Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

# Predicted essential proteins of *Plasmodium falciparum* for potential drug targets

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ARTICLE INFO

*Article history:*
Received 15 January 2012
Received in revised form 15 March 2012
Accepted 15 April 2012
Available online 20 May 2012

*Keywords:*
*Plasmodium falciparum*
Essential proteins
Database of essential genes
Druggability
Potential drug targets

ABSTRACT

**Objective:** To identify novel drug targets for treatment of *Plasmodium falciparum*. **Methods:** Local BLASTP were used to find the proteins non-homologous to human essential proteins as novel drug targets. Functional domains of novel drug targets were identified by InterPro and Pfam, 3D structures of potential drug targets were predicated by the SWISS-MODEL workspace. Ligands and ligand-binding sites of the proteins were searched by Ef-seek. **Results:** Three essential proteins were identified that might be considered as potential drug targets. AAN37254.1 belonged to 1-deoxy-D-xylulose 5-phosphate reductoisomerase, CAD50499.1 belonged to chorismate synthase, CAD51220.1 belonged to FAD binging 3 family, but the function of CAD51220.1 was unknown. The 3D structures, ligands and ligand-binding sites of AAN37254.1 and CAD50499.1 were successfully predicated. **Conclusions:** Two of these potential drug targets are key enzymes in 2-C-methyl-d-erythritol 4-phosphate pathway and shikimate pathway, which are absent in humans, so these two essential proteins are good potential drug targets. The function and 3D structures of CAD50499.1 is still unknown, it still need further study.

## 1. Introduction

Malaria is a common and severe tropical disease. It is caused by a protozoan belonging to the genus *Plasmodium*. Malaria is attributed to high mortality and morbidity in tropical countries and affects around 100 countries of the world. Nearly 1 million people die and almost 300 million symptomatic illnesses occur due to malaria annually. Over the past 50 years, the resistance to commonly used anti-malarial agents has significantly hindered malaria control[1]. The problem of antimalarial resistance is more pronounced with *Plasmodium falciparum*[2]. So the development of anti-malaria drugs is urgently needed to meet the prevailing challenges. Identification of potential drug targets is the first step in the process of modern drug development.

Whole genome sequences of a number of organisms and the completion of the human genome project provide methods for predicting potential drug targets against threatening human pathogens. The search for potential drug targets relies on the assumption that the potential target must play an essential role in the pathogen´s survival, because chemical inhibition of non-essential genes is unlikely to result in the death of the infectious agent. At the same time, this target should have non-human orthologous targets. So this would have little or no effect on human[3]. Zhang and Lin[4] have compiled information on experimentally proven essential genes from some prokaryotes and eukaryotes via Database of Essential Genes (DEG). This resource facilitates drug targets development by seeking non-human targets in the DEG. Based on the criteria that a target should be essential protein for a given pathogen, and should be no-homologous to human host, putative drug targets have been successfully identified in *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Burkholderia pseudomallei* and *Aeromonas hydrophila*[5].

This study aims to identify novel drug targets for treatment of *Plasmodium falciparum*.

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Foundation project: It is supported by Science and Technology Innovation Fund of Guangdong Medical College (No. STIF 201107).

## 2. Materials and methods

### 2.1. Comparative genome analysis

Protein sequences of *Homo sapiens* and *Plasmodium falciparum* 3D7 were downloaded from the National Center for Biotechnology Information (NCBI, ftp://ftp.ncbi.nlm.nih.gov/genomes/). The essential proteins of *Arabidopsis thaliana*, *Aspergillus fumigatus*, *Caenorhabditis elegans*, *Danio rerio*, *Drosophila melanogaster*, *Mus musculus*, Saccharomyces cerevisiae were downloaded from DEG (http://tubic.tju.edu.cn/deg/). The *Plasmodium falciparum* protein sequences were subjected to local BLASTP analysis against DEG essential protein sequences to identify essential proteins at an E-value cutoff of $10^{-10}$. Bit score and percentage of identity at amino acid level were considered 100% and >35%, respectively. The results were subjected to local BLASTP analysis against protein sequences of *Homo sapiens* at an expectation E-value cutoff of $10^{-3}$ to identify non-homologous sequences.

### 2.2. Identified functional domains

Functional domains were identified by InterPro (http://www.ebi.ac.uk/interpro/InterPro)[6] and Pfam (http://pfam.sanger.ac.uk/ Pfam)[7].

### 2.3. Homology modeling for 3D structure of potential drug targets

The 3D structures of potential drug targets were predicated by SWISS-MODEL workspace (http://swissmodel.expasy.org/workspace). The generated structural models were visualized using the Pymol viewer software. The quality of the model was evaluated using the PROCHECK program (http://nihserver.mbi.ucla.edu/SAVES_3/) and assessed using the Ramachandran plot. Ligands and ligand-binding sites of the proteins were searched by Ef-seek (http://ef-site.hgc.jp/eF-seek/).

## 3. Results

### 3.1. Essential non-human homologs proteins of Plasmodium falciparum

A total of 561 essential proteins and 3 proteins (AAN37254.1, CAD50499.1, CAD51220.1) belonged to non-human homologue essential proteins were identified.

### 3.2. Protein functional analysis

The InterPro and Pfam search indicated that AAN37254.1 belonged to 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), CAD50499.1 belonged to chorismate synthase, CAD51220.1 belonged to FAD binging 3 family, but the function of CAD51220.1 was unknown.

### 3.3. Structure modeling and validation of the generated model

The 3D structures of AAN37254.1 and CAD50499.1 were predicated as Figure 1. The quality of the model was evaluated using the PROCHECK program and assessed using the Ramachandran plot. It was evident from the Ramchandran plot that the predicted model of AAN37254.1 had 91.6%, 7.3%, and 0.3% residues in the most favorable regions, the allowed regions, and the disallowed regions, respectively. The predicted model of CAD50499.1 had 81.0%, 13.8%, and 1.5% residues in the most favorable regions, the allowed regions, and the disallowed regions, respectively. Such percentage distribution of the protein residues determined by Ramachandran plot showed that the predicted models were of good qualities. No homologous protein with a 3D structure of CAD51220.1 was found, so the 3D structure of CAD51220.1 couldn't be predicted.

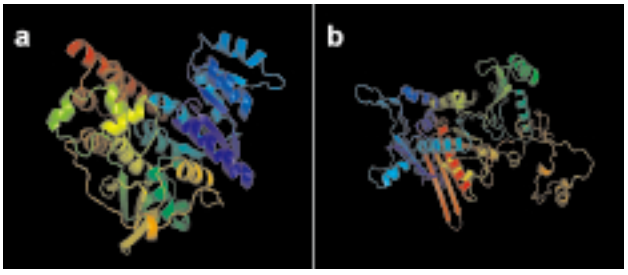


**Figure 1.** Predicted 3D structure.
a: AAN37254.1, b: CAD50499.1.

### 3.4. Ligands and ligand-binding sites

A total of 3 top ligand-binding residue sites in AAN37254.1 were identified using Ef-seek. All had the ligands of NDP, and ligand-binding residues were very similar in AAN37254.1 (Figure 2). In CAD50499.1, these 3 top ligands were C8E, PIE and CLA, and ligand-binding residues and ligands were showed as Figure 3. The ligands of CLA was not buried in the protein but exposed outside. It was large in size and could not stay comfortably in the incommodious pocket.

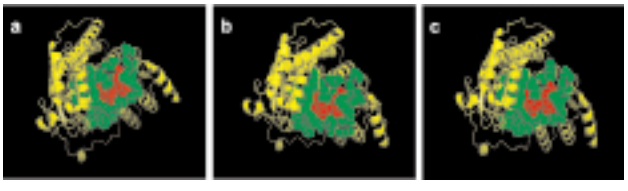


**Figure 2.** The ligands and ligand-binding sites of AAN37254.1.
The backbone of protein is shown as cartoon. The ligand (NDP) is shown as spheres in red, The ligand-binding sites are shown as spheres in green.
(a) ligand-binding sites: 84aa, 86-89aa, 92aa, 113-117aa, 136aa 162aa, 180-183aa, 185aa, 203-206aa, 231-233aa, 296-302aa, 305aa, 311aa, 314aa, 315aa, 360aa; (b) ligand-binding sites: 84aa, 86-89aa, 91aa, 92aa, 113-117aa, 120aa, 136aa, 137aa, 162aa, 180-182aa, 185aa, 203-206aa, 231-233aa, 296-302aa, 311aa, 315aa, 360aa; (c) ligand-binding sites: 84aa, 86-89aa, 92aa, 113-117aa, 136aa, 162aa, 180-183aa, 185aa, 188aa, 302-206aa, 231-233aa, 296-302aa, 305aa, 311aa, 314aa, 315aa, 360aa.

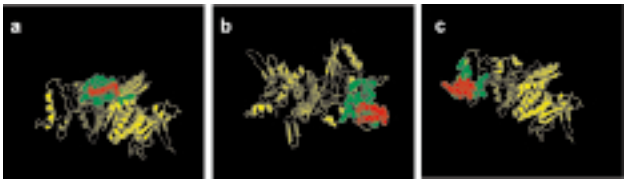


**Figure 3.** The ligands and ligand-binding sites of CAD50499.1. The backbone of protein is shown as cartoon. The ligand is shown as spheres in red, The ligand-binding sites is shown as spheres in green. (a) ligand: C8E . ligand-binding sites:326aa, 330aa, 338-340aa, 342-344aa, 346aa, 347aa, 349aa, 351aa, 354aa, 355aa, 436aa, 437aa; (b) ligand: PIE. ligand-binding sites: 382aa, 383aa, 385-39aa, 399 400; (c) ligand: CLA. ligand-binding sites: 379aa, 380aa, 382aa, 383aa, 386aa, 387aa, 389aa, 400-404aa, 407aa.

## 4. Discussion

Novel drug targets are required in order to design new drugs against parasites. The search for potential drug targets has increasingly relied on genomic approaches. The potential drug targets should be a molecule that is essential to the parasites and have no non-human orthologous targets, for which inhibition of essential genes would kill the parasite but have no effect on human homologue. Based on this criteria, we had found 3 potential drug targets of *Plasmodium falciparum*.

Isoprenoids, one of the largest groups of natural compounds, have a variety of roles in respiration, photosynthesis, membrane structure, allelochemical interactions, and growth regulation[8,9]. Isopentenyl diphosphate is a precursor of various isoprenoids and is produced by the 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway in plastids of plant and protozoa[10,11]. The MEP pathway is absent from humans, and DXR is a key enzyme in the MEP pathway. DXR has reported to be the target of fosmidomycin[12], so it will be a promising drug target for anti-malarial. The shikimate pathway is essential in algae, plants, bacteria, protozoa and fungi, and is also absent from mammals. The absence of the pathway in mammals makes them potential targets for development of new therapy against infectious diseases[13], such as *Plasmodium falciparum*. The enzymes of shikimate pathway are good candidates for development of new therapies against *Plasmodium falciparum*. The last enzyme of this pathway is chorismate synthase, which catalyzes the conversion of the 5-enolpyruvylshikimate-3-phosphate to chorismate. So chorismate synthase (CAD50499.1) of *Plasmodium falciparum* will be a good candidate for drug targets. Although CAD51220.1 is of unknown function, further bioinformatics analysis on this protein might shed some light on a new drug targets.

3D structure and/or structure complex with ligands and ligand-binding sites would enhance the druggability value by facilitating a structure based drug design strategy. Prediction of ligand-binding sites is a fundamental step in order to investigate the molecular recognition mechanism and function of a protein. Structural analysis and computational modeling of ligand-binding sites bring key information to designing drugs and annotating proteins[14,15]. We had found the ligands and the ligand-binding sites of DXR and chorismate synthase, the ligand-binding sites would be the sites for the drugs binding. All of these information about the drug targets would help the design of the drugs.

Targeting an essential protein for the parasite may provide an effective way to control infection. Comparative genome analysis is a highly efficient approach for identifying potential proteins that can be used as potential targets for effective drug designing against pathogenic organisms. Here, we have identified three potential drug targets of *Plasmodium falciparum*, and analyzed the function and ligands and ligand-binding sites of DXR and chorismate synthase. DXR and chorismate synthase fit for drug targets, and the druggability of CAD51220.1 needs further study. These targets should be experimentally validated for their role in *Plasmodium falciparum* drug targets.

## Conflict of interest statement

We declare that we have no conflict of interest.

## References

[1] Trape JF. The public health impact of chloroquine resistance in Africa. *Am J Trop Med Hyg* 2001; **64**: 12-17.
[2] Parija SC, Praharaj I. Drug resistance in malaria. *Indian J Med Microbiol* 2011; **29**(3): 243-248.
[3] Fritz B, Raczniak GA. Bacterial genomics: potential for antimicrobial drug discovery. *Bio Drugs* 2002; **16**(5): 331-337.
[4] Zhang R, Lin Y. DEG 5.0, a database of essential genes in both prokaryotes and eukaryotes. *Nucleic Acids Res* 2009; **37**: 455-458.
[5] Barh D, Kumar A. In silico identification of candidate drug and vaccine targets from various pathways in *Neisseria gonorrhoeae*. *In Silico Bio* 2009; **I9**: 225-231.
[6] Mulder NJ, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, et al. New developments in the InterPro database. *Nucleic Acids Res* 2007; **35**: 224-228.
[7] Finn RD, Tate J, Mistry J, Coggill PC, Sammut SJ, Hotz HR, et al. The Pfam protein families database. *Nucleic Acids Res* 2008; **36**: 281-288.
[8] Bouvier F, Rahier A, Camara B. Biogenesis, molecular regulation, and function of plant isoprenoids. *Prog Lipid Res* 2005; **44**: 357-429.
[9] Sacchettini JC, Poulter CD. Creating isoprenoid diversity. *Science* 1997; **277**(5333): 1788-1789.
[10] Takenoya M, Ohtaki A, Noguchi K, Endo K, Sasaki Y, Ohsawa K, et al. Crystal structure of 1-deoxy-d-xylulose 5-phosphate reductoisomerase from the hyperthermophile *Thermotoga maritima* for insights into the coordination of conformational changes and an inhibitor binding. *J Struct Biol* 2010; **170**: 532-529.
[11] Rungsihirunrat K, Chaijaroenkul W, Siripoon N, Seugorn A, Thaithong S, K Na-Bangchang. Comparison of protein patterns between *Plasmodium falciparum* mutantclone T9/94-M1-1(b3) induced by pyrimethamine and the original parent clone T9/94. *Asian Pac J Trop Biomed* 2012; **1**(1): 66-69.
[12] Odom AR, Van Voorhis WC. Functional genetic analysis of the *Plasmodium falciparum* deoxyxylulose 5-phosphate reductoisomerase gene. *Mol Biochem Parasitol* 2010; **170**: 108-111.
[13] Dias MV, Borges JC, Ely F, Pereira JH, Canduri F, Ramos CH, et al. Structure of chorismate synthase from *Mycobacterium* tuberculosis. *J Struct Biol* 2006; **154**: 130-143.
[14] Kahraman A, Morris RJ, Laskowski RA, Thornton JM. Shape variation in protein binding pockets and their ligands. *J Mol Biol* 2007; **368**: 283-301.
[15] Gunasekaran K, Nussinov R. How different are structurally flexible and rigid binding sites? Sequence and structural features discriminating proteins that do and do not undergo conformational change upon ligand binding. *J Mol Biol* 2007; **365**: 257-273.