Chapter 8

Structure and Functional Differentiation of PfCRT Mutation in Chloroquine Resistance (CQR) in *Plasmodium falciparum* Malaria

Pratap Parida, Kishore Sarma,
Biswajyoti Borkakoty and
Pradyumna Kishore Mohapatra

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64659

Abstract

Approximately one million deaths are attributed to malaria every year. Latest reports of multi-drug treatment failure of falciparum malaria underscore the desideratum to understand the molecular substratum of drug resistance. The mutations in the digestive vacuole transmembrane protein *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) are mainly responsible for chloroquine resistance (CQR) in *Plasmodium falciparum*. Multiple mutations in the PfCRT are concerned in chloroquine resistance, but the evolution of intricate haplotypes is not yet well understood. *P. falciparum* resistance to chloroquine is the standard antimalarial drug and is mediated primarily by mutant forms of the PfCRT. In this chapter, we present the mechanism of action of the chloroquine, the structural changes of the gene after the mutations as well as different haplotypes of the PfCRT.

Keywords: antimalarial resistance, haplotype, homology modeling, mutations, PfCRT

1. Introduction

The rapid advancement and spread of malaria parasite along with antimalarial resistance is becoming a critical disaster to the world health. Chloroquine resistance (CQR) originated in Southeast Asia and South America, more or less simultaneously, in late 1950s and subsequently spread to several other malaria-endemic countries [1]. PfCRT, a candidate gene for

CQR, is present at the digestive vacuole membrane and it holds 10 putative transmembrane domains [2, 3]. Mutations in two genes, namely, the *Plasmodium falciparum* CQ resistance transporter (PfCRT) and multidrug resistance transporter-1 (pfmdr1), have been reported as responsible for CQR in *P. falciparum*. In addition, the polymorphisms of the PfCRT gene produce two different forms of PfCRT based on the drug response class, such as Chloroquine Sensitive (CQS) and CQR. The point mutations of the PfCRT codons are 72–76, 271, 326, 356, and 371, whereas two codons responsible for pfmdr1 are 86 and 1246 as molecular markers of CQ resistance [4]. The position of 72–76 of the PfCRT are considered as molecular markers used for detecting CQR malaria parasites due to the mutations in the positions 72–76 of the PfCRT, which were observed as a majority of *P. falciparum* endemic areas. There are five polymorphisms that form different haplotypes vary among the *P. falciparum* endemic regions. There are three major haplotypes based on specific mutations such as CVIETIHSESI, CVIETIHSESTI, and SVMNTIHSQDLR [2, 5–7].

Chloroquine was used as a synthetic drug in the early 1950s and 1970s [8]. The resistance to this drug was reported in Palian area of Cambodia nearer to Thai-Cambodia border as well as in Latin America. Subsequently the reported resistance to chloroquine in South Asia moved westward and during 1973, it was found to be present in North East region of India. Subsequently it spread to rest of India and beyond [9]. In 1950s, before the chloroquine resistance became widespread, it was the main drug which was cheap and having least toxicity as well as highly effective schizonticidal drug and also was effective against all the types of parasite species affecting human. But appearance of widespread resistance to chloroquine has contributed to resurgence of malaria in many countries of Asia including India [10].

By late 1980s, chloroquine became more or less obsolete for treating *P. falciparum* infections globally. However, due to high economical burden in introducing alternate treatment such as artesunate combination therapy, etc. for treatment of *Plasmodium vivax* restricted many countries like India to continue only with chloroquine for treatment of *P. vivax* [11]. This dual drug policy for the treatment of *P. falciparum* and *P. vivax* resulted in further consolidation of *P. falciparum*-resistant population in various southeastern regions of the Asia, especially in India [10].

In this chapter, we estimated certain scores of mutations such as (limbo, tango, and waltz score) to understand the changes that occur to the PfCRT protein after mutation with the help of homology modeling and single-nucleotide polymorphism (SNP).

2. *P. falciparum* CRT as a target for antimalarial drug design

Earlier, the parasite proteins involved in the resistance mechanism of malaria were unknown, but currently, it is well understood that the mutations in the PfCRT gene are causally involved in various methods such as *in vitro* and *in vivo* resistance as well as altered drug accumulation [2, 3, 12]. Identification of PfCRT gene, which encodes a putative transporter or channel protein, was a major achievement in the search for the genetic basis of CQR in *P. falciparum* [2]. PfCRT is a 48-kDa protein having 424 amino acids, 13 exon gene spanning 36 kb of chromosome 7

and 10 predicted transmembrane-spanning domains and is confined in a small area to the Digestive Vacuole (DV) membrane in erythrocytic stage parasites [2, 3]. The polymorphisms of PfCRT segregate precisely with two distinct drug response, which is considered either CQS or CQR. Fifteen polymorphic amino acid positions in PfCRT are associated with CQR in field isolates. These vary significantly depending on the geographic location and selection history, while CQS strains maintain an invariable wild-type allele [5–6, 13, 14]. K76T and S163R mutations in $PfCRT^{CQR}$ are primary and necessary for the resistance phenotype, which is the most reliable molecular marker of resistance among the various PfCRT mutations [10, 15, 16]. The endogenous role of PfCRT in the malaria parasite has not been clear yet despite the wealth of epidemiological and *in vitro* drug response data demonstrating the critical role of PfCRT mutations in producing CQR. An understanding of the natural role of PfCRT in a normally functioning cell is indeed needed to provide a clearer picture of how drug resistance works in the malaria parasite. Muhamad et al. studied the polymorphic pattern of PfCRT, which may be applied surveillance of chloroquine [17].

3. Mechanism of PfCRT

The mechanism involved in resistance against quinoline containing compound CQ in *P. falciparum* is still unclear [18, 19] and indeed the mode of action of this antimalarial chemotherapeutic agent is not beyond the debate. Chloroquine is thought to accumulate in high levels in the food vacuole of the asexual erythrocytic malaria parasites, where it acts by interfering with the polymerization of heme (hematin) into the hemozoin. The malaria parasite feeds by degrading hemoglobin of host cell, producing free ferriprotoporphyrin IX (FP) as a by-product. Ferriprotoporphyrin IX (FP) is highly toxic to the parasite and is neutralized via a process of biomineralization (polymerization) to form innocuous hemozoin crystals (β-hematin) known as malaria pigment. Chloroquine is thought to inhibit parasite growth by inhibiting the detoxification of ferriprotoporphyrin IX (FP) [20–23]. Chloroquine (CQ) appears to trap FP in a μ-oxodimeric form and prevents the formation of the β-hematin dimers that are required for hemozoin formation [24]. Thus, CQ causes a buildup of toxic FP molecules that eventually destroy the integrity of malaria parasite protein and membranes [25].

Current studies highlight an important gene connected to the resistance, *P. falciparum* chloroquine resistance transporter (PfCRT). It encodes a new transporter and also differentiates between the global selective sweeps of different haplotypes having the mutation, K76T [26]. The PfCRT gene which produces the transport proteins on the plasma membrane of the parasite's food vacuole, have been confirmed of involving with the resistance of the parasite to antimalarial drugs [27].

The mechanisms involved in the development of CQ resistance are also unclear. It is postulated that CQ resistance could arise as a consequence of any phenotypic and genotypic alteration(s), which reduces the concentration of the drug in the food vacuole of the parasite. This leads to a change in parasite biology and can lead to reduced uptake of the drug or enhanced CQ efflux from the cell or a combination of both resulting in reduced accumulation of drug inside the

digestive vacuole of the parasite. It has been established that in CQ-resistant parasites, the accumulation of chloroquine inside the vacuole is significantly less than that in CQ-sensitive parasites [28, 29]. It was originally thought that this lack of accumulation was due to the result of an efflux mechanism, and P-glycoprotein was implicated as the pump responsible for the efflux. However subsequent studies have suggested that efflux rates of CQ-resistant and CQ-sensitive strains are similar. So it appears that CQ resistance involves a diminished level of drug uptake rather than, or as well as, enhanced efflux. Chloroquine-resistant parasites are known to get rid themselves of the drugs 40–50 times faster than sensitive parasites [30] but the biochemical basis of this efflux is not clear. The efflux of chloroquine and in fact the entire chloroquine-resistant phenotype can be reversed with Ca^{+} channel blockers such as verapamil and diltiazem [31]. This phenomenon is biologically very similar to multi-drug resistance (MDR) phenotype of mammalian tumor cells, where a wide spectrum of chemotherapeutic agents is expelled from the cells by a verapamil-sensitive pump [32–35]. Verapamil (VPL), which inhibits P-glycoprotein (encoded by mdr gene) mediated multi-drug resistance (MDR) in mammalian tumor cells, also partly reverses chloroquine resistance in malaria parasites grown *in vitro* [31]. Because this reversal phenomenon is in some ways analogous to the reversal of MDR in mammalian tumor cell lines, CQ resistance has been postulated to involve an energy driven P-glycoprotein pump similar to that encoded by the mammalian mdr gene [31]. Two *P. falciparum* genes, which are homolog to mammalian mdr, have been identified and mapped to chromosome 5 and named pfmdr1 and pfmdr2 [36–38]. Associations have been reported between chloroquine resistance and mutations or amplification of the mdr-like gene pfmdr1, which encodes P-glycoprotein homolog-1 (Pgh1) [36, 39], which has been found to be located in the food vacuole membrane of the erythrocytic stages of the parasite [40], suggesting that it could be involved in drug transport across this membrane [41]. DNA sequencing of the pfmdr1 gene from reference strains and field isolates has revealed several point mutations that correlated with CQ resistance [39]. In earlier studies, several point mutations or nucleotide changes at position 754, 1049, 3598, 3622, and 4234 in pfmdr1 gene resulting in amino acid change at codons 86, 184, 1034, 1042, and 1246, respectively, have been shown to be associated with CQ resistance [42, 43]. But the role of these mutations in pfmdr1 in CQ resistance is controversial [44–51]. It has been observed that most of the southeast Asian CQ-resistant isolates (K1 genotype, on the basis of 3′ polymorphism in pfmdr1) have the mutation at nucleotide 754 resulting in amino acid change at codon 86 from asparagines to tyrosine (N86Y) while CQ-resistant South American isolates (7G8 genotype based on 3′ polymorphism in pfmdr1) have not shown mutation at codon 86 but mutational change at codon 184, 1034, 1042, and 1246 have been shown. Out of these several mutations described, the mutation at codon 86 appears to be an important, as this may be involved in substrate specificity of the gene product (P-glycoprotein) and may alter the transport activity of the protein [52]. Moreover, mutation at codon 86 has also been correlated to CQ resistance in parasites selected *in vitro* for CQ resistance [53]. However, studies from different geographical areas of the world have given the controversial picture of this mutation. Some field studies have confirmed the presence of this mutation in chloroquine-resistant isolates from Malaysia [42], Nigeria [54] Guinea-Bissau [55], and sub-Saharan Africa [56]. No association with this mutation, however, was found in isolates from Sudan [44], Thailand [45], and Cambodia [46]. Moreover, genetic studies found

no linkage between chloroquine resistance and the pfmdr1 gene [57]. In India, Bhattacharya and Pillai have found strong association between chloroquine resistance and certain mutations in the pfmdr1 gene of *P. falciparum* isolates [58]. However, that study was conducted in small number *P. falciparum* strains. Gómez-Saladín et al. found the involvement of tyr86 mutation of pfmdr1 gene in CQ resistance but no significant association between tyr86 mutation and level of resistance with chloroquine [59]. Majority of pfmdr1 studies reported till date have indicated conflicting pieces of evidence, which suggest that CQ resistance in *P. falciparum* involves multiple transport mechanisms and multiple genes.

Another locus governing chloroquine resistance in a *P. falciparum* genetic cross has been mapped on chromosome 7 and has been named as cg2 (candidate gene) [57, 60]. A protein specified by this gene has size variation in three repeat regions (k, ϕ, and ω) several non-silent point mutations and size variation in a central poly-Asn tract. Recently, field studies have identified a series of mutations in cg2 gene in chloroquine-resistant *P. falciparum* strains suggesting that polymorphism in cg2 gene was highly associated with CQ resistance [61, 62]. Chloroquine-resistant strains have been found to consist of 16 tandem repeat units in omega repeat region (Dd2 type) of cg2 gene, while the chloroquine-sensitive strains have either ≤15 or ≥17 repeat units [54]. A significant but incomplete association has been found between the presence of the cg2 Dd2-like omega repeat length polymorphism and *in vitro* resistance and between the tyr86 allele of pfmdr1 and *in vitro* resistance [49]. Adagu and Warhurst have reported the ala281 mutation in cg2 gene and Dd2-type kappa (k) repeats in CQ-resistant isolates of northern Nigeria [62]. They have also reported the significant association between tyr86 mutation in pfmdr1 and Dd2-type kappa (k) repeat and ala 281 mutation of cg2 gene.

Recently PfCRT, a gene with 13 exons, has been identified near cg2 on chromosome 7 [2]. This gene encodes, a transmembrane protein "PfCRT" in the digestive vacuole of malaria parasites. Sets of point mutations in PfCRT were associated with *in vitro* chloroquine resistance in clinical isolates and laboratory lines of *P. falciparum* from Africa, South America, and southeast Asia [7, 12, 14, 63–66]. One mutation, the substitution of lysine (K76) by threonine (T76) at position 76 (K76T), was present in all resistant isolates and absent in all sensitive isolates tested *in vitro* [12, 14, 64, 66–69]. A significant association was found between an allele of the *P. falciparum* chloroquine resistance transporter gene (PfCRT-T76) with both *in vitro* and *in vivo* resistance and a significant association between pfmdr1-tyr86 and PfCRT-T76 has also been observed among resistant isolates, which suggests a joint action of the two genes in high-level CQ resistance [65]. Furthermore, it has also been observed that although the PfCRT-K76T mutation was present in all CQ-resistant isolates, yet this mutation was also present in patients who recovered clinically after CQ therapy [68, 70]. Hence, it has been suggested that other mechanism(s) might be involved in modulating outcome of therapy in such cases [64].

The nature of these genetic polymorphisms and their relationship with drug-resistant strains has not been studied in Indian strains of *P. falciparum*. Further, a specific marker capable of diagnosing a chloroquine resistance is yet not available. It was therefore, important to investigate whether the CQ-resistant *P. falciparum* parasites could be identified directly from a blood sample by a rapid, sensitive, and specific method like PCR, which may be of great help in prompt treatment of falciparum malaria, particularly the severe and complicated one.

Therefore, the present study was undertaken to investigate the mutation(s) and genetic polymorphism(s) in the gene PfCRT.

There is a divergence in the literature for the exact mechanism of PfCRT-producing CQR. One theory is that by using energy to transport CQ out of the DV, the protein mediates active drug efflux (similar to that of HuMDR1), which makes it away of its targets [71]. Mutations in the protein may alter its substrate specificity by using this model, which leads to greater CQ affinity for mutant isoforms.

One more hypothesis is that PfCRT facilitates the diffusion of the charged drug species (which is also known as "charged drug leak" hypothesis; [72, 73]). Inside the acidic DV, the drug molecules present are charged as compared to the outside of the DV, which neglects the drug binding. The charged molecules require some kind of carrier as they cannot pass through the hydrophobic environment of a membrane. A benefit of this suggestion is that it provides an explanation for the significance of the K76T mutation. In this mutation, the lysine in wild-type CQS isoforms has a basic side group having positive charge that repels protonated CQ, while the neutral threonine allows for an open pore through which charged CQ may pass.

Another mechanism is there based on the pH alterations of the DV, which may be influenced by PfCRT. It has been shown that CQR parasites have a more acidic DV than CQS parasites by calculating the pH of the DV [74], which is very surprising because at low pH weak base partitioning would predict increased drug accumulation. However, the rates of hematin aggregation and hemozoin formation are increased at acidic pH; as a result, it would reduce the quantity of target available for CQ binding [2]. The amount of surplus unbound drug may alter the equilibrium of passive drug accumulation [73] or may be transported out of the DV by mutant PfCRT [75].

4. Structure of *P. falciparum* CRT and its mutants

The PfCRT gene identified as the determinant gene for CQR gene since the genetic cross between a CQR clone of Indochina (Dd2) and a CQS clone of Honduras (HB3) [57]. The PfCRT protein is of 48.6 kDa, which contains 424 amino acids encoded by a 13 exon gene in the chromosome having 36-kb segment (**Figure 1**) [2]. It may catalyze chloroquine quinine flux

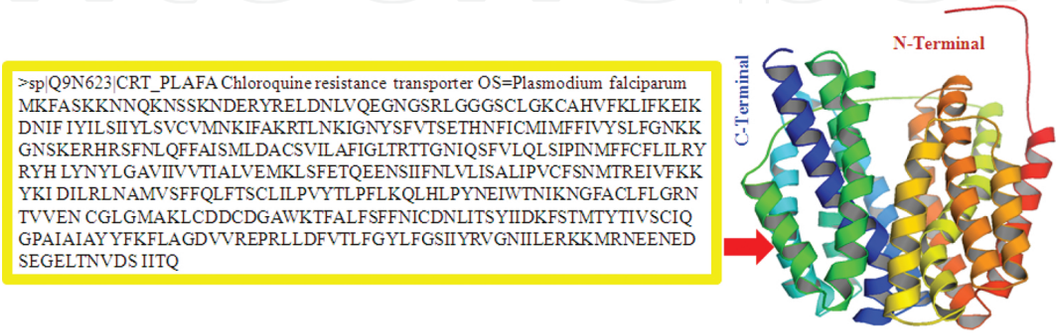

Figure 1. The homology model of the protein using the genebank sequence.

http://dx.doi.org/10.5772/64659

with H^+ across the digestive vacuole membrane having with 10 putative TMSs [76]. Nessler et al. observed activate various endogenous transporters in frog oocytes, which helps in transporting quinoline drugs including quinine and quinidine [77]. The drug specificity that determines levels of accumulation is because of the mutations in TMSs 1, 4, and 9 alter, which builds an idea of these TMSs play a role in substrate binding [78]. The substrates responsible for PfCRT mutants are chloroquine-resistant reversers [79].

Rapid progressions of chloroquine resistance (CQR) have activated the identification of some other genetic target(s) in genome of *P. falciparum* such as the mutation in K76T of PfCRT gene including three other positions 72, 74, and 75 [80]. The three mutations may present high resistance to CQ than the K76T mutant [81].

The protein is a member of the drug metabolite transporter (DMT) superfamily (TC #2.A.7) [82]. PfCRT contains drug/metabolite transporter domain. This domain is found in protein which is engaged in pectinase, cellulase, and blue pigment regulation. In plasmodium species, the PfCRT is situated at the intra-erythrocytic digestive vacuole. Mutations in this protein present verapamil-reversible chloroquine resistance to *P. falciparum*. The mutations in PfCRT result in increased compartment acidification. PfCRT-cognate vicissitudes in chloroquine replication involve altered drug flux across the parasite digestive vacuole membrane. Bray et al., concluded that PfCRT is mediated by the efflux of chloroquine from the digestive vacuole [72].

5. Analysis of single nucleotide variants scores

Three parameters have been considered for the estimation of structural changes of the PfCRT gene such as aggregation prediction (TANGO), amyloid prediction (WALTZ), and chaperone-binding prediction (LIMBO). SNPeffect 4.0: online prediction of molecular and structural effects of protein-coding variants was used for the study [83]. The three different positions of PfCRT gene were mutated manually in the protein sequence, that is, at 74, 75, and 76 positions. The mutated and the wild-type proteins were further processed using the SNPeffect software to calculate the difference in TANGO, WALTZ, and LIMBO score. The TANGO score obtained for the mutation of M74I, N75E, and K76T is given in **Figure 2**. The WALTZ scores of the wild type and the mutants are given in **Figure 3**. The LIMBO scores are given in **Figure 4**.

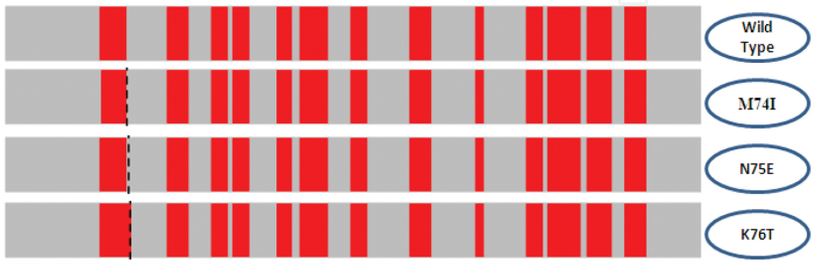

Figure 2. The aggregation prediction showing the differences of LIMBO scores of the wild-type PfCRT with the mutants.

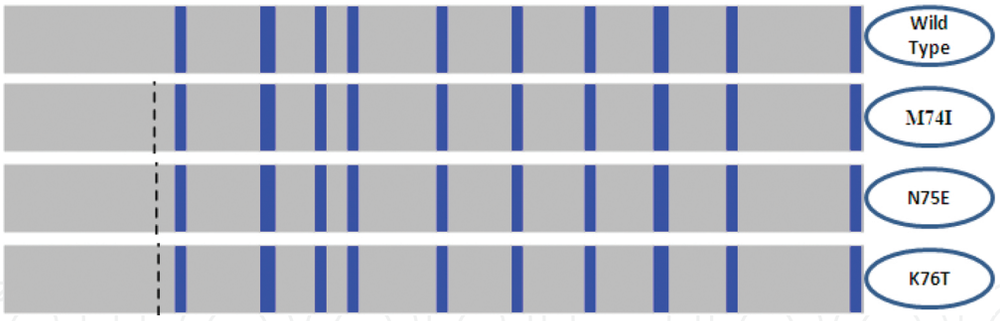

Figure 3. The amyloid prediction showing the differences of LIMBO scores of the wild-type PfCRT with the mutants.

Figure 4. The chaperone-binding prediction showing the differences of LIMBO scores of the wild-type PfCRT with the mutants.

6. The multiple haplotypes of PfCRT

In South America and southeast Asia, the CQR *P. falciparum* was first came into sight in the late 1950s and early 1960s, leading to the suggestion of Su et al., that resistance rises from two independent basic events [61]. Further studies have been done to analyze a huge number of geographically diversed PfCRT alleles and microsatellite genotypes from parasite isolates have identified at least three additional independent foci of resistance [5, 6]. CQR creation has been discovered in the Thai-Cambodian border region still now (eventually spreading westward into Africa), Papua New Guinea, the Philippines, Colombia, and Peru [84].

Cooper et al. revealed that 21 exclusive CQR PfCRT protein sequences are identified from field isolates and two additional haplotypes have been created using CQ selective pressure on the 106/1 parasite line in laboratory [3]. It is impossible to differentiate the CQR foci or the genetic variations of the subsequent involved in one origin without understanding performing the whole analysis of the PfCRT sequence and its surrounding loci by means of microsatellite typing. Johnson et al. developed four unique CQS haplotypes using the drug selection procedures of a laboratory [85].

http://dx.doi.org/10.5772/64659

Based on different geographical locations, the existence of the three PfCRT haplotypes revealed. According to Su et al. and Wootton et al. the first and the oldest resistant haplotype is CVIETIHSESII (amino acids 72–73–74–75–76–77–97–220–271–326–356–371), which exists in the FCB line of southeast Asia and is found in African isolates such as RB8, with consistency of spreading the CQR from Asia to Africa [5, 61]. The second haplotype is CVIETIHSESTI, which is found in the 102/1 Sudan strain, illustrated the characteristics of the isolates such as Dd2 from Thailand, and is newly explained the PH4 isolate from Morong, Philippines [2, 6]. An older PfCRT haplotype is CVIET found in South America, which implies that it may be because of the traveler who recently traveled to the location [86]. The third haplotype is detected in the INDO19, FCQ22, and 7G8 isolate line from Thailand, Papua New Guinea, and Brazil, respectively, and is reported as SVMNTIHSQDLR [2, 6, 7].

Different mutations in the PfCRT gene which change the nucleotide sequence into different genes and form different haplotypes are very general with the incidence of chloroquine resistant (CQR) [87].

7. Conclusion

The haplotype variations of PfCRT broadly classified into three groups, namely southeast Asian, Latin America, and Papua new guinea. This is used as a marker in the study of *P. falciparum* population diversity along with other markers. It is noteworthy to point out that PfCRT plays an important role in CQ transport and intracellular pH (pHi) regulation in parasite and the problem of drug resistance to antimalarials. In order to study the functions of wild-type PfCRT and mutants of PfCRT, calculation of different structural estimation score were generated where it showed that there is a notable variation in LIMBO, TANGO, and WALTZ scores.

Conflicts

The authors declared that there is no conflict of interest.

Author details

Pratap Parida, Kishore Sarma, Biswajyoti Borkakoty and Pradyumna Kishore Mohapatra*

*Address all correspondence to: Mohapatrapk@icmr.org.in

Regional Medical Research Centre, NE Region, Indian Council of Medical Research, Dibrugarh, Assam, India

References

[1] R.G. Ridley, "Medical need, scientific opportunity and the drive for anti-malarial drugs," *Nature*, Vol. 415, pp. 686–93, 2002.

[2] D.A. Fidock, T. Nomura, A.K. Talley, R.A. Cooper, S.M. Dzekunov, M.T. Ferdig, L.M. Ursos, A.B. Sidhu, B. Naudé, K.W. Deitsch, X.Z. Su, J.C. Wootton, P.D. Roepe and T.E. Wellems, "Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance," *Mol Cell*, Vol. 6, pp. 861–71, 2000.

[3] R.A. Cooper, M.T. Ferdig, X.Z. Su, L.M. Ursos, J. Mu, T. Nomura, H. Fujioka, D.A. Fidock, P.D. Roepe and T.E. Wellems, "Alternative mutations at position 76 of the vacuolar transmembrane protein PfCRT are associated with chloroquine resistance and unique stereospecific quinine and quinidine responses in *Plasmodium falciparum*," *Mol Pharmacol*, Vol. 61, pp. 35–42, 2002.

[4] W.M. Atroosh, H.M. Al-Mekhlafi, M.A. Mahdy and J. Surin, "The detection of pfcrt and pfmdr1 point mutations as molecular markers of chloroquine drug resistance, Pahang, Malaysia," *Malar J*, Vol. 11, pp. 251, 2012.

[5] J.C. Wootton, X. Feng, M.T. Ferdig, R.A. Cooper, J. Mu, D.I. Baruch, A.J. Magill and X.Z. Su, "Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*," *Nature*, Vol. 418, pp. 320–323, 2002.

[6] N. Chen, D.E. Kyle, C. Pasay, E.V. Fowler, J. Baker, J.M. Peters and Q. Cheng, "pfcrt Allelic types with two novel amino acid mutations in chloroquine-resistant *Plasmodium falciparum* isolates from the Philippines, *Antimicrob Agents Chemother*, Vol. 47, pp. 3500–3505, 2003.

[7] N. Chen, B. Russell, J. Staley, B. Kotecka, P. Nasveld and Q. Cheng, "Sequence polymorphisms in pfcrt are strongly associated with chloroquine resistance in *Plasmodium falciparum*," *J Infect Dis*, Vol. 183, pp. 1543–1545, 2001.

[8] R.M. Packard, "The origins of antimalarial-drug resistance," *N Engl J Med*, Vol. 371, pp. 397–399, 2014.

[9] U. Arora, G.S. Sonal, G.P. Dhillon, H.G. Thakor, "Emergence of drug resistance in India," *J Indian Med Assoc*, Vol. 106, pp. 678–681, 2008.

[10] N.K. Shah, G.P. Dhillon, A.P. Dash, U. Arora, S.R. Meshnick, N. Valecha, "Antimalarial drug resistance of *Plasmodium falciparum* in India: changes over time and space," *Lancet Infect Dis*, Vol. 11, pp. 57–64, 2011.

[11] N.M. Douglas, N.M. Anstey, B.J. Angus, F. Nosten, R.N. Price, "Artemisinin combination therapy for vivax malaria," *Lancet Infect Dis*, Vol. 10, pp. 405–416, 2010.

[12] A. Djimdé, O.K. Doumbo, R.W. Steketee and C.V. Plowe, "Application of a molecular marker for surveillance of chloroquine-resistant falciparum malaria," *Lancet*, Vol. 358, pp. 890–891, 2001.

[13] W. Plummer, L.M. Pereira and C.V. Carrington, "Pfcrt and pfmdr1 alleles associated with chloroquine resistance in *Plasmodium falciparum* from Guyana, South America," *Mem Inst Oswaldo Cruz*, Vol, 99, pp. 389–392, 2004.

[14] V. Durrand, A. Berry, R. Sem, P. Glaziou, J. Beaudou and T. Fandeur, "Variations in the sequence and expression of the *Plasmodium falciparum* chloroquine resistance transporter (Pfcrt) and their relationship to chloroquine resistance in vitro," *Mol Biochem Parasitol*, Vol. 136, pp. 273–85, 2004.

[15] V. Lakshmanan, P.G. Bray, D. Verdier-Pinard, D.J. Johnson, P. Horrocks, R.A. Muhle, G.E. Alakpa, R.H. Hughes, S.A. Ward, D.J. Krogstad, A.B. Sidhu and D.A. Fidock, "A critical role for PfCRT K76T in *Plasmodium falciparum* verapamil-reversible chloroquine resistance," *EMBO J*, Vol. 24, pp. 2294–2305, 2005.

[16] C.V. Plowe, "Monitoring antimalarial drug resistance: making the most of the tools at hand," *J Exp Biol*, Vol. 206, pp. 3745–3752, 2003.

[17] P. Muhamad, W. Chaijaroenkul, P. Phompradit, R. Rueangweerayut, P. Tippawangkosol and K. Na-Bangchang, "Polymorphic patterns of pfcrt and pfmdr1 in *Plasmodium falciparum* isolates along the Thai-Myanmar border," *Asian Pac J Trop Biomed*, Vol. 3, 931–935, 2013.

[18] S. Karcz and A.F. Cowman, "Similarities and differences between the multidrug resistance phenotype of mammalian tumor cells and chloroquine resistance in *Plasmodium falciparum*," *Exp Parasitol*, Vol. 73, pp. 233–240, 1991.

[19] P.G. Bray and S.A. Ward, "Malaria chemotherapy: resistance to quinoline containing drugs in *Plasmodium falciparum*," *FEMS Microbiol Lett*, Vol. 113, pp. 1–7, 1993.

[20] A.F. Slater and A. Cerami, "Inhibition by chloroquine of a novel haem polymerase enzyme activity in malaria trophozoites," *Nature*, Vol. 355, pp. 167–169, 1992.

[21] A. Dorn, R. Stoffel, H. Matile, A. Bubendorf and R.G. Ridley, "Malarial haemozoin/beta-haematin supports haem polymerization in the absence of protein," *Nature*, Vol. 374, pp. 269–71, 1995.

[22] K. Raynes, M. Foley, L. Tilley and L.W. Deady, "Novel bisquinoline antimalarials. Synthesis, antimalarial activity, and inhibition of haem polymerization," *Biochem Pharmacol*, Vol. 52, pp. 551–559, 1996.

[23] D.J. Sullivan, "Theories on malarial pigment formation and quinoline action," *Int J Parasitol*, Vol. 32, pp. 1645–1653, 2002.

[24] L.M. Ursos and P.D. Roepe, "Chloroquine resistance in the malarial parasite, *Plasmodium falciparum*," *Med Res Rev*, Vol. 22, pp. 465–491, 2002.

[25] N. Campanale, C. Nickel, C.A. Daubenberger, D.A. Wehlan, J.J. Gorman, N. Klonis, K. Becker and L. Tilley, "Identification and characterization of heme-interacting proteins in the malaria parasite, *Plasmodium falciparum*," *J Biol Chem*, Vol. 278, pp. 27354–27361, 2003.

[26] R.A. Cooper, C.L. Hartwig and M.T. Ferdig, "pfcrt is more than the *Plasmodium falciparum* chloroquine resistance gene: a functional and evolutionary perspective," *Acta Trop*, Vol. 94, pp. 170–180, 2005.

[27] J. Marfurt, I. Müller, A. Sie, O. Oa, J.C. Reeder, T.A. Smith, Beck HP, Genton B, "The usefulness of twenty-four molecular markers in predicting treatment outcome with combination therapy of amodiaquine plus sulphadoxine-pyrimethamine against falciparum malaria in Papua New Guinea," Malar J, Vol. 7, pp. 61, 2008.

[28] C.D. Fitch, "*Plasmodium falciparum* in owl monkeys: drug resistance and chloroquine binding capacity," *Science*, Vol. 169, pp. 289–290, 1970.

[29] P.G. Bray, M. Mungthin, R.G. Ridley and S.A. Ward, "Access to hematin: the basis of chloroquine resistance," *Mol Pharmacol*, Vol. 54, pp. 170–179, 1998.

[30] D.J. Krogstad, I.Y. Gluzman, D.E. Kyle, A.M. Oduola, S.K. Martin, W.K. Milhous and P.H. Schlesinger, "Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance," *Science*, Vol. 238, pp. 1283–1285, 1987.

[31] S.K. Martin, A.M. Oduola and W.K. Milhous, "Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil," *Science*, Vol. 235, pp. 899–901, 1987.

[32] J.L. Biedler and H. Riehm, "Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radioautographic, and cytogenetic studies," *Cancer Res*, Vol. 30, pp. 1174–1184, 1970.

[33] V. Ling and L.H. Thompson, "Reduced permeability in CHO cells as a mechanism of resistance to colchicines," *J Cell Physiol*, Vol. 83, pp. 103–116, 1974.

[34] T. Skovsgaard, "Mechanisms of resistance to daunorubicin in Ehrlich ascites tumor cells," *Cancer Res*, Vol. 38, pp. 1785–1791, 1978.

[35] T. Tsuruo, H. Iida, K. Naganuma, S. Tsukagoshi and Y. Sakurai, "Promotion by verapamil of vincristine responsiveness in tumor cell lines inherently resistant to the drug," *Cancer Res*, Vol. 43, 808–813, 1983.

[36] S.J. Foote, J.K. Thompson, A.F. Cowman and D.J. Kemp, "Amplification of the multi-drug resistance gene in some chloroquine-resistant isolates of *P. falciparum*," *Cell*, Vol. 57, pp. 921–930, 1989.

[37] C.M. Wilson, A.E. Serrano, A. Wasley, M.P. Bogenschutz, A.H. Shankar and D.F. Wirth, "Amplification of a gene related to mammalian mdr genes in drug-resistant *Plasmodium falciparum*," *Science*, Vol. 244, pp. 1184–1186, 1989.

[38] M.G. Zalis, C.M. Wilson, Y. Zhang and D.F. Wirth, "Characterization of the pfmdr2 gene for *Plasmodium falciparum*," *Mol Biochem Parasitol*, Vol. 63, pp. 311, 1994.

[39] S.J. Foote, D.E. Kyle, R.K. Martin, A.M. Oduola, K. Forsyth, D.J. Kemp and A.F. Cowman, "Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*," *Nature*, Vol. 345, pp. 255–258, 1990.

[40] A.F. Cowman, S. Karcz, D. Galatis and J.G. Culvenor, "A P-glycoprotein homologue of *Plasmodium falciparum* is localized on the digestive vacuole," *J Cell Biol*, Vol. 113, pp. 1033–1042, 1991.

[41] S.R. Karcz, D. Galatis and A.F. Cowman, "Nucleotide binding properties of a P-glycoprotein homologue from *Plasmodium falciparum*," *Mol Biochem Parasitol*, Vol. 58, 269–276, 1993.

[42] J. Cox-Singh, B. Singh, A. Alias and M.S. Abdullah, "Assessment of the association between three pfmdr1 point mutations and chloroquine resistance in vitro of Malaysian *Plasmodium falciparum* isolates," *Trans R Soc Trop Med Hyg*, Vol. 89, pp. 436–437, 1995.

[43] M.T. Duraisingh, C.J. Drakeley, O. Muller, R. Bailey, G. Snounou, G.A. Targett, B.M. Greenwood and D.C. Warhurst, "Evidence for selection for the tyrosine-86 allele of the pfmdr 1 gene of *Plasmodium falciparum* by chloroquine and amodiaquine," *Parasitology*, Vol. 114, pp. 205–211, 1997.

[44] F.M. Awad-el-Kariem, M.A. Miles and D.C. Warhurst, "Chloroquine-resistant *Plasmodium falciparum* isolates from the Sudan lack two mutations in the pfmdr1 gene thought to be associated with chloroquine resistance," *Trans R Soc Trop Med Hyg*, Vol. 86, 587–589, 1992.

[45] C.M. Wilson, S.K. Volkman, S. Thaithong, R.K. Martin, D.E. Kyle, W.K. Milhous and D.F. Wirth, "Amplification of pfmdr1 associated with mefloquine and halofantrine resistance in *Plasmodium falciparum* from Thailand," *Mol Biochem Parasitol*, Vol. 57, pp. 151–160, 1993.

[46] L.K. Basco, P.E. de Pecoulas, J. Le Bras, C.M. Wilson, "*Plasmodium falciparum*: molecular characterization of multidrug-resistant Cambodian isolates," *Exp Parasitol*, Vol. 82, pp. 97–103, 1996.

[47] M.T. Duraisingh, P. Jones, I. Sambou, L. von Seidlein, M. Pinder and D.C. Warhurst, "The tyrosine-86 allele of the pfmdr1 gene of *Plasmodium falciparum* is associated with increased sensitivity to the anti-malarials mefloquine and artemisinin," *Mol Biochem Parasitol*, Vol. 108, pp. 13–23, 2000.

[48] M.T. Duraisingh, C. Roper, D. Walliker and D.C. Warhurst, "Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the pfmdr1 gene of *Plasmodium falciparum*," *Mol Microbiol*, Vol. 36, pp. 955–961, 2000.

[49] M.T. Duraisingh, L.V. von Seidlein, A. Jepson, P. Jones, I. Sambou, M. Pinder and D.C. Warhurst, "Linkage disequilibrium between two chromosomally distinct loci associ-

ated with increased resistance to chloroquine in *Plasmodium falciparum*," *Parasitology*, Vol. 121, pp. 1–7, 2000.

[50] R.N. Price, A.C. Uhlemann, A. Brockman, R. McGready, E. Ashley, L. Phaipun, R. Patel, K. Laing, S. Looareesuwan, N.J. White, F. Nosten and S. Krishna, "Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number," *Lancet*, Vol. 364, pp. 438–447, 2004.

[51] M.T. Duraisingh and A.F. Cowman, "Contribution of the pfmdr1 gene to antimalarial drug-resistance" *Acta Trop*, Vol. 94, pp. 181–190, 2005.

[52] K.H. Choi, C.J. Chen, M. Kriegler and I.B. Roninson, "An altered pattern of cross resistance in multidrug resistant cells results from spontaneous mutation in mdr1 (p-glycoprotein) gene," *Cell*, Vol. 53, pp. 519–529, 1988.

[53] S.A. Peel, P. Bright, B. Yount, J. Handy and R.S. Baric, "A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (pfmdr) of *Plasmodium falciparum* in vitro, *Am J Trop Med Hyg*, Vol. 51, pp. 648–658, 1994.

[54] I.S. Adagu, D.C. Warhurst, W.N. Ogala, I. Abdu-Aguye, L.I. Audu, F.O. Bamgbola and U.B. Ovwigho, "Antimalarial drug response of *Plasmodium falciparum* from Zaria, Nigeria," *Trans R Soc Trop Med Hyg*, Vol. 89, pp. 422–425, 1995.

[55] I.S. Adagu, F. Dias, L. Pinheiro, L. Rombo, V. do Rosario and D.C. Warhurst, "Guinea Bissau: association of chloroquine resistance of *Plasmodium falciparum* with the Tyr86 allele of the multiple drug-resistance gene Pfmdr1," *Trans R Soc Trop Med Hyg*, Vol. 90, pp. 90–91, 1996.

[56] L.K. Basco, P. Ringwald, R. Thor, J.C. Doury and J. Le Bras, "Activity in vitro of chloroquine, cycloguanil, and mefloquine against African isolates of *Plasmodium falciparum*: presumptive evidence for chemoprophylactic efficacy in Central and West Africa," *Trans R Soc Trop Med Hyg*, Vol. 89, pp. 657–658, 1995.

[57] T.E. Wellems, L.J. Panton, I.Y. Gluzman, V.E. do Rosario, R.W. Gwadz, A. Walker-Jonah and D.J. Krogstad, "Chloroquine resistance not linked to mdr-like genes in a *Plasmodium falciparum* cross," *Nature*, Vol. 345, pp. 253–255, 1990.

[58] P.R. Bhattacharya and C.R. Pillai, "Strong association, but incomplete correlation, between chloroquine resistance and allelic variation in the pfmdr-1 gene of *Plasmodium falciparum* isolates from India," *Ann Trop Med Parasitol*, Vol. 93, pp. 679–84, 1999.

[59] E. Gómez-Saladín, D.J. Fryauff, W.R. Taylor, B.S. Laksana, A.I. Susanti, Purnomo, B. Subianto, and T.L. Richie, "*Plasmodium falciparum* mdr1 mutations and in vivo chloroquine resistance in Indonesia," *Am J Trop Med Hyg*, Vol. 61, 240–244, 1999.

[60] T.E. Wellems, A. Walker-Jonah and L.J. Panton, "Genetic mapping of the chloroquine-resistance locus on *Plasmodium falciparum* chromosome 7," *Proc Natl Acad Sci U S A*, Vol. 88, pp. 3382–3386, 1991.

[61] X. Su, L.A. Kirkman, H. Fujioka and T.E. Wellems, "Complex polymorphisms in an approximately 330 kDa protein are linked to chloroquine-resistant *P. falciparum* in Southeast Asia and Africa," *Cell*, Vol. 91, pp. 593–603, 1997.

[62] I.S. Adagu and D.C. Warhurs, "Association of cg2 and pfmdr1 genotype with chloroquine resistance in field samples of *Plasmodium falciparum* from Nigeria," *Parasitology*, Vol. 119, pp. 343–348, 1999.

[63] L.K. Basco and P. Ringwald, "Molecular epidemiology of malaria in Yaounde, Cameroon V. analysis of the omega repetitive region of the *plasmodium falciparum* CG2 gene and chloroquine resistance," *Am J Trop Med Hyg*, Vol. 61, pp. 807–813, 1999.

[64] L.K. Basco and P. Ringwald, "Point mutations in the *Plasmodium falciparum* cg2 gene, polymorphism of the kappa repeat region, and their relationship with chloroquine resistance," *Trans R Soc Trop Med Hyg*, Vol. 95, pp. 309–314, 2001.

[65] P.P. Vieira, M. Das Gracas Alecrim, L.H. DA Silva, I. Gonzalez-Jimenez and M.G. Zalis, "Analysis of the PfCRT K76T mutation in *Plasmodium falciparum* isolates from the Amazon region of Brazil," *J Infect Dis*, Vol. 183, pp. 1832–33, 2001.

[66] P. Lim, S. Chy, F. Ariey, S. Incardona, P. Chim, R. Sem, M.B. Denis, S. Hewitt, S. Hoyer, D. Socheat, M.P. Odile and F. Thierry, "Pfcrt polymorphism and chloroquine resistance in *Plasmodium falciparum* strains isolated in Cambodia," *Antimicrob Agents Chemother*, Vol. 47, pp. 87–94, 2003.

[67] H.A. Babiker, S.J. Pringle, A. Abdel-Muhsin, M. Mackinnon, P. Hunt and D. Walliker, "High-level chloroquine resistance in Sudanese isolates of *Plasmodium falciparum* is associated with mutations in the chloroquine resistance transporter gene pfcrt and the multidrug resistance Gene pfmdr1," *J Infect Dis*, Vol. 183, pp. 1535–1538, 2001.

[68] G. Dorsey, M.R. Kamya, A. Singh and P.J. Rosenthal, "Polymorphisms in the *Plasmodium falciparum* pfcrt and pfmdr-1 genes and clinical response to chloroquine in Kampala, Uganda," *J Infect Dis*, Vol. 183, pp. 1417–1420, 2001.

[69] R. Durand, S. Jafari, J. Vauzelle, J. Delabre, Z. Jesic and J. Le Bras, "Analysis of pfcrt point mutations and chloroquine susceptibility in isolates of *Plasmodium falciparum*," *Mol Biochem parasitol*, Vol. 114, pp. 95–102, 2001.

[70] A.G. Mayor, X. Gómez-Olivé, J.J. Aponte, S. Casimiro, S. Mabunda, M. Dgedge, A. Barreto and P.L. Alonso, "Prevalence of the K76T mutation in the putative *Plasmodium falciparum* chloroquine resistance transporter (pfcrt) gene and its relation to chloroquine resistance in Mozambique," *J Infect Dis*, Vol. 183, pp. 1413–1416, 2001.

[71] C.P. Sanchez, J.E. McLean, P. Rohrbach, D.A. Fidock, W.D. Stein and M. Lanzer, "Evidence for a pfcrt-associated chloroquine efflux system in the human malarial parasite *Plasmodium falciparum*," *Biochemistry*, Vol. 44, pp. 9862–9870, 2005.

[72] P.G. Bray, R.E. Martin, L. Tilley, S.A. Ward, K. Kirk and D.A. Fidock, "Defining the role of PfCRT in Plasmodium falciparum chloroquine resistance," *Mol Microbiol*, Vol. 56, pp. 323–333, 2005.

[73] H Zhang, M Paguio and P.D. Roepe, "The antimalarial drug resistance protein *Plasmodium falciparum* chloroquine resistance transporter binds chloroquine," *Biochemistry*, Vol. 43, pp. 8290–8296, 2004.

[74] T.N. Bennett, A.D. Kosar, L.M. Ursos, S. Dzekunov, A.B. Singh Sidhu, D.A. Fidock and P.D. Roepe, "Drug resistance-associated pfCRT mutations confer decreased *Plasmodium falciparum* digestive vacuolar pH," *Mol Biochem Parasitol*, Vol. 133, 99–114, 2004.

[75] E.M. Howard, H. Zhang and P.D. Roepe, "A novel transporter, Pfcrt, confers antimalarial drug resistance," *J Membr Biol*, Vol. 190, pp. 1–8, 2002.

[76] T.E. Wellems, "Plasmodium chloroquine resistance and the search for a replacement antimalarial drug," *Science*, Vol. 298, pp. 124–126, 2002.

[77] S. Nessler, O. Friedrich, N. Bakouh, R.H. Fink, C.P. Sanchez, G. Planelles and M. Lanzer, "Evidence for activation of endogenous transporters in Xenopus laevis oocytes expressing the *Plasmodium falciparum* chloroquine resistance transporter, PfCRT," *J Biol Chem*, Vol. 279, pp. 39438–39446, 2004.

[78] R.A. Cooper, K.D. Lane, B. Deng, J. Mu, J.J. Patel, T.E. Wellems, X. Su and M.T. Ferdig, "Mutations in transmembrane domains 1, 4 and 9 of the *Plasmodium falciparum* chloroquine resistance transporter alter susceptibility to chloroquine, quinine and quinidine," *Mol Microbiol*, Vol. 63, pp. 270–282, 2007.

[79] A.M. Lehane and K. Kirk, "Efflux of a range of antimalarial drugs and 'chloroquine resistance reversers' from the digestive vacuole in malaria parasites with mutant PfCRT," *Mol Microbiol*, Vol. 77, pp. 1039–1051, 2010.

[80] G. Awasthi and A. Das, "Genetics of chloroquine-resistant malaria: a haplotypic view," *Mem Inst Oswaldo Cruz*, Vol. 108, pp. 947–961, 2013.

[81] N. Takahashi, K. Tanabe, T. Tsukahara, M. Dzodzomenyo, L. Dysoley, B. Khamlome, J. Sattabongkot, M. Nakamura, M. Sakurai, J. Kobayashi, A. Kaneko, H. Endo, F. Hombhanje, T. Tsuboi and T. Mita, "Large-scale survey for novel genotypes of *Plasmodium falciparum* chloroquine-resistance gene pfcrt," *Malar J*, 11, pp. 92, 2012.

[82] C.V. Tran and M.H. Saier Jr, "The principal chloroquine resistance protein of *Plasmodium falciparum* is a member of the drug/metabolite transporter superfamily," *Microbiology*, Vol. 150, pp. 1–3, 2004.

[83] G. De Baets, J. Van Durme, J. Reumers, S. Maurer-Stroh, P. Vanhee, J. Dopazo, J. Schymkowitz and F. Rousseau, "SNPeffect 4.0: on-line prediction of molecular and structural effects of protein-coding variants," *Nucleic Acids Res*, Vol. 40, pp. D935–D939, 2012.

[84] K. Hayton and X.Z. Su, "Genetic and biochemical aspects of drug resistance in malaria parasites," *Curr Drug Targets Infect Disord*, Vol. 4, pp. 1–10, 2004.

[85] D.J. Johnson, D.A. Fidock, M. Mungthin, V. Lakshmanan, A.B. Sidhu, P.G. Bray and S.A. Ward, "Evidence for a central role for PfCRT in conferring *Plasmodium falciparum* resistance to diverse antimalarial agents," *Mol Cell*, Vol. 15, pp. 867–877, 2004.

[86] P.P. Vieira, M.U. Ferreira, Md. Alecrim, W.D. Alecrim, L.H. da Silva, M.M. Sihuincha, D.A. Joy, J. Mu, X.Z. Su and M.G. Zalis, "pfcrt Polymorphism and the spread of chloroquine resistance in *Plasmodium falciparum* populations across the Amazon Basin." *J Infect Dis*, Vol. 190, pp. 417–424, 2004.

[87] G. Awasthi, G.B. Satya Prasad and A. Das, "Pfcrt haplotypes and the evolutionary history of chloroquine-resistant *Plasmodium falciparum*," *Mem Inst Oswaldo Cruz*, Vol. 107, pp. 129–134, 2012.

IntechOpen