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# Genetic Polymorphism of *Plasmodium falciparum* Candidate Genes: A Global Problem in Malaria Control

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## Abstract

Malaria is one of the deadliest infectious diseases worldwide. This review elucidates the genetic cause of treatment failure in *Plasmodium falciparum* infection. One hundred ninety eight (198) million of malaria cases are reported globally and estimated 584000 deaths occur in 2013. Single point mutation of asparagine to tyrosine in codon 86 (N86Y) of pfmdr-I gene and some other polymorphisms, like 184Y, 1034N, N1042D and D1246Y is associated with CQ resistance. Different point mutations including K76T in pfcrt gene were highly associated with chloroquine resistance. Antifolate Sulfadoxine/pyrimethamine (SP) combination has been used as a second-line therapy against chloroquine-resistant *Plasmodium falciparum* malaria. Polymorphism in pfdhfr codon 108 (S108N) and other point mutation in pfdhfr N51I and C59R confer higher levels of resistance. Mutation in pfdhps A437 is associated with sulfadoxine resistance, while additional changes (S436A, K540E, A581G, A613T/S) appear to increase the degree of resistance. Perseverance of SP resistance in relation to treatment outcome is visualized when at least two pfdhfr and one pfdhps mutation occurs. Increasing failure of predominantly used chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) has been a serious obstacle towards the global malaria control. The combination of artesunate (AS) and sulfadoxine-pyrimethamine (SP) (ACT) has replaced the single use of CQ and SP. Artemisinin resistance is strongly associated with an increase in parasite clearance half-life (PCHL), which imitates the reduced susceptibility of ring-stage parasites. Different codon of pfkelch13 gene point mutation (441codon and other) was highly correlated with ACT failure as well as increased PCHL and RSA thereby resistance to AS. Genetic polymorphism of different candidate genes leads to drug resistance in malaria parasite which makes a global problem to eradicate malaria.

**Keywords:** *Plasmodium falciparum*; Polymorphism; Chloroquine resistance; Sulfadoxine/pyrimethamine (SP) resistance; Artemisinin combination therapy (ACT)

## Introduction

The emergence of anti-malarial drug resistance is dependent on the occurrence that causes spontaneous change genetically (mutation or gene amplification) in a malaria parasite. These genetic alterations of the parasitic gene cause susceptibility to a particular drug [1]. The resistance property of *Plasmodium falciparum* against available anti-malarial drugs is a major challenge towards malaria control in different endemic areas of the globe [2]. Combinations of different antimalarial drugs having different molecular targets, ultimately helps to delay the emergence of resistance [3]. Therefore recent malaria control programmes precisely focus on the adaption of different combination medicine against plasmodium infection [4]. Single use of antimalarial drugs now has been banned by the WHO, especially against *P. falciparum*. Mutations in different target genes can be used as molecular markers to detect the drug-resistant parasite [5].

Onset of chloroquine (CQ) resistant falciparum malaria was reported from Thailand in 1957 [6] and from Cambodia in 1960, followed by sub-Saharan Africa and gradually it emerged in different parts the Southeast Asia and Africa [7]. In 1980s, CQ-resistant parasite rapidly increased in South and Central America [8]. Without replacing a particular drug having such low cost and reliability as CQ, the morbidity and motility resurged among the children in Africa [9]. The substitution of lysine to threonine (K76T) at the codon position 76 of the *Plasmodium falciparum* chloroquine resistance transporter (pfcrt) gene is associated with *in vivo* as well as *in vitro* CQ resistance in Africa, South America as well as in Southeast Asia [10, 11]. Further it was proved that the point mutations associated with the entire pfcrt gene were found to be highly co related to *in vitro* CQ resistance among *P. falciparum* isolates from Africa, South America, and Southeast Asia. In India, CQ resistant *P. falciparum* was reported for first time in the North-Eastern state of Assam, in 1973 at Karbi-anglang subdivision [12]. Soon after it spread to many other areas in India [13].

The point mutation in different position of a gene on a chromosome (locus) (C72S, M741, N75E, K76T, A220S, Q271E, N326S, 1456T, R371I) of pfcrt gene, a transporter that is located

at chromosome 7, may distinguish between CQ-resistance and CQ-sensitive strain. Various molecular analyses confer CQ resistance by allowing enhanced efflux of CQ from the digestive vacuole (DV) due to the genetic alteration in pfcrt gene [14]. The analysis of K76T mutation in pfcrt gene reveals its 100% association with the *in vitro* CQ resistance. Various nucleotide mutation that alters the amino acid sequence of a protein (non-synonymous) polymorphisms at codon 76 of pfcrt gene are found (K76I, K76N or K76T), with greater CQ efflux. A specific combination of pfcrt and pfmdrl alleles, resulting in varying responses to CQ, seems geographically restricted, which may explain why some field studies reported that there is an association between *Plasmodium falciparum* multidrug-resistance gene (pfmdr1) polymorphisms and CQ resistance. Significant association between the pfmdr1 86Y and the EC of CQ among clones with the pfcrt 76T allele suggests the possible role of both mutations in development of CQ resistance.

Pfmdr1 is one of the principal genes that associate CQ resistance [15]. Mutation in the pfmdr-1 gene, located on chromosome 5 has some significant role in *P. falciparum* resistance towards various antimalarials, such as CQ, mefloquine, quinine, and artemisinin derivatives. In an earlier study, significant association (linkage disequilibrium, LD) between the alleles pfmdr1 86Y and pfcrt 76T has been observed [15]. Studies conducted in different geographical areas of the world have suggested that the point mutation of tyrosine at codon 86 (N-86 to 86-Y) is related to CQ resistance [16]. Several other pfmdr1 polymorphisms 184-phe, 1034-cys, 1042-asp and 1246-tyr are being implicated to vary the degrees in CQ resistance [16, 17]. In India pfmdr1 86-tyr and 184-phe are commonly observed mutant allele with high *in vitro* IC50 for CQ [18]. In some parts of the world, CQ resistance occurs due to polymorphisms in both pfcrt and pfmdr1 gene [19]. In India *in vivo*, CQ treatment failure and *in vitro* CQ resistance are linked with pfcrt 76-thr but not for pfmdr1 gene [18].

Dihydrofolatereductase (DHFR) is an important enzyme that converts Dihydrofolate (DHF) to Tetrahydrofolate (THF) at the presence of NADPH, which is used as H+ donor [20]. Folate biosynthesis is very important for *P. falciparum* parasite because it is a precursor of purine essential for DNA replication. To prevent parasite DNA replication antifolate drug is used which can inhibit these proteins. Though, sulfadoxine and pyrimethamine (SP) is combined drugs but the mode of action of each component is different. Sulfadoxine actively inhibits dihydropteroate synthase (DHPS), the enzyme is involved in folate synthesis, mediating the synthesis of dihydropteroate which results reduction of dihydropteroate. The reduction of dihydropteroate subsequently decreases dyhydrofolate synthesis, which lowers the substrate of dhfr and the activity of dhfr-inhibitor increases. In this way, concomitant blockage of dihydropteroate synthase (dhps) and the inhibition of Dihydro folate reductase (dhfr) results in the synergistic action and interruption of the DNA replication process. Pyrimethamine is a potent inhibitor of DHFR, which plays three main roles in the folate pathway in *P. falciparum*. It controls de novo folate synthesis by catalyzing the synthesis of tetrahydrofolate (THF). It also mediates the salvage of exogenous folate derivatives, 7, 8-dihydrofolate (DHF) and the completely oxidized folate, by reducing them to tetrahydrofolate. Due to lack of folic acid, parasite multiplication is stopped and gradually parasites are destroyed [21]. *In vitro* resistance to pyrimethamine is known to be associated with the key dhfr mutation at S108N codon. Additional mutations in dhfr N51I, C59R and I164L confer higher levels of resistance [22]. Mutation of dhfr profile demonstrated several varients over wild type allele. dhfr 108-asn is very common followed by mutation polymorphism at 51-Ile and 59-arg.

Sulfadoxine competes with the substrate that binds to the parasite enzyme *P. falciparum* dihydropteroate synthetase (pfdhps). A mutation at several amino acid positions of this enzyme reduces binding efficiency with the drug. For that reason, a higher amount of drug is required to inhibit the mutated pfdhps enzyme and the growth of parasite [23]. There are five different amino acid positions (436, 437, 540, 580 and 613) which undergo polymorphism in its mutated form and highly associates sulfadoxine resistance. The amino acid sequence of wild type pfdhps allele at these positions is the SAKAA haplotype. Mutation in pfdhps may start settling amino acid positions at 436 or 437, followed by the mutations at other amino acid positions. The higher the number of mutations in pfdhps gene is proportional to the higher level of drug resistance property shown by the parasite [24]. *In vitro* resistance to sulpfadoxine has been associated with the key mutation of dhps A437G; additional mutations in dhps S436A, K540E, A581G and A613T⁄S confer higher levels of resistance [25]. Previous studies reported the presence of 11 different genotypic variant of pfdhps in the Indian *P. falciparum* population [24]. The wild-type SAKAA allele of pfdhps gene was highly leading in parasite populations of all regions of India except Andaman and Nicobar Island [26].

In Artemesinin Combination Therapy (ACT), the artemisinin compounds rapidly reduce the parasite number whereas the typically long half-life drugs clear the remaining parasite population [27]. If the parasite becomes resistant to any of these compounds, both Parasite clearance time (PCT) and median Parasite clearance half-life (PCHL) increase leading to ACT failure [28]. Early ACT Failure indicates the parasite line is less susceptible to AS, while Delayed Parasite Clearance is usually associated with the reduced activity of fast-acting drug (artemisinins) and recrudescence is associated with the less efficacy of the long acting drugs (Anti-folate drugs). Artemisinin resistance is characterized by slow parasite clearance in Thailand and Cambodia [29, 30]. Clearance (assessed by microscopy) of sensitive *P. falciparum* parasite is achieved within 2 days in 95% of patients whereas artemisinin-resistant infections remain slide-positive for 3 or more days; treatment failure is more common in such infections after ACT [31]. Another cause of great concern is this artemisinin compounds are regarded as the last and final order of antimalarials.

*Plasmodium falciparum* kelch 13 (pfk13) polymorphisms after 441 codon has been accounted for reduced susceptibility to artemisinin *in vitro* (ring stage survivality>10% and increase IC50 of artemisinin) as well as with increase in parasite clearance half-life (PCHL>5 h) thereby resulting *in vivo* ACT treatment failure in different parts of the world [32]. In African rodent malaria,

polymorphisms at 739F and 770F codon of ubiquitin binding protein 1 (ubp-1) gene associated reduce susceptibility of artemisinin. Although ubp-1 polymorphism have not been observed in human beings yet. Recent study revealed that ubiquitin binding protein plays together with Kelch protein. Pfnhe1 another gene, located on chromosome 13, contains a predicted $Na^+$-$H^+$ exchanger. It is one of the new implicated putative transporters that modulate parasite response to antimalarials [33]. Analysis of microsatellite variations provides a significant association between DNNND repeats in the C-terminal cytoplasmic domain of pfnhe1 and *in vitro* response to quinine (QN). Sometime cg2, a putative transporter that modulates intraparasitic drug concentrations [34]. Recently it has been proved that polymorphism in COXI, pfmrp and cytochrome P450 partially modulates the different antimalarial drug activity *in vitro* as well as *in vivo*.

Malaria parasite changes its genetic arrangement (polymorphism) of different candidate gene (pfcrt, pfmdr1, pfdhfr, pfdhps and pfkelch13) which lead to inactivation of antimalarial drug. This phenomenon makes a major problem to eradicate malaria globally.

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