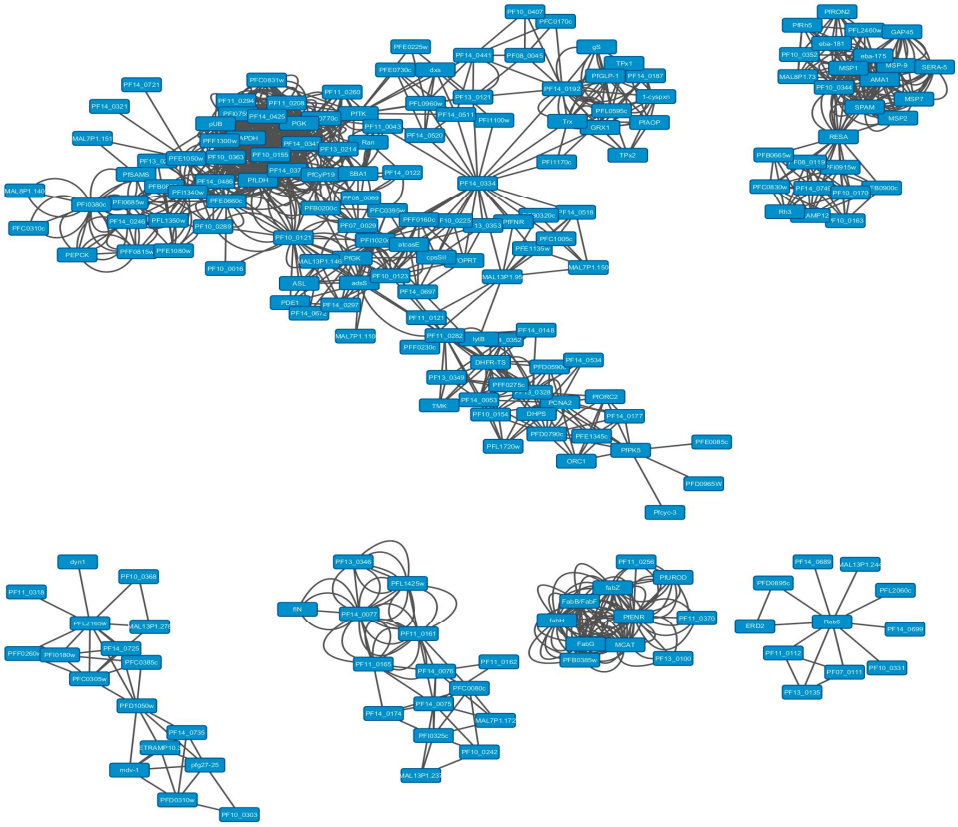

Figure 1: Protein protein interaction network of malaria drug targets and their interacting partners. Blue bars represent nodes and black lines represent edges.

Network Analyzer plug in was used to compute network parameters that are helpful in their analysis and to understand the characteristics of a network and important network topologies. Network parameters provide a detailed description of the network. One of the important network parameters is clustering coefficient of a node which describes how well connected the neighbours of the node are. It was found to be 0.674 which indicates high density of connections in the network. Network parameters are summarized in table 2. Degree refers to the number of interactions to a single node with all other nodes. Degree distribution gives a relationship between numbers of nodes and edges as shown in fig. 2.

Table No. 2 : "Network parameters"

Clustering coefficient	0.674	No. Of nodes	206
Network diameter	8	No. Of edges	1215
Network radius	1	Network density	0.034
Shortest paths	16904 (40%)	Network heterogeneity	0.664
Characteristic path length	3.469	Isolated nodes	0
Average number of neighbors	6.883	No. Of self loops	0

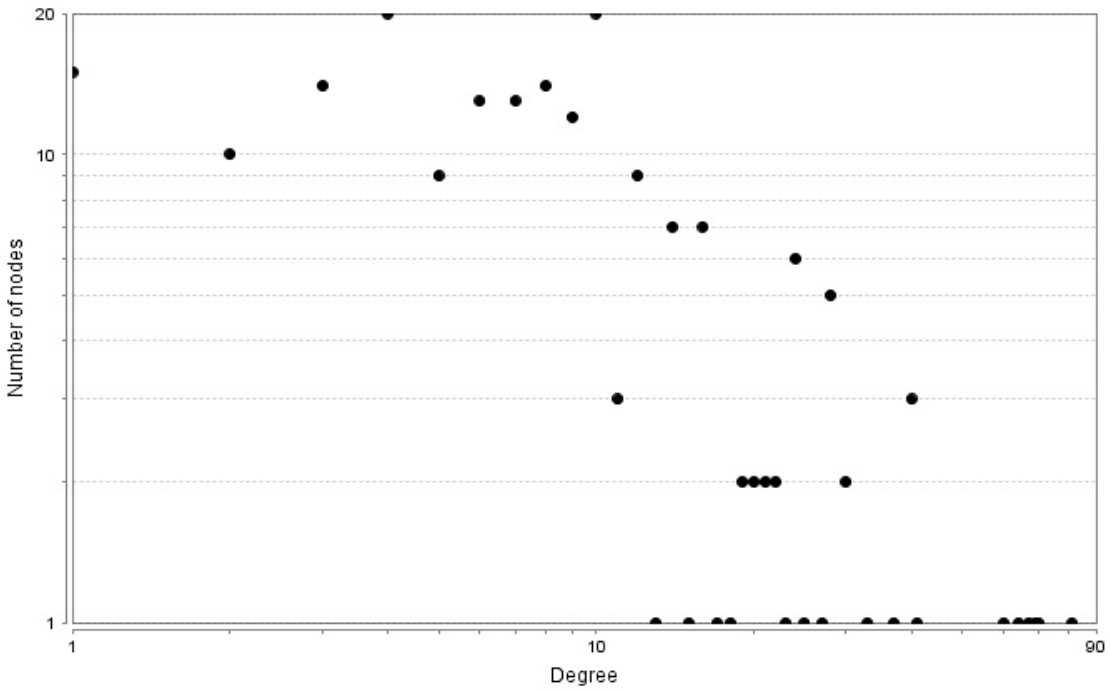

Figure 2: Graph depicting node degree distribution

A subset of densely connected networks within the interactome was constructed with the help of Cluster One plug in with minimum size of the cluster being 10. This resulted in a total of 8 clusters. Top five clusters were studied in detail. Cluster 1 represents the proteins involved in pathogenesis and membrane invasion (table 3). This includes apical membrane antigen that binds to host cell surface[9]. Several studies have indicated the potential of apical membrane antigen as a target for therapeutic intervention[9,10]. Another component of this cluster is ring infected erythrocyte surface antigen that is known to bind and stabilize spectrin tetramers in the host cell membrane and further enhances the cell resistance to mechanical as well as thermal degradation[11]. The antigen is known to be responsible for invagination of RBCs cell membrane. Another antigen included in this cluster is Erythrocyte binding antigen-175 (Eba-175) that binds to glycophorin A in a sialic acid dependent manner and facilitates invasion of erythrocytes[12]. Rhoptry neck protein included in cluster 1 is known to bind apical membrane antigen 1 and this complex formation is essential for erythrocyte invasion process of *P. falciparum*[13], thereby making a suitable candidate for drug development.

Table No. 3 : "Components of cluster 1"

Target id	Name of protein	Target id	Name of protein
PfL2460w	Coronin	PF10_0344	Glutamate-rich protein
AMA 1	Apical membrane antigen-1	MSP1	Merozoite surface protein-1
RESA	Ring infected erythrocyte surface antigen	PfRON2	Rhoptry neck protein
SERA	Serine – repeat antigen protein	PfRh5	*P.falciparum* Reticulocyte Binding Protein Homologue 5
Eba – 175	Erythrocyte binding antigen-175	MSP7	Merozoite surface protein -7
MAL8P1.73	Conserved protein with unknown function	GAP45	Glideosome associated protein 45
SPAM	Secreted polymorphic antigen associated with the merozoite	MSP2	Merozoite surface protein-2
PF10_0352	Merozoite Surface Protein - 3.7	MSP9	Merozoite surface protein-9

Cluster 1 includes MAL8P1.73, a conserved *Plasmodium* protein whose function is unknown. However, this protein shows strong interactions with other parasitic proteins responsible for pathogenesis that indicates a potential role of MAL8P1.73 in this process. The top most cluster also contains merozoite surface proteins (MSP) – MSP1, MSP2, MSP7 and MSP9. MSPs are the key proteins required in the initial attachment of the parasite to the host erythrocyte cell membrane that begins the process of invasion. Recent studies have shown that antibodies against *Plasmodium* MSP1 and MSP2 proteins inhibit the growth of parasites and provide protection from malaria[14]. Thus MSP1 and MSP2 might constitute potential candidates for malaria treatment. Knockout studies have shown that the deletion of *Plasmodium* MSP7 gene reduces the invasion of red blood cells by the parasite[15]. Another component of cluster 1 is coronin that binds to actin filaments and is responsible for motility of malaria parasite[16]. Glideosome associated protein 45 (GAP45) is an essential component of glideosome that provides the force needed to invade the host and also assist in gliding motility of the motile forms of the parasites[17]. Another component of cluster 1 is SPAM which is referred to as merozoite surface protein-3 that is located on the surface of the merozoite and inhibits its invasion[18].

Table No. 4 : "Components of cluster 3"

Target id	Name of protein	Target id	Name of protein
ATCase	Aspartate carbonyl transferase	PF13_0353	Putative cytochrome b5 reductase
Pf10_0225	Orotidine 5'-phosphate decarboxylase	cpsSII	Carbamoyl phosphate synthetase
Pf10_0121	Hypoxanthin phosphoribosyl transferase	PfOPRT	*P.falciparum* Orotate phosphoribosyltransferases
Pf10_0123	GMP synthase	PFF0160c	Dihydroorotate dehydrogense
Pf14_0697	Dihydroorotase, putative	PFC0395w	Putative asparagine synthetase
MAL3P1.146	AMP deaminase, putative	PF14_0672	Putative phosphodiesterase
Pfi1020c	Inosine-5'-monophosphate dehydrogenase	PF14_0297	Putative apyrase
ASL	Adenylsuccinate lyase	PDE1	Phosphodiesterase-1
Adss	Adenylosuccinate synthatase	PfGK	*P. falciparum* Glycerol kinase
MAL7P1.110	Inosine triphosphate pyrophosphatase	PFI1020c	Inosine-5'-monophosphate dehydrogenase

Cluster 2 includes enzymes of glycolysis such as phosphofructokinase, Pyruvate Kinase, Triose phosphate isomerise, Glyceraldehydes-3-phosphate dehydrogenase, Fructose-bisphisphate aldolase. These enzymes are also found in the mammalian systems, thereby making them unsuitable drug targets. Cluster 3 represents the protein involved in cell division and growth component synthesis process (table 4). It includes aspartate transcarbamoyl transferase (AspAT) that catalyzes the conversion of aspartate and α–ketoglutarate into oxaloacetate and glutamate and takes part in de novo nucleotide biosynthetic process. Although plasmodial enzyme aspartate transcarbamoyl transferase shares homology with other AspATs however, structural studies have shown significant divergences in the conformation of N-terminal residues. Deletion of N-terminal residues has resulted

in inhibition of plasmodial enzyme in vitro as well as in cell lysate isolated from cultured parasites but the activity of human AspAT was unaffected[19]. This study suggests that plasmodial AspAT is a potential therapeutic drug target. Other components of cluster 3 are Orotidine 5'-phosphate decarboxylase, Hypoxanthin phosphoribosyl transferase, GMP synthase, Inosine-5'-monophosphate dehydrogenase, Adenylsuccinate lyase, Adenylosuccinate synthetase.

Cluster 4 includes enzymes involved in DNA replication. Cluster 5 consists of proteins responsible for cell redox homeostasis. Peroxiredoxins are important components of cluster 5. Gene targeting strategies have suggested a key role of peroxiredoxins in protecting the parasite from oxidative and nitrosative stress[20]. Thioredoxin peroxidases are integral part of thioredoxin system of *P. falciparum* that represents the major defence strategy against peroxides[21]. Studies involving development of monoclonal antibodies against thioredoxin peroxidase have indicated a pivotal role of these enzymes as a biomarker to diagnose malaria[22]. Cluster 5 also contains glutaredoxin and thioredoxin that perform several functions including redox metabolism, antioxidant defence, cellular signalling[23].

CONCLUSION

In this study, protein protein interaction map of *P. falciparum* proteins was constructed. This map includes the malaria drug targets and their interacting partners in the whole proteome of the parasite. Only strong interactions were taken into consideration. Within the map, subclusters which are densely connected and overlapping were computed. Such analysis revealed some proteins that were not reported as drug targets in the targetDB. However, since these proteins are strongly connected to drug targets and hence possess the potential of probable therapeutic drug targets. Thus, this approach has identified core proteins that are crucial for survival of the parasite. Cluster analysis has showed that ring infected erythrocyte surface antigen shows interactions with proteins involved in pathogenesis and cell membrane invasion. Previous studies have suggested the pivotal role of ring infected erythrocyte surface antigen in invagination of RBCs cell membrane. Thus, this protein is a promising drug target for malaria treatment. Other proteins responsible for causing malaria were identified by this approach. However, these proteins were not found in targetDB. These include Erythrocyte binding antigen-175, Rhoptry neck protein, coronin, Glideosome associated protein 45. Similarly other promising drug targets identified by this approach are plasmodial enzyme aspartate transcarbamoyl transferase, peroxiredoxins, thioredoxin peroxidases, glutaredoxin and thioredoxin. The analysis reported here has led to the identification of potential drug targets that can be employed

for designing drugs against malaria. This strategy holds implication in identifying new drug targets and hence can be used for other diseases as well.

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