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Prediction of Antimalarial Target Proteins in *Plasmodium falciparum* Using Network Pharmacology Approach

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ABSTRACT

Background and Purpose: Resistance has developed to all known antimalarial medicines. Therefore, tracking the drug sensitivities of clinical infections is crucial for treatment strategies. This study was designed to predict antimalarial target proteins of *Plasmodium falciparum* that are commonly cited in the STRING database using the network pharmacology approach.

Methods: Cytoscape (v3.9.1) and StringApp (v2.0.1) were used to construct and analyse *Plasmodium falciparum* protein interaction networks related to antimalarial resistance, based on a PubMed query via STRING. A confidence score of 0.7 and a limit of 50 proteins were applied, with results ranked by text-mining scores to identify candidate target proteins.

Results: The PubMed query yielded 15,614 results, of which 9999 were downloaded, including target proteins from *P. falciparum* species that were often cited in published papers. The outcome displayed target proteins for *P. falciparum*, including PfCRT, PfMDR1, DHPS, PfMRP, MSP2, MSP1, PF14_0077, PF10_0344, DHFR-TS, and PfMDR2 with high text-mining scores arranged in descending order. The text-mining score showed how frequently the target proteins are mentioned in the STRING database, with the most mentioned (PfCRT) given the highest score.

Conclusion: Using network Pharmacology technique, PfCRT, PfMDR1, DHPS, PfMRP, MSP2, MSP1, PF14_0077, PF10_0344, DHFR-TS, and PfMDR2 were identified as target proteins with high text-mining evidence for *P. falciparum* resistance. This can influence experimental and clinical decisions.

INTRODUCTION

Malaria is a public health issue, with an estimated 219 million infections in 87 countries and 435,000 malaria-related fatalities anticipated in 2017 (WHO, 2018). Africa has the highest prevalence of the disease (WHO, 2018). The five major Plasmodium species that infect humans include *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale, and P. knowlesi* (Kotepui *et al*., 2020). *P. falciparum* causes the most virulent malaria infections in people and accounts for most malaria-related deaths (Amesa *et al*., 2024). Resistance has developed to all known antimalarials; therefore, tracking drug sensitivities of clinical infections is crucial for treatment strategies as well as mass medication administration programs aimed at eliminating the disease (Haldar *et al.*, 2018).

Amplification and/or single nucleotide polymorphisms in target catalytic enzymes or efflux pumps have been shown to cause resistance to numerous antimalarials (Cheuka *et al*., 2024). So far, not all molecular targets of currently used antimalarials have been characterised. Antimalarial drug resistance can be caused by a direct catalytic mechanism or by amplification of the gene coding the target enzyme or transporter, which pumps the medication out of the parasite (Cheuka *et al*., 2024). Furthermore, resistance can be mediated by processes that decrease the toxicity caused by the drug (Haldar *et al*., 2018). Genome-wide association studies (GWAS) can discover genetic variations that are related to clinical antimalarial resistance phenotypes (Haldar *et al*., 2018). Proteins are involved in a range of functional partnerships and interactions that are essential to cellular processing, and their thorough characterization serves to offer context in molecular systems biology (Szklarczyk *et al*., 2015). However, known and probable interactions are spread over numerous resources, and the available data vary significantly in terms of quality and completeness. The STRING database (http://string-db.org) seeks to provide a critical examination and integration of protein-protein interactions, encompassing both direct (physical) and indirect (functional) relationships (Szklarczyk *et al*., 2015). Undoubtedly, the most useful networks for functional evaluations are those that include all types of interaction, such as information relay, transient binding, stable physical interactions, substrate chaining, and so on. The global STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) focuses on the functional interactions between proteins (Szklarczyk *et al*., 2015). The statistical analysis of microbial protein- protein interactions across many species (meta-interactomes) has provided significant insights into the roles of proteins and cellular processes (Ahmadi *et al*., 2022). This present study was designed to use the network pharmacology approach to predict the antimalarial target proteins in *P. falciparum*, rank the proteins according to their text-mining scores, and provide an array of genes that could be identified following genome sequencing techniques.

MATERIALS AND METHODS

Database Mining and Network Construction

An ethical waiver with clearance number PTAC/Sp/Arp/(RV)/65-24 dated 28th January 2024 was obtained from the ethical clearance committee of Usmanu Danfodiyo University, Sokoto, Nigeria. Cytoscape (v3.9.1) was used for network construction and analysis. The StringApp (v2.0.1) was installed from the Cytoscape App Store. To import protein interaction networks, the option 'Network from Public Database' was selected under the 'Import' menu. STRING was chosen as the data source with the input method set to 'PubMed query'. The species was specified as Plasmodium falciparum, and the search term used was "Antimalarial Resistance Plasmodium falciparum." A confidence score threshold of 0.7 was applied, and the number of resistance-associated P. falciparum proteins was limited to 50. The retrieved proteins were ranked by text-mining scores, generating a prioritised list of candidate genes potentially associated with antimalarial resistance for downstream genomic analysis.

RESULTS

String Network for *P. falciparum* Target Proteins

The PubMed query yielded 15,614 results, of which 9999 were downloaded, including target proteins from *P. falciparum* species that were often cited in published papers. Nodes in networks represent proteins, whereas edges indicate protein interactions. Figure 1 depicts the network of *P. falciparum* target proteins created from the string database. Proteins have been coloured based on text-mining evidence linked to antimalarial targets.

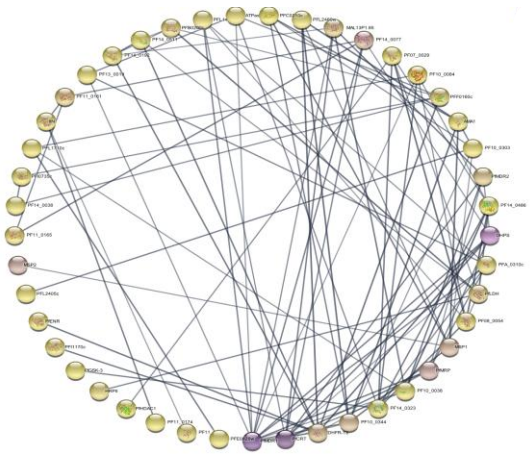

Figure 1: Merging string data into a network to uncover relationships between *P. falciparum* target proteins. The colour purple signifies nodes with high text-mining scores, while gold represents nodes with low text-mining scores.

Ranking of *P. falciparum* Target Protein

Table 1 shows the ranking of the text-mining scores of *P. falciparum* target proteins from highest to lowest using Cytoscape software. PfCRT, PfMDR1, DHPS, PfMRP, MSP2, MSP1, PF14_0077, PF10_0344, DHFR-TS, and PfMDR2 have high text-mining scores. The text-mining score indicates how frequently the *P. falciparum* target proteins were referenced in the downloaded abstract, with the most mentioned protein (PfCRT) receiving the highest score.

Table 1: Text-mining scores of *P. falciparum* target protein.

S/N*	Display Names	Descriptions	Text Mining Score	S/N*	Display Names	Descriptions	Text Mining Score
1	PfCRT	Putative chloroquine resistance transporter	68.389244	**26**	flN	Falcilysin	3.693968
2	PfMDR1	Multidrug resistance protein	62.832684	**27**	AMA1	Apical membrane antigen 1, AMA1	3.62024
3	DHPS	Dihydropteroate synthetase	24.926888	**28**	PF14_0323	Calmodulin	3.565047
4	PfMRP	Multidrug resistance protein 1	16.588675	**29**	PF14_0511	Glucose-6-phosphate 1-dehydrogenase	3.544167
5	MSP2	Merozoite surface antigen 2	15.950306	**30**	PFL2460w	Coronin	3.5
6	MSP1	Merozoite surface protein 1	13.780494	**31**	PFE0825w	Metabolite/drug transporter	3.482202
7	PF14_0077	Plasmepsin-2	13.77503	**32**	PF13_0238	Kelch protein	3.448938
8	PF10_0344	Glutamate-rich protein	12.472457	**33**	PF13_0271	ABC transporter	3.448938
9	DHFR-TS	Dihydrofolate reductase-thymidylate synthase	10.829157	**34**	PF10_0303	25 kDa ookinete surface antigen (Pfs25)	3.335538
10	PfMDR2	Multidrug resistance protein 2	9.677572	**35**	PF10_0084	Tubulin beta chain	3.259807
11	MAL13P1.95	Ferredoxin	8.425204	**36**	PFI0735c	NADH dehydrogenase	3.25
12	PF11_0161	Falcipain-2	7.942647	**37**	PF13_0019	Sodium/hydrogen exchanger	3.184857
13	PfLDH	L-lactate dehydrogenase	7.022597	**38**	PFL2405c	PFG377 protein	3.125683
14	PFF0160c	Dihydroorotate dehydrogenase (quinone)	6.443931	**39**	ERD2	ER lumen protein retaining receptor	3.066639
15	PFB0200c	Aspartate aminotransferase	5.452822	**40**	PFL1710c	TetQ GTPase	3.046927
16	HRPII	Histidine-rich protein PFHRP-II	5.283121	**41**	PfGSK-3	Glycogen synthase kinase 3	2.969468
17	PFA_0310c	P-type calcium transporting ATPase	5.242973	**42**	PfHDAC1	Histone deacetylase	2.964424
18	PF11_0165	Falcipain-2A;	4.666065	**43**	PF07_0029	Heat shock protein 86	2.94861
19	PFL1410c	ABC transporter (CT family)	4.564875	**44**	ERC	Membrane-associated calcum-binding protein	2.943386
20	PF10_0038	40S ribosomal protein S20e, putative	4.564875	**45**	PfENR	Enoyl-acyl carrier reductase	2.930894
21	PFI1170c	Thioredoxin reductase 2	4.35678	**46**	SPP	Signal peptide peptidase	2.861938
22	ATPase4	Non-SERCA-type Ca^{2+}-transporting P-ATPase	4.157638	**47**	PF11_0174	Cathepsin C	2.800708
23	PF11_0162	Falcipain-3	3.981097	**48**	PF14_0486	Elongation factor 2	2.78679
24	PF08_0054	Heat shock 70 kDa protein	3.843272	**49**	PF14_0192	Glutathione reductase;	2.74383
25	PF14_0038	Cytochrome c, putative	3.717327	**50**	PFC0210c	Circumsporozoite protein	2.669867

*According to text-mining score from highest to lowest. S/N = Serial Number.

DISCUSSION

Protein-protein interactions originate from the STRING database (https://www.stringdb.org). STRING was chosen because it covers a large range of microbial species and may be extended to accommodate new species (Ahmadi *et al*., 2022). In this study, we ranked *P. falciparum* target proteins from highest to lowest in terms of text-mining scores using the Cytoscape software, with PfCRT, PfMDR1, DHPS, PfMRP, MSP2, MSP1, PF14_0077, PF10_0344, DHFR-TS, and PfMDR2 having high text-mining scores. The text-mining score indicated how frequently *P. falciparum* target proteins were referenced in the downloaded abstract, with PfCRT having the highest score. For decades, malaria treatment was based on chloroquine (CQ), a safe and inexpensive 4-aminoquinoline that was very successful against intra-erythrocytic asexual blood-stage parasites, until resistance emerged in Southeast Asia and South America and spread globally. Chloroquine targets the polymerization of free haem in the parasite's food vacuole where haemoglobin taken up from the host is digested into amino acids, which are needed for the synthesis of parasite proteins and Fe^{2+}-containing haem. Fe^{2+}-containing haem oxidises to Fe^{3+}-containing protoporphyrin IX (FPIX), which is poisonous to the parasite and is thus transformed into the polymer haemozoin (malaria's black pigment). Chloroquine inhibits haemozoin production. The primary chloroquine resistance mechanism is drug efflux via the *P. falciparum* chloroquine-resistant transporter (encoded by PfCRT) situated in the food vacuole. K76T, a single nucleotide polymorphism (SNP) in PfCRT, was uniformly associated with chloroquine resistance in Africa, and other changes in PfCRT are associated with the development of chloroquine resistance globally (Kim *et al*., 2019)**.**

PfMDR1, like PfCRT, is located on the food vacuole (FV) membrane, although transport is expected to be directed inward towards the FV. Topologically, PfMDR1 is similar to a normal P-glycoprotein-type ABC transporter, with two membrane-spanning homologous domains that each include six predicted helices and a hydrophilic nucleotide-binding pocket. This binding domain appears to be situated on the FV's cytosolic side, where it could initially interact with antimalarials. Reducing PfMDR1 copy number makes parasites more susceptible to mefloquine, halofantrine, lumefantrine, quinine, and artemisinin derivatives. The amino acid alteration N86Y, seen in many African strains, alters medication susceptibility by boosting parasite resistance to chloroquine and amodiaquine while increasing susceptibility to mefloquine, lumefantrine, and dihydroartemisinin. The combination of artesunate and amodiaquine has been shown to select for PfMDR1 N86Y and D1246Y in parasites that emerge following therapy, as well as lower sensitivity to mono-desethyl-amodiaquine, an active metabolite. PfMDR1 mutations in 7G8 South American parasites had a greater impact on chloroquine resistance than in Asian Dd2 or African GB4 parasites, indicating important isoform-specific interactions between PfCRT and PfMDR1. Drugs must have optimal access to their target to be highly active. Mutations in PfMDR1 apparently limit antimalarial transport from the cytosol to the FV, lowering the concentration of heme-targeting medicines like chloroquine and amodiaquine in the FV. Antimalarials, including mefloquine, lumefantrine, and halofantrine, which are anticipated to inhibit targets outside the FV, become more powerful when their transport via PfMDR1 away from the cytosol is inhibited (Wicht *et al*., 2020)

Sulfadoxine and pyrimethamine block the *P. falciparum* enzymes dihydropteroate synthase (PfDHPS) and dihydrofolate reductase (PfDHFR), which are involved in the folate cycle. Resistance to these antimalarials is caused by dominant mutations in catalytic sites and/or amplification of the PfDHPS and PfDHFR genes. Multiple mutations in PfDHPS and PfDHFR provide resistance to sulfadoxine-pyrimethamine in endemic areas, while parasites with quintuple mutations (PfDHFRN51I, C59R, S108N, PfDHPSA437G, and K540E) have been related to sulfadoxine-pyrimethamine resistance in Africa. Sulfadoxine-pyrimethamine has been combined with the artemisinin-containing compound artesunate. Double, triple, and quadruple mutations in PfDHPS and PfDHFR genes resulted in the replacement of this medication combination with a second ACT constituted of the artemisinin-based compound artemether and the companion medicine lumefantrine in North East India (Haldar *et al*., 2018; Rahman *et al.*, 2018; Bazie *et al.*, 2020)**.**

During the blood stage, the parasite expresses a variety of proteins, including merozoite surface protein 1 (MSP1) and MSP2. These proteins are involved in erythrocyte invasion and are targeted by immune responses, making them potential vaccine targets. The MSP1 and MSP2 genes are likewise highly polymorphic and, hence, play an essential role in the identification of genetically different *P. falciparum* parasite subpopulations. The MSP1 gene is situated on chromosome 9 and consists of 17 blocks of sequences. Block 2 is the most polymorphic and is divided into three allelic families: MAD20, K1, and RO33. The MSP2 gene is situated on chromosome 2 and is divided into 5 blocks, with block 3 being the most polymorphic. The MSP1 alleles are divided into two groups (FC27 and IC1/3D7). Many studies have looked into the potential roles of MSP1 and MSP2 alleles in the modulation of malaria clinical symptoms. In French Guyana, a study on the MSP1 and var genes revealed that MSP1 K1 allele, var genes, and

D allele overexpression are linked to severe malaria (Haldar *et al.*, 2018; Jamil *et al.*, 2021; Arzika *et al.*, 2023).

PfMDR1 SNPs and/or increased copy number have been extensively explored as indicators of parasite resistance to multiple antimalarial drugs. The ABCC family of ABC proteins, commonly known as Multidrug Resistance Transporters (MRPs), is understudied but has the potential to be important MDR players. MRPs have been observed in many phylogenetically varied populations as broad-range transporters, with substrates comprising essential endogenous compounds like glutathione and its conjugates, as well as a variety of medicines with diverse structures (Gil and Fançony, 2021; Rout and Mahapatra, 2019).

CONCLUSION

Using *in silico* network Pharmacology technique, we identified PfCRT, PfMDR1, DHPS, PfMRP, MSP2, MSP1, PF14_0077, PF10_0344, DHFR-TS, and PfMDR2 as proteins with high text-mining evidence for *P. falciparum* resistance. The identified genes could influence experimental and clinical decision-making.

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Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization, methodology, validation, and manuscript writing were jointly carried out by OFA and MUT.; Supervision, O.F.A.

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AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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