BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 8(22)

Current opinion on an emergence of drug resistant strains of *Plasmodium falciparum* through genetic alterations

Kondapalli Kasturi*, Donepudi Siva Mallika, Seelam Jeevan Amos, Pavithra Venkateshaiah & KRS Sambasiva Rao

Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India; Kondapalli Kasturi – Email: kasturi.is.kodapalli21@gmail.com; *Corresponding author

Received August 15, 2012; Revised September 05, 2012; Accepted October 17, 2012; Published November 13, 2012

Abstract:
The human malarial parasite *Plasmodium falciparum* is one of the world's most devastating pathogen. Its capability to regulate its genes under various stages of its life cycle as well as under unfavourable environmental conditions has led to the development of vaccine resistant strains. Similarly, under drug pressure it develops mutations in the target genes. These mutations confer mid and high-level resistance to the antimalarial drugs. Increasing a resistance of malaria parasites to conventional antimalarial drugs is an important factor contributing to the persistence of the disease as a major health threat. This article reviews current knowledge of stage specific malarial targets, antimalarial drugs and the mutations that have led to the emergence of resistant strains.

Keywords: Malaria, *Plasmodium falciparum*, Antimalarial drugs, Mutations, Stage specific protein targets.

Background:
Malaria is a life threatening disease, which is transmitted to public from one another exclusively by female Anopheles mosquitoes. The life cycle of Plasmodium is extraordinarily complex involving an invertebrate vector (mosquito) and a vertebrate host (human). Among the other, *Plasmodium falciparum* causes severe symptoms such as cerebral malaria and finally death. This disease is present in 106 countries and among these 99 have ongoing transmission of malaria. According to WHO (World Health Organization) estimation there were 216 million malaria cases was reported worldwide and among this 6, 55,000 persons were dead in 2010. 86% of the victims were children under 5 years of age, and 91% of malaria deaths occurred in the WHO African Region. Malaria mortality rates have fallen by 5.3% in global context in 2010 **[1]** http://www.who.int/malaria/world_malaria_report_2011/WMR2011_factsheet.pdf. The global incidence of malaria is mainly caused by four Plasmodium species. They are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*.

Life cycle in brief
Life cycle of malarial parasite can be dividing into three stages (1) Exoerythrocytic stage; (2) Intraerythrocytic stage and (3) Sporogonic stage. Among these first two stages are completed in humans and next one is in mosquitoes. Infection of Plasmodium is initiated as porozoites (Exoerythrocytic Stage), they are injected into peripheral blood stream through the saliva of a feeding mosquito. These sporozoites are carried to the liver and invade hepatocytes through the circulatory system. After that it forms merozoites and enters into the blood stream and infects the erythrocytes (Intraerythrocytic), which is a pathogenic stage of malaria. Digestion of Hemoglobin is the major catabolic process of Plasmodium sps in merozoitic stage **[2],** mature merozoites can form tropozoites and these can form gamatocytes or again invade into fresh erythrocytes. Plasmodium completes its life cycle in humans and enters into mosquitoes gut by blood meal for sexual cycle which is sporogonic and enters into salivary gland, ready to infect another human being.

© 2012 Biomedical Informatics

Stage specific protein targets

The protein targets of *P. falciparum* from sporozoites (the infectious form injected by the mosquito), merozoites (the invasive stage of the erythrocytes), trophozoites (the form multiplying in erythrocytes), and gametocytes (sexual stages) of the human malaria parasite are tabulated **Table 1 (see supplementary material)**. Genes involved in virulence and antigenic variation (for example, var, vir and rif genes) in *Plasmodium falciparum* are located in the subtelomeric regions of the chromosomes **[3, 4].**

Anti malarial drug- target- resistance

A limited number of chemotherapeutic agents for the treatment of malaria are available but the growing problem of drug resistance makes the treatment of malaria increasingly difficult **[5].** Its control is hampered by increasing resistance of malaria to the available drugs **[6].** Some of the anti malarial drugs available in market are mainly Quinine derivatives, antifolates and arylalcohols. Quinoline antimalarials, i.e. an active ingredient of Cinchona bark, which was used as antimalarial drug since seventeenth century **[7].** Quinine requires thrice daily administration over 7 days; this can result in poor compliance. The dependence on raw material for its extraction and the availability of structural elucidation techniques leads to the development of fully synthetic chloroquine and later amodiaquine **[8],** which are inexpensive and more effective for a long time. Amodiaquine use has been limited since the mid 1980s, as it shows a high degree of efficacy **[9].** The other antimalarial drugs structurally similar to chloroquine are Mefloquine and halofantrine, but resistance to these drugs was rapidly developed than chloroquine **[10].** Additional limitations to these drugs usage of these drugs are occasionally neuropsychiatric disturbances for mefloquine and halofantrine, it can cause harm to the people with a history of heart disease **[9].**

Artemisinin the active constituent of the Chinese herb (Artemisia annua), which was used traditionally for treating several types of fevers, have been used increasingly over the past two decades **[9, 11].** The derivatives of this include artemether, arteether and artesunate, they are metabolized to dihydroartemisinin the main active agent in the body to treat malaria. These drugs can act against gametocytes, the sexual stages of the parasite that infect mosquitoes. Lumefantrine is an aryl alcohol similar to mefloquine and halofantrine, and neurotoxicity was not reported in animal toxicology studies **[12].** Antifolates are not derived from plants and synthesized through knowledge of cell biology and synthetic medicinal chemistry. Fully reduced folate cofactors are essential for nucleotide biosynthesis and amino-acid metabolism **[13].** Antifolate used to treat malaria is 2, 4-diaminopyrimidine (Pyrimethamine) and Sulphadoxine, (sulphonamide) an inhibitor of dihydrofolate reductase (DHFR), and dihydroopteroate synthase (DHPS) respectively. These enzymes are the part of folate pathway. Atovaquone/proguanil is another combination similar to that of sulphadoxine/ pyrimethamine **[13].** The hydroxynaphthoquinone atovaquone interferes with mitochondrial electron transport. Common antibiotics acting against bacterial protein synthesis such as tetracycline, doxycycline and clindamycin inhibit parasite growth and are being used increasingly in combination with other antimalarial treatments to augment their activity **[9].**

Resistance

Chloroquine, which served as a cheap and reliable drug for decades, is becoming ineffective against *Plasmodium falciparum* in most tropical areas. Thus, there is a need to develop novel antimalarial agents that are effective against drug-resistant malarial parasites **[14].** Drug development efforts generally aim for structurally unrelated compounds that work through new, independent mechanisms of action to existing antimalarial agents. Chloroquine and other derivatives such as amodiaquine, mefloquine, quinidine, quinacrine are potent inhibitors of heme polymerization. Target for the Chloroquine is the lysosome or food vacuole of the parasite; it interferes in the detoxification of haemoglobin. Pfmdr1 gene located on chromosome 5 of P. falciparum, encodes a P glycoprotein of 160 kDa which plays a role in drug efflux **[15].** Pfmdr1 gene at codons 86, 184, 1034, 1042 and 1246 have been proposed to be associated with chloroquine resistance **[16].** N86Y mutation in pfmdr1 seems to be playing some role in the chloroquine resistance but it is unclear. pfcrt is located on chromosome 7 and encodes for a protein named as *P. falciparum* chloroquine resistance transporter protein (PfCRT), this 36 kb membrane protein localizes to the parasite digestive vacuole (DV), the site of CQ action K76T mutation in pfcrt has been found in all the chloroquine resistant parasites. Besides K76T mutation in pfcrt, mutations at codon 72, 74, 75, 97, 220, 271, 326, 356 and 371 have also been found to be associated with chloroquine resistance **[17].** Concomitantly associated with increased sensitivity to mefloquine and halofantrine **[18-38]** because of the 1042D and 1034C/1042D/1246Y alleles are associated with increased resistance to quinine and increased sensitivity to mefloquine and artemisinin drugs. The increased copy number of the Pfmdr1 gene in *plasmodium falciparum* leads to treatment failure with mefloquine **[19, 20].**

After the failure of chloroquine, Sulphadoxine-pyrimethamine is used as a second line of drug to treat uncomplicated chloroquine resistant falciparum malaria cases. Use of SP is limited for pregnant women during the early trimester **[21, 22].** Sulphadoxine and pyrimethamine inhibit the enzymes dihydropteroate synthase and dihydrofolate reductase, respectively, involved in folate pathway. The mutation at codon 108 of *P. falciparum* DHFR S108N reduces the sensitivity of the pyrimethamine. The parasite isolates which showed pyrimethamine resistance, were found to contain this mutation and other mutations at codons 51 (N51I), 59 (C59R) and 164 (I164L)] were associated with S108N mutation. Majority of Indian isolates were found to contain double DHFR mutations (C59R+ S108N) **[24].** Most of the mutation in dihydropteroate synthase are at codons 436 (Ser to Ala/Phe), 437 (Ala to Gly), 540 (Lys to Glu), 581 (Ala to Gly) and 613 (Ala to Ser/ Thr). A437G is the key point mutation in DHPS; it is similar to S108N mutation in DHFR. A single DHFR mutation or double DHFR mutations alone will not cause SP treatment failure but that double DHFR mutations plus a single DHPS mutation or triple DHFR mutations alone can cause higher level of SP resistance **[24, 25].** Artemisinin was another antimalarial drug adopted by several countries; it inhibits the uptake of host hemoglobin. *P. falciparum* degrades host hemoglobin in an acidic digestive vacuole in a mid ring stage and reaches a peak in the mid trophozoite stage **[17].** Artemisinin distributes like BODIPYthapsigargin to membranous structures in the cytoplasm of parasites, and does not localize to the parasite

© 2012 Biomedical Informatics

food vacuole **[26].** Artemisinin resistance would be calamitous for global malaria control. Artemisinin resistance is observed because of the polymorphisms in the *Plasmodium falciparum* merozoite genes encoding the surface proteins of (MSP-1 and MSP-2) and the glutamate-rich protein (GLURP). The point mutations in the PfMDR1 gene at positions N86Y, S1034C, N1042D, and D1246Y, but the mutant codon Y184F was found to be responsible for resistance againt Artemisinin. Sporadic point mutations noted in the sequence of PfSERCA gene revealed that mutations at I89T, N465S, and E847K are also seen in several field isolates. The PfSERCA L263E and S769N polymorphisms, proposed to confer artemisinin resistance, but these two mutations were not detected in fields **[27] Table 2 (see supplementary material).**

Amodiaquine, artemisinin-based combination therapy (ACT), introduced in 2001has the World Health Organization recommended ACTs as the first-line treatment for uncomplicated malaria. The mode of action of the combination therapy appears to be similar, and known to be concentrated in the parasite's lysosome like Chloroquine(CQ) **[28].** Most countries have now adapted to either Artemether Lumefantrine (AL) or ArteSunate (AS) and AmodiaQuine (AQ) as their first-line ACT, AQ is a 4-aminoquinoline related to chloroquine (CQ), and Although CQ resistance is now widespread across the world, the efficacy of AQ is variable in treatment trials **[29].** A mutation from 76K-T of the *P. falciparum* chloroquine resistance transporter (Pfcrt) gene is associated with resistance to both CQ and AQ **[30].** Amplification of Pfmdr1gene modulate susceptibility to the artemether-lumefantrine ACT, leading to poor treatment response

Drugs that are potent inhibitors of heme polymerization, such as amodiaquine, mefloquine, quinidine, quinacrine, and chloroquine, competed well, although quinine competed to a lesser extent, Mefloquine is a lipophilic quinoline alcohol which is in wide clinical use. Like chloroquine and quinidine, it also binds to heme polymer in a heme-dependent fashion and diminishes binding of quinolines associates with hemozoin. Mefloquine and a protease inhibitor blocks heme release in the food vacuole **[31].** Chloroquine accumulates in food vacuole and is bound to free heme and results in the inhibition of formation of hemozoin. Over expression of pfmdr1 related to the mafloquine resistance because it is also sharing same mechanism as other quinine derivatives **[32].** Polymorphisms in the *P. falciparum* multidrug-resistant 1 (Pfmdr1) gene have concluded that its protein product, Pgh1, plays a role in modulating levels of resistance to several structurally unrelated drugs **[18, 38].** All resistant parasites had the K76T mutation in the PfCRT gene. Some antimalarials, including artemisinin, are reported to inhibit hemoglobin uptake **[34],** but it is not clear if this is a direct effect, or a consequence of parasite killing. Artemisinin is highly potent against hemoglobin-digesting stages of malaria parasites **[34, 37]** than other hemoglobin-degrading drugs, but it is much less potent against other pathogens **[35].** Falcipain-2 is the only cysteine protease hemoglobinase expressed early in erythrocytic parasite development, and knockout parasites have markedly diminished hemoglobinase activity in the early trophozoite stage **[36].** These parasites exhibit a swollen vacuole phenotype, consistent with defective haemoglobin digestion, but the deletion is not lethal, apparently due to rescue by the expression of falcipain-2 and falcipain-3 beginning at the mid trophozoite stage **[36].**

Conclusion:

Antimalarial drugs play very important role in controlling of malaria at individual as well as at epidemiological level. Careful treatment can slow down the development of resistance against the available drugs, mostly due to regular usage leads to development of mutations in the target gene. It is advisable to check the efficacy of the drug at regular intervals so as to take a policy decision in advance on its continued usage in the field. Molecular surveillance can give an advanced indication that a particular drug is going to lose its efficacy in near future. However, molecular markers for several antimalarial drugs are yet to be identified and much improvement is required on the currently used method.

Acknowledgement:

Authors thank the Department of Biotechnology, Govt. of India for the financial assistance provided to carry out the project work. The authors would also like to thank Acharya Nagarjuna University, Guntur, India for providing the congenial environment and also for extending the lab facilities to carry out the work.

Reference:

[1] World Health Organisation. The World malaria report; Geneva, Switzerland. 2011
[2] Klemba M & Goldberg DE, *Biochem.* 2002 **71**: 275
[3] Hernandez-Rivas *et al. Mol Cell Biol*. 1997 **17**: 604 [PMCID: PMC231785]
[4] Del Portillo HA *et al. Nature*. 2001 **410**: 839 [PMID: 11298455]
[5] World Health Organisation 2000 Report of the WHO expert Committee on malaria. WHO Tech.Rep.Ser.1-74.
[6] Greenwood BM *et al. Malaria*. 2005 **365**: 1487 [PMCID: PMC15850634]
[7] Croft S, *Drug Discov Today*. 2001 **15:** 1151
[8] Ridley RG & Hudson AT, *Exp Opin Ther Patents*. 1998 **8**: 121
[9] Report No. 2001 WHO/CDS/RBM/2001.33
[10] Wellems T & Plowe CV, *J Infect Dis*. 2001 **184**: 770 [PMID: 11517439]
[11] Li Y & Wu YL, *Med Trop*. 1998 **58**: 9 [PMID: 10212890]
[12] Skelton-Stroud P & Mull R, *Med Trop*. 1998 **58**: 77 [PMID: 10212907]
[13] Sherman I, *Malaria: Parasite Biology*. 1998 **177**: 184
[14] Oaks SC Jr *et al. Malaria: Obstacles and Opportunities.* National Academy Press, Washington, DC. 1991.
[15] Foote SJ *et al. Cell.* 1989 **57**: 921 [PMID: 2701941]
[16] Fidock DA *et al. Mol Cell*. 2000 **6**: 861 [PMID: 11090624]
[17] Slomianny C, *Blood Cells*. 1990 **16**: 369 [PMID: 2096983]
[18] Price RN *et al. Clin Infect Dis*. 2006 **42:** 1570 [PMID: 16652314]
[19] Pickard AL *et al. Antimicrob Agents Chemother*. 2003 **47**: 2418 [PMID: 12878499]
[20] Price RN *et al. Lancet*. 2004 **364**: 438 [PMID: 15288742]
[21] Korsinczky M *et al. Antimicrob Agents Chemother*. 2004 **48**: 2214 [PMID: 15155224]
[22] Leartsakulpanich U *et al. Mol Biochem Parasitol*. 2002 **119:** 63 [PMID: 11755187]

 © 2012 Biomedical Informatics

[23] Ahmed A *et al. Antimicrob Agents Chemother*. 2004 **48:** 879 [PMID: 14982779]
[24] Wang P *et al. Mol Biochem Parasitol*. 1995 **71**: 115 [PMID: 7630375]
[25] Plowe CV *et al. J Infect Dis*. 1997 **176**: 1590 [PMID: 9395372]
[26] Eckstein-Ludwig U & Webb RJ, *et al. Nature*. 2003 **424**: 957[PMID: 12931192]
[27] Dondorp AM *et al. N Engl J Med*. 2009 **361**: 455 [PMID: 19641202]
[28] Famin O & Ginsburg H, *Biochem Pharmacol*. 2002 **63**: 393 [PMID: 11853690]
[29] Olliaro, P & Mussano P, *Cochrane Database Syst Rev*. 2003 2: CD000016 [PMID: 12804382]
[30] Djimde A *et al. N Engl J Med*. 2001 **344:** 257 [PMID: 11172152]
[31] Sullivan DJ Jr *et al. J Biol Chem*. 1998 **273**: 31103 [PMID: 9813011]
[32] Nishiyama Y *et al. Nucleic Acids Symp Ser (Oxf)*. 2004 **48:** 163 [PMID: 17150529]
[33] Hoppe HC *et al. Antimicrob Agents Chemotherapy*. 2004 48: 2370 [PMID: 15215083]
[34] O'Neill PM *et al. Molecules*. 2010 **15**: 1705 [PMID: 20336009]
[35] Kaiser M *et al. Antimicrob Agents Chemother*. 2007 **51**: 2991 [PMID: 17562801]
[36] Sijwali PS *et al. Mol Biochem Parasitol*. 2006 **150:** 96 [PMID: 16890302]
[37] Klayman DL, *Science.* 1985 **228**: 1049 [PMID: 3887571]
[38] Duraisingh MT *et al. Mol Biochem Parasitol*. 2000 **108**: 13 [PMID: 10802315]
[39] Florens L & Washburn MP, *et al. Nature*. 2002. **419**: 520 [PMID: 12368866]
[40] Pinder JC, *et al. J Cell Sci*. 1998 **111:** 1831 [PMID: 9625746]
[41] Holder AA, aMulti-immune Response andMulti-stage Perspective 77–104 (ASM Press, Washington, 1996).
[42] Coppel RL *et al. Nature.* 1983 **306**: 751 [PMID: 6361573]
[43] Taylor HM *et al. Infect Immun*. 2001 **69:** 3635 [PMID: 11349024]
[44] Kaneko O *et al. Mol Biochem Parasitol*. 2001 **118:** 223 [PMID: 11738712]
[45] Dessens JT *et al. EMBO J.* 1999 **18**: 6221 [PMID: 10562534]
[46] Deitsch, KW & Wellems TE, *Mol Biochem Parasitol.* 1996 **76**: 1 [PMID: 8919990]
[47] Kyes SA *et al. Proc Natl Acad Sci* USA. 1999 **96**: 9333 [PMID: 10430943]

Edited by P Kangueane
Citation: Kasturi *et al*. Bioinformation 8(22): 1114-1118 (2012)
License statement: This is an open-access article, which permits unrestricted use, distribution, and reproduction in any medium, for non-commercial purposes, provided the original author and source are credit

BIOINFORMATION

open access

Supplementary material:

Table 1: Stage specific Protein Targets in *Plasmodium falciparum*

Stage	Protein Type	Protein Name	References
Merozoite	Cell recognition and invasion	MCP1, MTIP	**40**
	Membrane Surface proteins	MSP1, MSP2, GPI	**41**
		MSP3, MSP6	
	Erythrocyte Binding Protein	EBA175, EBA140 / BAEBL	**39**
	Recticulocyte Binding Protein	PfRH3	**43**
	Vacuolar Proteins	ABRA, SERA,	**42**
	Rhoptry associated protein	RAP1,2 and 3, RhopH1,2,and 3, RESA,	**44**
	Apical Organellar proteins*	AMA1,MAEBL,	**39**
Sporozoite	Membrane Surface Proteins	MSP8	
	Circumsporozoit Protein	CSP	
	Sporozoit Surface Protein	SSP2 / TRAP,CTRP	**46**
	var Gene Proteins	PfEMP1 25 isoforms	**47**
	rif Gene Proteins	*rifins21* isoforms	
Tropozoite	Erythrocyte Membrane Protein	EMP2 and 3, Skeleton Binding Protein 1	**45**
	Exporting proteins	EXP1 and EXP2	

*Proteins which are expressed in both merozoite and sporozoite stages

Table 2: Target for the available drugs for *Plasmodium falciparum*: Responsible mutations

*Year	Drug	Class	Target	Mutation	References
1945	Chloroquine	4, amino-quinolines	*pfcrt*	K76T SNP's at 72, 74, 75, 97, 220, 271, 326, 356 & 371	16
			pfmdr1	N86Y, SNP's at 184, 1034, 1042 & 1246	
1961	Pyrimethamine	Diamino pyramidine	*pfdhfr*	S108N SNP's F50L, N51I, C59R and I164L	24, 25
				Double mutation: C59R+ *S108N	
				Triple Mutation: N51T + C59R+ *S108N	
	Sulphadoxine	Sulfanamides	*pfdhps*	A437G, S436A / F, L540 E, A581G and A613 S / T	
1984	Mefloquine	Amino alcohols	*pfcrt*	K76T	19, 18
			pfmdr1	N86Y, N1042D & D1246Y	
1994	Artemisinin	Sesquiterpene lactones	*pfmdr1*	Y184F N86Y, S1034C, N1042D, and D1246Y	26
			PfSERCA	I89T, N465S, E847K #L263E and #S769N	27
1999	Artemether	Amino alcohols	*pfcrt*	K76T	19
			pfmdr1	N86Y, N1042D & D1246Y	
	Lumefantrine	Sesquiterpene lactones	*pfcrt*	76K	16
			pfmdr1	86N	

*Key mutations play a role in Drug resistance. # Proposed responsible mutations but not seen in fields.
*W H O had declared the drug as first-line treatment for the malaria in particular Year

© 2012 Biomedical Informatics