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PRE-binding protein of *Plasmodium falciparum* is a potential candidate for vaccine design and development: An *in silico* evaluation of the hypothesis

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ABSTRACT

Immunity to *Plasmodium falciparum* is contributed by many known and unknown factors. Lack of effective vaccine and rise in drug-resistant *Plasmodium* parasites leads to the major challenge in controlling the parasite. Determination of surface or secreted proteins which are essential for the survival of the pathogen is a very important step in finding potential vaccine candidates. Despite the discovery of several vaccine candidates against *Plasmodium* over the last decades, an effective vaccine is not available. The present study hypothesized that the PRE-binding protein (PREBP) could be a potential vaccine candidate against malaria pathogenesis. PREBP is highly conserved among the class Aconoidasida and exhibit high antigenicity and accessibility as a secretory protein. The present hypothesis was tested by employing *in-silico* translational genomics wherein the antigenicity, localization and the conservancy were determined.

Introduction

Malaria is a life threatening disease caused by *Plasmodium* species. In 2017, there were 219 million cases of malaria and 435,000 malaria deaths globally [1]. *Plasmodium falciparum* causes the most severe and fatal cases of malaria [2]. Despite decades of research, no effective vaccine for malaria is available [3]. *Plasmodium* parasite has a complex life cycle expressing varying immunodominant and polymorphic antigens during the multi staged life cycle [4]. The availability of multiple proteins to accomplish one or more similar function as an alternative pathway has also been reported, such as the erythrocyte binding ligand (EBL) and the reticulocyte binding homologues (RH) protein families involved in essential functions like RBC invasion. There is an increasing burden of the emergence of various drug resistant *Plasmodium* strains towards chloroquine and Artemisinin combination therapies (ACTs) [5,6]. Therefore, there is an urgent requirement of alternative approach to control the disease. Immunogenic vaccination strategies are providing strong base in minimizing malaria burden making vaccination an ultimate approach to control malaria [7,8]. Conserved epitopes can be targeted to design broad-spectrum vaccines to inhibit parasite invasion [9]. Therefore, finding of conserved parasite antigens is a necessity and further efforts are needed to identify parasitic molecules, proteins or pathways that could be targeted for the development of an effective vaccine.

Reverse vaccinology (RV) is an advanced genomic strategy for the design and selection of vaccine candidate [10]. RV approach uses bioinformatics tools to identify the potential vaccine candidates [11]. Epitope-based vaccines, therapeutic antibodies, and diagnostic tools can be developed by further determining the epitopes [12]. Therefore, identifying unknown proteins crucial for parasite survival might help in designing new and effective vaccines. A number of potential vaccine candidates (Merozoite surface protein-1, Erythrocytic binding antigen-175 and Apical membrane antigen-1) have been reported and studied over the decades [13,14]. Despite the discoveries of many potential vaccine candidates only one vaccine candidate, “RTS,S,” has advanced to Phase 3 clinical trials providing partial protection [15,16]. Considering the genetic diversity and the complex life cycle of the parasite, vaccines development targeting single strain or only one stage might be a reason behind the failures in developing an effective vaccine [15–17]. Therefore, an effective malaria vaccine is likely to be a multi-component targeting the different parasitic stage and strains [17,18]. Identification of more potential candidates might help in the development of an effective vaccine.

Hypothesis

The present study hypothesized that the highly conserved *P. falciparum* protein, PREBP, could be a potential vaccine candidate against malaria pathogenesis. The protein is highly conserved among the class Aconoidasida, and has high antigenicity and accessibility as a secretory

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protein. PREBP expressed in the merozoite stage of the *Plasmodium*, has been reported to be the target of monoclonal antibodies inhibiting parasite invasion [19] but not evaluated as a vaccine candidate. The present hypothesis was tested by employing *in-silico* translational genomics wherein the antigenicity, localization and the conservancy were determined using combination of bioinformatics tools.

Evaluation of the hypothesis

Methodology

Identification of essential genes of P. falciparum expressed during intra-erythrocytic stage

A systematic multi-steps strategy was designed involving PlasmoDB, several bio-informatical tools and important parameters with the objective of identifying essential *P. falciparum* protein that can be used as a vaccine candidate. PlasmoDB- the genome database for the genus *Plasmodium* (http://plasmodb.org/plasmo/) [20], was used as the primary source for data mining and identifying essential proteins. By applying essential gene prioritizing parameters, cross-species analysis was carried out to identify evolutionary conserved genes of *P. falciparum* 3D7 which are absent in human host and expressed in the blood stage [21]. Genes can be essential if 1) they are evolutionary conserved, with orthologues in close and distant relatives and 2) paralogues are absent [21]. Parasite genes homologous to the human host were removed to avoid any unwanted cross reactions as well as immune tolerance (Flochart 1.). Conserved genes of *P. falciparum* 3D7 across the class Aconoidasida were identified by running BLAST. These orthologous genes are evolutionarily conserved and are essential for *P. falciparum*. Genes with paralogues were removed if there was any and the resulted essential genes were further analyzed for genes expressed in the blood stage of the parasite life cycle.

In-silico antigenic profiling

The corresponding protein sequences were retrieved from PlasmoDB in the FASTA format and were used for the determination of antigenicity by employing VaxiJen, with a threshold value of 0.4 [22]. VaxiJen (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) is an online webserver for alignment-independent prediction of protective antigens that classifies antigen based on the physicochemical properties of proteins.

Prediction of subcellular localization

Considering the fact that surface and secreted proteins are good targets for therapeutic interventions, the subcellular localization of the proteins were predicted using DeepLoc and Loctree. DeepLoc (http://www.cbs.dtu.dk/services/DeepLoc/) used deep neural networks to predict protein subcellular localization depending on the sequence information of the proteins [23]. Whereas, Loctree (https://rostlab.org/services/loctree3/) used support vector machines by searching the k-consecutive residues with experimental proteins annotations [24].

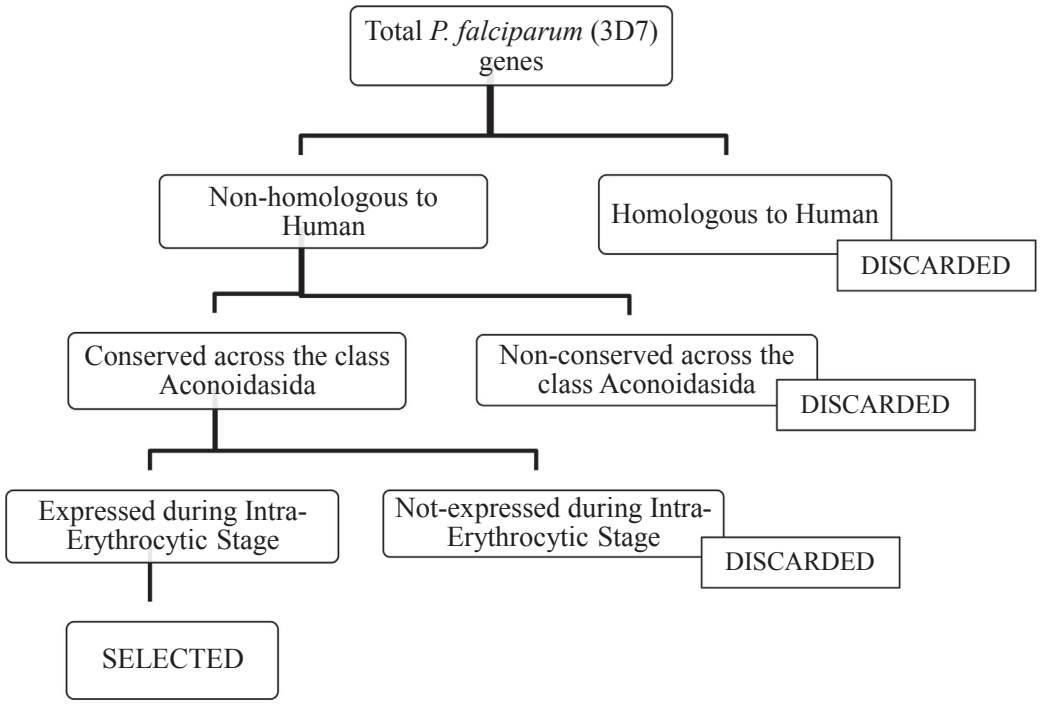
Determination of transmembrane helix

Number of helix present in a protein is an important factor in selecting vaccine candidates. Transmembrane helices were predicted using Trans-membrane Hidden Markov Model program (TMHMM) [25] and the Consensus Constrained TOPology prediction (CCTOP) server [26]. TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) is one of the best performing transmembrane prediction program based on a hidden Markov model [25,27] and CCTOP (http://cctop.enzim.ttk.mta.hu/) server is a web-based application based on 10 state-of-the-art topology prediction methods with high accuracy in topology prediction [26].

Results

Identification of essential P. falciparum genes expressed during intra-erythrocytic stage

Comparative analysis of the parasite genome with the human genome identified 3743 parasite genes that lack orthologs in the host genome, out of the 5712 genes recorded in PlasmoDB. 348 genes were further identified from the 3743 non-homologous genes from the



Floatchart 1. Strategy for the screening of essential genes expressed in blood stage of parasite life cycle.

Table 1
Essential proteins from *P. falciparum* derived from PlasmoDB expressed during intra-erythrocytic stage of the parasite life cycle.

S/N	Gene ID	Chromosome no.	Proteins	Protein Length (aa)	Molecular Weight (Da)	Ortholog count
1	PF3D7_0316100	03	Mitochondrial ribosomal protein L27 precursor, putative	298	35,411	29
2	PF3D7_0506900	05	Rhomboid protease ROM4	759	86,653	29
3	PF3D7_0605300	06	Serine/threonine protein kinase	353	40,907	29
4	PF3D7_0628200	06	Eukaryotic translation initiation factor 2-alpha kinase	3072	364,328	29
5	PF3D7_0709000	07	Chloroquine resistance transporter	424	48,674	29
6	PF3D7_1011800	10	PRE-binding protein	1139	131,625	29
7	PF3D7_1027300	10	Peroxiredoxin	393	43,929	28
8	PF3D7_1115200	11	Histone-lysine N-methyltransferase SET7	793	94,292	31
9	PF3D7_1133400	11	Apical membrane antigen 1	622	72,041	29
10	PF3D7_1211500	12	Mitochondrial ribosomal protein S18 precursor, putative	387	47,172	29
11	PF3D7_1222700	12	Glideosome-associated protein 45	204	23,631	29
12	PF3D7_1239100	12	Mitochondrial ribosomal protein L23 precursor, putative	269	31,762	29
13	PF3D7_1246400	12	Myosin A tail domain interacting protein	204	23,491	29
14	PF3D7_1310900	13	Mitochondrial ribosomal protein S15 precursor, putative	303	35,711	29
15	PF3D7_1311800	13	M1-family alanyl aminopeptidase	1085	126,061	29
16	PF3D7_1342800	13	Phosphoenolpyruvate carboxykinase	583	66,208	29
17	PF3D7_1363700	13	Conserved Plasmodium protein, unknown function	790	92,785	29
18	PF3D7_1431000	14	Mitochondrial ribosomal protein L17-2 precursor, putative	257	31,146	29
19	PF3D7_1445300	14	Mitochondrial ribosomal protein S29 precursor, putative	604	72,301	30
20	PF3D7_1454600	14	Mitochondrial ribosomal protein S11 precursor, putative	229	26,527	29

**aa-amino acid and Da-Daltons.

previous step to be conserved across the class Aconoidasida, indicating its essentiality in the parasite's survival. The sequential selection resulted in 20 genes expressed during the intra-erythrocytic stage (blood stage) of the parasite's life cycle (Table 1). These putative proteins are absent in human but conserved in all the other species of the class Aconoidasida indicating its uniqueness and essentiality to its specific class. These essential proteins are of great significance and could serve as potential targets for vaccine development.

Determination of antigenicity by VaxiJen

Merozoite surface protein 1 (MSP1), Erythrocytic binding antigen 175 (EBA175) and Apical membrane antigen 1 (AMA) were taken as positive control for the antigenicity prediction. The FASTA format of PREBP along with AMA1, MSP1 and EBA175 were subjected to find the antigenicity using VaxiJen server taking a threshold value of 0.4. Proteins having scores greater than the threshold value (0.4) are considered antigenic in nature. PREBP was predicted to have a score of 0.7914 which is greater than the scores of AMA1, MSP1 and EBA175 indicating its high antigenicity (Table 2).

Subcellular localization and trans-membrane helix

Surface or secreted proteins are exposed to immune surveillance and are good vaccine candidates. Loctree predicted PREBP as a secreted protein, the result of the analysis is provided in the Table 2. According to the predicted localization, PREBP is a secreted protein that is exposed and considering its accessibility, it is a potential vaccine candidate. The number of trans-membrane helices (TMHs) of a protein is an important criterion during protein expression and isolation. TMH of the protein was analyzed by CCTOP and TMHMM program. PREBP was predicted to have only one TMH, which makes it a good candidate.

Discussion

The malaria parasite has a multi-staged complex life cycle and during these different stages the parasite expresses different proteins on the surface [4,28]. Immune response against the erythrocytic stage is mainly governed by humoral immunity since RBCs are devoid of antigen processing mechanisms [29]. However both the humoral and cell mediated immunity contribute major role in combating the clinical symptomatic stage of the disease [30]. DCs plays an important role in the development of the protective immune responses by regulation both innate and adaptive immunity [31,32]. Infected RBCs and the merozoites trigger innate immune responses by activating DCs, which produce pro-inflammatory cytokines that further activate the T cells initiating the adaptive immune responses [33,34]. $CD4^+$ T cells play a crucial role in the development of blood stage immunity [35,36]. Although full protective immunity is never achieved, acquired immunity provides protection and immunity is developed with repeated exposure to the parasite [29,35]. The blood-stage of infection is accountable for the malaria disease [29], therefore, understanding and identification of parasite proteins that are the targets of protective immunity during this stage might lead to effective vaccine development.

In immuno-informatics study, RV has become a popular approach in designing and development of vaccines, since the successful development of vaccine against meninggococcus [37]. RV approach has been applied to many other pathogens: *Streptococcus pneumonia* [38], *Bacillus anthracis* [39], *Cryptosporidium Species* [40] and *Campylobacter jejuni* [41]. Present study adopted the RV approach employing a multi-step computational strategy of genomic and proteomic comparison for the prediction of essential proteins of *P. falciparum* that can be used as potential vaccine candidates. Essentiality implies conservancy over the evolutionary phases, uniqueness, lack of paralogs and orthologs to the host genome [21]. These essential proteins are important for the survival of the parasite and can act as potential vaccine candidates [42].

Table 2
Proteins with their respective predicted antigenicity scores and localization.

S/N	Gene ID	Proteins	Antigenicity score	Localization
1.	PF3D7_1011800	PRE-binding protein	0.7914	Secreted
2.	PF3D7_1133400	Apical membrane antigen 1	0.5743	Secreted/Membrane
3.	PF3D7_0930300	Merozoite surface protein 1	0.6369	Membrane
4.	PF3D7_0731500	Erythrocytic binding antigen 175	0.5706	Membrane

An ideal vaccine candidate must be accessible to the immune system inducing protective immune responses which lead to the activation of T and B lymphocyte, resulting in the generation of memory cells [43,44]. The protein has to be either secreted or surface protein so that it is accessible to the immune system [45]. Sorting out proteins with high number of TMHs is also important as they are not good candidates due to its difficulty in isolation [46].

The present study predicted and demonstrated that PREBP is more antigenic scoring 0.7914 which was the highest in comparison with the three control candidates: MSP1, EBA175 and AMA1. This makes PREBP a potential candidate than the previously reported antigens in terms of antigenicity. Previously PREBP was not explored as a vaccine candidate though it was reported as a merozoite protein with high binding affinity with two parasite growth inhibiting monoclonal antibodies [19]. The work by Epping et al [19] is in the favor of the current hypothesis, enhancing the credibility of PREBP as a potential vaccine candidate. Considering all the important aspects of previous work and current bioinformatics analysis, it can be concluded that PREBP is a potential candidate for the malaria vaccine development. The present work is an encouraging study to utilize bio-informatics tool for the identification of potential vaccine candidate in *Plasmodium species*.

Conclusion

Present study identified PREBP as an essential protein of the parasite expressed in the intra-erythrocytic stage through a systematic genomic comparison approach. From all the analysis of the current study, we concluded PREBP as a potential vaccine candidate and thus, can be useful in developing vaccines or as a subunit for epitope-based vaccines by identifying best epitopes of the protein.

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Conflict of interest statement

The authors declare no conflicting interest in publishing the article.

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