Review Article

Assessing the Roles of Molecular Markers of Antimalarial Drug Resistance and the Host Pharmacogenetics in Drug-Resistant Malaria

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Malaria caused by the Plasmodium parasites is a major public health concern in malaria-endemic regions with P. falciparum causing the most severe form of the disease. The use of antimalarial drugs for the management of the disease proves to be one of the best methods to manage the disease. Unfortunately, P. falciparum has developed resistance to almost all the current in-use antimalarial drugs. Parasite development of resistance is primarily caused by both parasite and host genetic factors. The parasite genetic factors involve undergoing mutation in the drug target sites or increasing the drug target gene copy number to prevent the intended action of the antimalarial drugs. The host pharmacogenetic factors which determine how a particular antimalarial drug is metabolized could result in variations of drug plasma concentration and consequently contribute to variable treatment outcomes and the emergence or propagation of resistant parasites. Since both host and parasite genomes play a role in antimalarial drug action, a key question often asked is, “which of the two strongly drives or controls antimalarial drug resistance?” A major finding in our recent study published in the Malaria Journal indicates that the parasite’s genetic factors rather than the host are likely to energize resistance to an antimalarial drug. However, others have reported contrary findings suggesting that the host genetic factors are the force behind resistance to antimalarial drugs. To bring clarity to these observations, there is the need for deciphering the major driving force behind antimalarial drug resistance through optimized strategies aimed at alleviating the phenomenon. In this direction, literature was systematically reviewed to establish the role and importance of each of the two factors aforementioned in the etiology of drug-resistant malaria. Using Internet search engines such as Pubmed and Google, we looked for terms likely to give the desired information which we herein present. We then went ahead to leverage the obtained information to discuss the globally avid aim of combating antimalarial drug resistance.

1. Introduction

Antimalarial drug resistance (ADR) continues to hinder global efforts to effectively manage and eradicate malaria disease [1, 2]. So far, of the Plasmodium species known to infect humans, P. falciparum has developed resistance to almost all antimalarials used for malaria treatment. ADR in the P. falciparum is known to emerge from low-transmission regions and spread to high-transmission areas [2, 3]. Parasite strains resistant to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) emerged from Southeast Asia (SEA) or South America before spreading to sub-Saharan Africa (sSA) [4, 5]. The high prevalence of CQ-resistant and SP-resistant parasites necessitated the introduction of artemisinin-based combination therapy (ACT) for the treatment of
uncomplicated malaria in malaria-endemic regions. The highly efficacious ACT regimens were quickly adopted by most malaria-endemic countries as their first-line treatment option for uncomplicated malaria [6]. Unfortunately, partial resistance to the artemisinin (ART) component of the ACT, which is defined as “slower clearance of malaria parasitemia in the first 3 days of ART monotherapy or ACT treatment,” was reported in the western part of Cambodia in 2008 and 2009 [7, 8] and in the Greater Mekong Subregion [9–11]. This situation is a setback to the efficacy of the ACT regimens and consequential to the management of malaria. These concerns have subsequently been aggravated by the selection of parasites with partial resistance to the ART partner drug(s). Reports of treatment failures with dihydroartemisinin-piperaquine (DHAP) in Cambodia [12–14] and artesunate-mefloquine (ASMQ) on the Thai-Myanmar border [15] support this assertion.

The early detection of resistant parasite strains is crucial in the fight against malaria, as it will allow prompt identification and containment of these resistant strains. For the early detection of resistant parasite strains to a particular antimalarial drug, there is the need to understand the mechanisms at play in *Plasmodium* spp. antimalarial drug resistance development [16].

Certain mutation in the parasite genome confers resistance to certain antimalarial drugs. Malaria treatment failure is not only dependent on drug-resistant *P. falciparum* bearing these mutations but also on other factors such as incorrect use or suboptimum drug dosage, noncompliance to a drug regimen, use of counterfeit or fake drugs, drug-drug interactions [17], and poor drug metabolism [18]. Suboptimal drug concentration in blood contributes to poor malaria treatment outcomes leading to the emergence and/or spread of parasite-resistant strains [18]. On the other hand, a high drug concentration in blood is more likely to result in increased toxicity. The pharmacokinetic profile of a drug (absorption, distribution, metabolism, and excretion) can differ substantially among individuals with different cytochrome (CYP) genes. These make the drug metabolism enzymes (e.g., cytochrome P450 enzymes) and transport proteins (e.g., P-glycoproteins) very important in the breakdown, absorption, distribution, and excretion of antimalarial drugs [19].

The genetic variations in the genes encoding these enzymes in an individual may be responsible for differences in individual responses to antimalarial drugs. This suggests that it is important to consider the pharmacogenetics of individual patients before administering any particular antimalarial drug [18, 20].

This evidence shows that the most important factors that are principal in determining the efficacy of antimalarial drugs are the parasite genetic factors and pharmacogenetics [3, 18, 21]. Hence, this review aims to highlight the parasite genetic factors and host pharmacogenetic factors that could affect the efficacy of an antimalarial drug and attempts to leverage this towards the management of antimalarial drug resistance.

### 2. Malaria: A Brief Account of the Current Situation

The World Health Organization (WHO) reported 241 million cases of malaria worldwide in 2020 [22]. This indicates a decline in cases compared to the 251 million malaria cases reported in 2010 and an increase in cases compared to the 231 million cases reported in 2017. The WHO African Region recorded 228 million malaria cases out of the total 241 million malaria cases in 2020, representing 95% of the total malaria cases. This was followed by the WHO Southeast Asia Region, which recorded 3% of all malaria cases [22]. The WHO Eastern Mediterranean Region recorded 2% of the malaria cases recorded in 2020 [22].

### 3. Molecular Markers of Antimalarial Drug Resistance

The use of molecular markers of resistance to monitor the emergence and spread of parasites resistant to antimalarial drugs proves to be a very effective method in monitoring ADR [2]. The identification and validation of these molecular markers have boosted our confidence in using these tools to monitor ADR in malaria-endemic areas [2]. Markers such as mutations in the *P. falciparum* chloroquine resistance transporter gene (*pfcrt*) [23], *P. falciparum* multidrug resistance protein 1 gene (*pfmdr1*) [24], and *P. falciparum* kelch 13 gene (*pfk13*) [25] have been linked to resistance to CQ, lumefantrine (LMF), and ART, respectively. The underlining mechanisms of *Plasmodium* spp. resistance to these antimalarial drugs include undergoing mutations in the parasite genome resulting in changing the original transporter protein conformation which leads to expelling the drug from the digestive vacuole at a faster rate, loss of binding affinity between the drug and its target, or increased in gene copy number in the case of pfmdr1 [26–28].

### 4. Cross-Resistance of *P. falciparum* to Antimalarial Drugs

*P. falciparum* has developed cross-resistance to some antimalarial drugs that are in the same class, chemically related, and/or have a similar mechanism of action. The development of resistance to one antimalarial drug can set the right precedent for the development of resistance to other antimalarial drugs [29]. Cross-resistance has been reported for two 4-aminoquinolines drugs, that is, amodiaquine and chloroquine. Cross-resistance to amodiaquine and chloroquine has been reported in both clinical and laboratory isolates. For the quinoline drugs, cross-resistance has been reported between MQ, QN, and HLF. There are high cases of cross-resistance reported between HLF and MQ, especially in MQ-resistant clinical isolates [30]. Cross-resistance has also been recorded between LMF and MQ, which is caused by a mutation in pfmdr1 N86Y [31]. In a few cases, resistance to one drug confers increased susceptibility to other drugs. For example, pfmdr1 N86Y causes decreased susceptibility to CQ but increased susceptibility to MQ, while the increased pfmdr1 copy number is associated with increased...
CQ sensitivity and decreased MQ susceptibility [32]. In antifolates, cross-resistance has been observed between cycloguanil and pyrimethamine [33].

5. Controversies Surrounding the Use of Molecular Markers in the Surveillance of Resistant Parasite Strain

The use of molecular markers of resistance to monitor the emergence and spread of parasite strains resistant to antimalarial drugs has proven to be very effective. Nonetheless, this comes with its challenges, especially when there is a lack of universality in a particular molecular marker of resistance used for monitoring ADR. For example, the major mutations that have been reported as molecular markers of resistance to ART and its derivatives in SEA are pfk13 C580Y, R539T, and Y493H [34], but this is not the case in most African countries. This could probably be due to low levels of resistance to ART in most African countries. In cases with delayed ART treatment outcomes in most African countries, pfk13 C580Y, R539T, and Y493H mutations were not observed. This finding highlights the fact that there is the absence of universality in the use of pfk13 C580Y, R539T, and Y493H for ART resistance surveillance in all WHO malaria-endemic regions [34]. This assertion is further strengthened after pfk13 M476I was selected for in a Tanzanian clinical isolate in the presence of in vitro ART drug pressure. This suggests the possibility of pfk13 M476I being used as an ART resistance marker in Tanzania and not pfk13 C580Y, R539T, and Y493H [34].

In SEA, an increase in pfpm2 and pfpm3 gene copy number is used as a molecular marker of resistance to PQ in clinical isolates [28]. However, this is not the case in Africa, as high proportions of clinical isolates have multiple copies of the pfmp2 gene which has an association with PQ resistance. For example, more than 30% of clinical isolates from Burkina Faso and Uganda had multiple copies of the pfmp2 gene [35]. The observed high prevalence of multiple gene copies of the pfmp2 gene in African isolates could be that isolates had multiple copies of the gene before introduction of PQ for the treatment of malaria. Therefore, the use of increased gene copy number in pfmp2 and pfpm3 genes as molecular markers of resistance in monitoring DHAP may not be accurate in Africa [35]. The above assertions point to the importance of the identification and validation of peculiar molecular markers of resistance to first-line antimalarial drugs used in a particular country for malaria treatment. This can ensure the accurate use of Plasmodium spp. molecular markers of resistance for antimalarial drug efficacy studies in malaria-endemic regions.

6. Drug Metabolism in the Human Host

The drug metabolism involves the enzymatic conversion of a therapeutic important chemical into a new molecule inside the human body for a specific activity [21]. The process of the enzymatic conversion may result in pharmacologically active, inactive, or toxic metabolites, depending on the genetic makeup of the individual [21]. The drug metabolic process involves two phases: the conversion of the therapeutic compound into a pharmacological active metabolite by the cytochrome P450 isoenzymes (CYP) and the transport of the pharmacologically active metabolite to their target site of action [21].

7. Cytochrome P450 Isoenzymes (CYP) in the Human Host

The main enzymes involved in the antimalarial drug metabolism are the cytochrome P450 (CYP) enzymes. Approximately, 40% of these enzymes are polymorphic. The CYP genes with polymorphisms include CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 [36]. The polymorphisms lead to three main phenotypes, which are classified as poor metabolizers, intermediate metabolizers, and extensive metabolizers. Poor metabolizers break down drugs slowly, which may lead to a more pronounced side effect. Additionally, poor metabolizers might experience treatment failure when administered with prodrugs that need to be bioactivated. Poor metabolizers will have problems in the bioactivation of proguanil to cycloguanil by the CYP2C19 gene [36]. Extensive metabolizers tend to metabolize the drugs more extensively which results in faster relief from the disease symptoms [36]. Intermediate metabolizers metabolize the drugs efficiently, resulting in the optimal concentration of the pharmacologically active metabolite in the plasma, with no toxicity or adverse drug effect being recorded [36].

Polymorphisms in CYP3A4 (the most abundant human CYP enzyme) have a major role in the expression and function of the gene, and this may lead to drug toxicity [37]. In CYP3A5, genetic variation accounts for the majority of its expression and function [36]. In CYP2C8, studies that incubated AQ with human liver microsomes and recombinant expressed CYP2C8 protein from cells observed a 50% reduced metabolic activity for CYP2C8*2 and an 85% reduced metabolic activity for CYP2C8*3 when compared to the wildtype [38]. For CYP2C19, CYP2C19*2 and CYP2C19*3 polymorphisms are null alleles which result in the complete absence of protein functions [39]. The CYP2C19*17 has been associated with the increased metabolism [40]. Among several polymorphisms in CYP2A6, only CYP2A6*2 and CYP2A6*7A have reduced 7-hydroxylation of coumarin [41].

8. Drug Transport in the Human Host

Transporters are membrane-bound proteins that help in the movement of compounds in and out of cells. Transporters play a very important role in the delivery of metabolized drugs to their target sites [42, 43]. Genetic variations in drug transporter genes in humans are very important in determining the concentration of metabolized drugs in the targeted cells which contribute to the variability of drug response among individuals [42, 44, 45]. The ABCB1 gene which encodes the human MDR1 (P-glycoprotein) protein functions as an efflux transporter and its polymorphic forms ABCB1 c.1236C>T, ABCB1 c.2677G>T/A, and ABCB1
enzyme can metabolize a single antimalarial drug. For example, piperaquine is metabolized primarily by CYP3A4 and to a lesser extent by CYP2C8 when compared to CYP3A4 [73]. Lumezantrine is metabolized by both CYP3A4 and CYP3A5 [64]. This suggests that mutation(s) in one of the CYP genes leading to a defective metabolism may be compensated for by the second CYP enzyme that can also metabolize the antimalarial drug. Hence, the chances of the poor antimalarial drug metabolism occurring in an individual is less. For some antimalarials such as AQ, both the parent drug and its N-desethlamodiaquine (DEAQ) metabolite are therapeutically active against the malaria parasite. This suggests that AQ can work effectively in the absence of the efficient metabolism by the patients [87, 88]. Due to the functional redundancy in some CYP enzymes and the therapeutical activeness of some parent drugs and their metabolites, it will be important for researchers to focus on the transporters that may play a role in transporting metabolized drugs to their target site of action. How these enzymes contribute to malaria treatment outcomes with the view of improving upon personalized medicine is discussed.

### 9. Typing of Polymorphisms in CYP gene as a Means to Personalize Medication in Malarial Infection: The Setbacks

One of the most effective ways of knowing how an individual will metabolize an antimalarial drug is by genotyping the CYP gene which encodes the enzyme mainly involved in the antimalarial drug metabolism. This makes it an easy approach to personalize medicine. Unfortunately, this is not true for some antimalarial drugs as more than one CYP enzyme can metabolize a single antimalarial drug. For example, piperaquine is metabolized primarily by CYP3A4 and to a lesser extent by CYP2C8 when compared to CYP3A4 [73]. Lumezantrine is metabolized by both CYP3A4 and CYP3A5 [64]. This suggests that mutation(s) in one of the CYP genes leading to a defective metabolism may be compensated for by the second CYP enzyme that can also metabolize the antimalarial drug. Hence, the chances of the poor antimalarial drug metabolism occurring in an individual is less. For some antimalarials such as AQ, both the parent drug and its N-desethlamodiaquine (DEAQ) metabolite are therapeutically active against the malaria parasite. This suggests that AQ can work effectively in the absence of the efficient metabolism by the patients [87, 88]. Due to the functional redundancy in some CYP enzymes and the therapeutical activeness of some parent drugs and their metabolites, it will be important for researchers to focus on the transporters that may play a role in transporting metabolized drugs to their target site of action. How these enzymes contribute to malaria treatment outcomes with the view of improving upon personalized medicine is discussed.
10. Sickle Cell Anemia and Malaria

Sickle cell anemia (SCA) is a major health problem in mostly sub-Saharan Africa (sSA) with over 250000 babies born annually with the disease [89]. In Africa, approximately 200000 babies are born with SCA annually and approximately 50% die before the age of five [90]. Individuals with SCA are four times more susceptible to malaria compared with individuals with sickle cell trait. This makes malaria a major contributor to morbidity and mortality in these individuals [90]. Malaria infection in SCA individuals results in severe anemia and painful crises, which can result in the death of these persons. In most malaria-endemic areas, crises due to malaria infection in individuals with SCA occur mostly in high malaria transmission seasons [91]. Due to this knowledge, presumptive malaria treatment is the ideal way of preventing malaria in individuals with SCA. The antimalarial drugs used mostly for presumptive malaria treatment are CQ and SP [92]. Due to the high level of CQ-resistant parasites recorded in most countries in sSA, the use of SP has a higher success rate in preventing malaria in SCA individuals [92]. The antimalarial drug SP is also preferred for presumptive malarial treatment in pregnant women with SCA [93]. The treatment of SCA is mostly by the use of hydroxyurea [94]. The recent use of hydroxyurea for SCA treatment means there is limited data on hydroxyurea and antimalarial drug-drug interactions; hence, the need for investigation in this aspect. Since CQ and SP are mostly used as presumptive treatments for malaria in SCA individuals, it will be ideal for future research to focus on hydroxyurea and CQ or SP drug-drug interactions [92, 94].

11. The Use of Genetic Factors of Parasite and Host to Curb Antimalarial Drug Resistance

Detection of *Plasmodium* spp. molecular markers of resistance to antimalarial drugs has proven to be an effective way of identifying potential ADR parasite phenotypes. The use of high throughput sequencing techniques has helped in the identification of molecular markers associated with resistance to antimalarial drugs in efficacy studies in most malaria-endemic countries [2].

The categorization of people by their genotype has proven to be effective in establishing the link between individual pharmacogenetics and antimalarial drug pharmacokinetics [95–97]. This has led to improved drug response in most individuals to antimalarial drugs. This suggests that there is the need to establish a comprehensive worldwide CYP gene polymorphism database, which will incorporate the antimalarial drug pharmacokinetic parameters associated with its CYP gene polymorphism(s) [98]. This will help improve personalized medicine and significantly reduce incidents of adverse drug effects that may be associated with taking antimalarial drugs [21]. For example, pharmacogenetic tests have been used to optimize warfarin doses, avoid tamoxifen treatment failure, and hypersensitivity drug effects associated with abacavir treatment [20]. A similar test can be performed on individuals before the prescription of antimalarial drug for malarial treatment. This will help to ascertain the best antimalarial drug to administer during malarial treatment.

Pharmacogenetic research has become very important due to the possibility of drug-drug interaction, as several drugs such as antiviral, antibacterial, and antimalarial drugs are given in combination to individuals in most malaria-endemic areas. These drugs are substrates, inducers, or inhibitors of CYP enzymes and MDR1 transporters. This makes the chances of drug-drug and/or drug-gene interactions resulting in adverse drug effects highly likely. Due to the abovementioned reasons, there is a need to develop comprehensive clinical data from a large number of patients to assess antimalarial drug pharmacokinetics in relation to dosage and clinical outcomes. The evaluation of individual pharmacogenetics in combination with the *Plasmodium* spp. genetic factors is crucial to ascertain the mechanism of ADR [21]. This assertion is supported by a study conducted by Hodoameda et al. (2020) where it was reported that *P. falciparum* genetic factors rather than host factors are likely to drive resistance to ACT in Ghana, while a study by Kiaco et al. (2017) report that the drug transporter ABCB1 c.3435C>T SNP influences AL treatment outcome in Angola. Results from both findings highlight the need to factor both the parasite’s genetic and host pharmacogenetics in the determination of malaria treatment outcomes. Knowledge of the prevalence of the *Plasmodium* spp. molecular markers of resistance to a particular antimalarial drug can inform policymakers as to which the antimalarial drug should be introduced for use in a particular country. This is also true for the knowledge of the prevalence of pharmacogenetics of individuals in a particular population, as this can help to inform which antimalarial drug will be metabolized effectively by individuals in a population.

12. What Is the Major Driver of Antimalarial Drug Resistance between the Factors, Parasite Genetic Factors and Host Pharmacogenetics: The Authors Take

One major puzzle the scientific community wants to bring a final closure to is to ascertain the major driver of antimalarial drug resistance, especially when both the parasite genetic factors and host pharmacogenetics [21] play vital roles in malaria treatment outcomes. Of the two factors, the parasite genetic factor is the major contributor to antimalarial drug resistance [3, 99]. During drug development, one major factor that is considered is the ability of an individual to metabolize the drug efficiently. This ensures that only antimalarial drugs that can be metabolized by the majority of individuals living in malaria endemic regions are developed [21]. Although polymorphism may exist in the CYP genes that can lead to the altered metabolism of a particular antimalarial drug, they are only present at a very low prevalence level in any given population [20, 21, 65]. Additionally, the ability of two or more CYP enzymes to metabolize a particular antimalarial drug results in most antimalarial drugs being metabolized effectively in most individuals [64, 73]. Also, for some antimalarial drugs, both the parent
drug and the active metabolite are therapeutically active against the malarial parasite. Due to this, the possibility of the host pharmacogenetics contributing to drug resistance is highly unlikely [3, 64, 73]. For this reason, the major factor which contributes to antimalarial drug resistance is the parasite genetic factor [3, 99]. Mutations in the parasite genome that confers resistance to antimalarial drugs do occur in nature. Although the natural proportion of such mutants is low, they become selected under drug pressure and subsequently become the dominant population over time [3, 100]. Additionally, changes in the parasite genome leading to the selection of resistant parasite strains can occur rapidly due to long exposure to antimalarial drugs. Subsequent redrawer of the antimalarial drug over some time can restore parasite susceptibility to the antimalarial drug [101]. These genetic changes occur in the form of point mutation(s) or increased gene copy number in the antimalarial drug target sites in response to antimalarial drug pressure. Additionally, the rapid spread of resistant parasite strains from one geographical location to the other contributes to global antimalarial drug failure in malaria-endemic regions [3]. The rapid genetic changes in the parasite genome due to drug pressure coupled with the global spread of ADR parasite strains result in antimalarial drug failure in malaria transmission regions within few decades. Hence, the parasite should be the primary focus in our quest to fight antimalarial drug resistance [2]. For this reason, there is the need to constantly search for mutations in the parasite genome to identify possible mutations in antimalarial drug target sites and validate these mutations as molecular markers of resistance or not. This will allow the early detection of resistant parasite strains leading to the rapid implementation of containment strategies to avoid the global spread of resistant parasite strains.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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**References**

[1] World Health Organization, “Medicines for Malaria Ventures,” 2019, https://www.mmv.org/newsroom/publications/world-malaria-report-2019.

[2] P. P. Hodoameda, “Falciparum and its molecular markers of resistance to antimalarial drugs,” *InPlasmodium Species and Drug Resistance*, 2021.

[3] N. J. White, “Antimalarial drug resistance,” *The Journal of clinical investigation*, vol. 113, no. 8, pp. 1084–1092, 2004.

[4] J. F. Trape, “The public health impact of chloroquine resistance in Africa,” *American Journal of Tropical Medicine and Hygiene*, vol. 64, no. 1, 2001.

[5] E. L. Korenromp, B. G. Williams, E. Gouws, C. Dye, and R. W. Snow, “Measurement of trends in childhood malaria mortality in Africa: an assessment of progress toward targets based on verbal autopsy,” *The Lancet Infectious Diseases*, vol. 3, no. 6, pp. 349–358, 2003.

[6] World Health Organization, “The Global Health Observatory,” 2018, https://www.who.int/gho/malaria/en/.

[7] H. Noedl, Y. Se, K. Schaecher, B. L. Smith, D. Socheat, and M. M. Fukuda, “Evidence of artemisinin-resistant malaria in western Cambodia,” *New England Journal of Medicine*, vol. 359, no. 24, pp. 2619–2620, 2008.

[8] A. M. Donduorp, F. Nosten, P. Yi et al., “Artemisinin resistance in Plasmodium falciparum malaria,” *New England Journal of Medicine*, vol. 361, no. 5, pp. 455–467, 2009.

[9] C. Amaratunga, S. Sreng, S. Suon et al., “Artemisinin-resistant Plasmodium falciparum in Pursat province, western Cambodia: a parasite clearance rate study,” *The Lancet Infectious Diseases*, vol. 12, no. 11, pp. 851–858, 2012.

[10] F. Huang, S. Takala-Harrison, C. G. Jacob et al., “A single mutation in K13 predominates in southern China and is associated with delayed clearance of Plasmodium falciparum following artemisinin treatment,” *The Journal of infectious diseases*, vol. 212, no. 10, pp. 1629–1635, 2015.

[11] E. A. Ashley, M. Dhorda, R. M. Fairhurst et al., “Spread of artemisinin resistance in Plasmodium falciparum malaria,” *New England Journal of Medicine*, vol. 371, no. 5, pp. 411–423, 2014.

[12] R. Leang, A. Barrette, D. M. Bouth et al., “Efficacy of dihydroartemisinin-piperazine for treatment of uncomplicated Plasmodium falciparum and Plasmodium vivax in Cambodia, 2008 to 2010,” *Antimicrobial Agents and Chemotherapy*, vol. 57, no. 2, pp. 818–826, 2013.

[13] M. D. Spring, J. T. Lin, J. E. Manning et al., “Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: an observational cohort study,” *The Lancet Infectious Diseases*, vol. 15, no. 6, pp. 683–691, 2015.

[14] C. Amaratunga, P. Lim, S. Suon et al., “Dihydroartemisinin-piperaquine resistance in Plasmodium falciparum malaria in Cambodia: a multisite prospective cohort study,” *The Lancet Infectious Diseases*, vol. 16, no. 3, pp. 357–365, 2016.

[15] V. L. Carrara, K. M. Lwin, A. P. Phyo et al., “Malaria burden and artemisinin resistance in the mobile and migrant population on the Thai–Myanmar border, 1999–2011: an observational study,” *PLoS Medicine*, vol. 10, no. 3, Article ID e1001398, 2013.

[16] S. Jana and J. Paliwal, “Novel molecular targets for antimalarial chemotherapy,” *International Journal of Antimicrobial Agents*, vol. 30, no. 1, pp. 4–10, 2007.

[17] J. K. Baird, “Effectiveness of antimalarial drugs,” *New England Journal of Medicine*, vol. 352, no. 15, pp. 1565–1577, 2005.

[18] K. L. Barnes, W. M. Watkins, and N. J. White, “Antimalarial dosing regimens and drug resistance,” *Trends in Parasitology*, vol. 24, no. 3, pp. 127–134, 2008.

[19] E. Pussard, M. Merzouk, and H. Barennes, “Increased uptake of quinine into the brain by inhibition of P-glycoprotein,” *European Journal of Pharmaceutical Sciences*, vol. 32, no. 2, pp. 123–127, 2007.

[20] D. A. Flockhart, T. Skaar, D. S. Berlin, T. E. Klein, and R. Kerb, R. Fux, K. M. Mörke et al., “Pharmacogenetics of chloroquine resistance in Plasmodium falciparum,” *Trend in Parasitology*, vol. 16, no. 3, e1001398, 2013.

[21] R. Kerb, R. Fux, K. Mörke et al., “Pharmacogenetics of antimalarial drugs: effect on metabolism and transport,” *European Journal of Pharmaceutical Sciences*, vol. 32, no. 2, pp. 123–127, 2008.
[22] World Health Organization, "World Malaria Report 2021," 2021, https://www.mmv.org/newsroom/publications/world-malaria-report.

[23] A. Ecker, A. M. Lhahane, J. Clain, and D. A. Fidock, "pCMT and its role in antimalarial drug resistance," Trends in Parasitology, vol. 28, no. 11, pp. 504–514, 2012.

[24] C. Sisowath, J. Petersen, M. I. Veiga et al., "In vivo selection of Plasmodium falciparum parasites carrying the chloroquine-susceptible pfcrk76 allele after treatment with artemether-lumefantrine in Africa," The Journal of infectious diseases, vol. 199, no. 5, pp. 750–757, 2009.

[25] K. K. Dayanand, R. N. Achur, and D. C. Gowda, "Epideomiology, drug resistance, and pathophysiology of Plasmodium vivax malaria," Journal of Vector Borne Diseases, vol. 55, no. 1, p. 1, 2018.

[26] S. G. Valderramos and D. A. Fidock, "Transporters involved in resistance to antimalarial drugs," Trends in Pharmacological Sciences, vol. 27, no. 11, pp. 594–601, 2006.

[27] C. Sisowath, J. Strömberg, A. Mårtensson et al., "In vivo selection of Plasmodium falciparum pfmdr1 86N coding alleles by artemether-lumefantrine (Coartem)," The Journal of infectious diseases, vol. 191, no. 6, pp. 1014–1017, 2005.

[28] S. Bopp, P. Magistrado, W. Wong et al., "Plasmepsin II–III copy number accounts for bimodal piperazine resistance among Cambodian Plasmodium falciparum," Nature Communications, vol. 9, no. 1, pp. 1–0, 2018.

[29] M. Foley and L. Tilley, "Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents," Pharmacology & Therapeutics, vol. 79, no. 1, pp. 55–87, 1998.

[30] B. Blasco, D. Leroy, and D. A. Fidock, "Antimalarial drug resistance: linking Plasmodium falciparum parasite biology to the clinic," Nature Medicine, vol. 23, no. 8, pp. 917–928, 2017.

[31] D. Menard and A. Dondorp, "Antimalarial drug resistance: a threat to malaria elimination," Cold Spring Harbor Perspectives in Medicine, vol. 7, no. 7, Article ID a025619, 2017.

[32] A. Gregson and C. V. Ploew, "Mechanisms of resistance of malaria parasites to antifolates," Pharmacological Reviews, vol. 57, no. 1, pp. 117–145, 2005.

[33] M. Ghorbal, M. Gorman, C. R. Macpherson, R. M. Martins, A. Scherf, and J. J. Lopez-Rubio, "Genome editing in the human malaria parasite Plasmodium falciparum using the CRISPR-Cas9 system," Nature Biotechnology, vol. 32, no. 8, pp. 819–821, 2014.

[34] D. Leroy, F. Macintyre, Y. Adoke et al., "African isolates show a high proportion of multiple copies of the Plasmodium falciparum plasmepsin-2 gene, a piperazine resistance marker," Malaria Journal, vol. 18, no. 1, p. 1, 2019.

[35] U. M. Zanger, M. Turpeinen, K. Klein, and M. Schwab, "Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation," Analytical and Bioanalytical Chemistry, vol. 392, no. 6, pp. 1093–1108, 2008.

[36] A. Westlund-Johnsson, R. Hermann, A. Huenemeyer et al., "Identification and characterization of CYP3A4+20, a novel rare CYP3A4 allele without functional activity," Clinical Pharmacology & Therapeutics, vol. 79, no. 4, pp. 339–349, 2006.

[37] D. Dai, D. C. Zeldin, J. A. Blaisdell et al., "Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid," Pharmacogenetics and Genomics, vol. 11, no. 7, pp. 597–607, 2001.

[38] R. Kerb, Implications of genetic polymorphisms in drug transporters for pharmacotherapy, Cancer Letters, vol. 234, no. 1, pp. 4–33, 2006.

[39] H. Koepsell, K. Lips, and C. Volk, Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications, Pharmacological Research, vol. 24, no. 7, pp. 1227–1251, 1994.

[40] A. T. Nies, M. Schwab, and D. Keppeler, "Interplay of conjugating enzymes with OAT uptake transporters and ABC/MRP efflux pumps in the elimination of drugs," Expert Opinion on Drug Metabolism and Toxicology, vol. 4, no. 5, pp. 545–568, 2008.

[41] Z. M. Zair, J. J. Eloranta, B. Stieger, and G. A. Kullak-Ublick, "Pharmacogenetics of OATP (SLC21A2), OAT and OATC (SLC22A) and PEPT (SLC15A) transporters in the intestine, liver and kidney," Pharmacogenomics, vol. 9, no. 5, pp. 597–624, 2008.

[42] Y. Chen, S. Li, C. Brown et al., "Effect of genetic variation in the organic cation transporter 2, OCT2, on the renal elimination of metformin," Pharmacogenetics and Genomics, vol. 19, no. 7, p. 497, 2009.

[43] A. T. Nies, H. Koepsell, S. Winter et al., "Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver," Hepatology, vol. 50, no. 4, pp. 1227–1240, 2009.

[44] I. S. Song, H. J. Shim, E. J. Shim et al., "Genetic variants of the organic cation transporter 2 influence the disposition of metformin," Clinical Pharmacology & Therapeutics, vol. 84, no. 5, pp. 559–562, 2008.

[45] A. B. Sidhu, S. G. Valderramos, and D. A. Fidock, "pfmdr1 mutations contribute to quinine resistance and enhance meloquine and artemisinin sensitivity in Plasmodium falciparum," Molecular Microbiology, vol. 57, no. 4, pp. 913–926, 2005.

[46] J. Mu, M. T. Ferdig, X. Feng et al., "Multiple transporters associated with malaria parasite responses to chloroquine and quinine," Molecular Microbiology, vol. 49, no. 4, pp. 977–989, 2003.

[47] J. Zhang, P. F. Coville, R. J. Walker, J. O. Miners, D. J. Birkett, and S. Wannimelkur, "Evidence for involvement of human CYP3A in the 3-hydroxylation of quinine," British Journal of Clinical Pharmacology, vol. 43, no. 3, pp. 245–252, 1997.
[53] R. A. Mirghani, Ü. Yasar, T. Zheng et al., “Enzyme kinetics for the formation of 3-hydroxyquine and three new metabolites of quinine in vitro; 3-hydroxylation by CYP3A4 is indeed the major metabolic pathway,” Drug metabolism and disposition, vol. 30, no. 12, pp. 1368–1371, 2002.

[54] A. B. Sidhu, A. C. Uhlemann, S. G. Valderramos, J. C. Valderramos, S. Krishna, and D. A. Fidock, “Decreasing pfmdr1 copy number in Plasmodium falciparum malaria heights susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin,” The Journal of infectious diseases, vol. 194, no. 4, pp. 528–535, 2006.

[55] B. Baune, J. P. Flinois, V. Furlan et al., “Halofantrine metabolism in micromoles in man: major role of CYP 3A4 and CYP 3A5,” Journal of Pharmacy and Pharmacology, vol. 51, no. 4, pp. 419–426, 1999.

[56] P. Muhamad, P. Phompradit, W. Sornjai et al., “Polymorphisms of molecular markers of antimarial drug resistance and relationship with artemesine-mefloquine combination therapy in patients with uncomplicated Plasmodium falciparum malaria in Thailand,” The American Journal of Tropical Medicine and Hygiene, vol. 85, no. 3, p. 568, 2011.

[57] R. N. Price, A. C. Uhlemann, A. Brockman et al., “Mefloquine resistance in Plasmodium falciparum and increased pfmdr1 gene copy number,” The Lancet, vol. 364, no. 9432, pp. 438–447, 2004.

[58] F. Fontaine, G. de Sousa, P. C. Burcham, P. Duchene, and R. Rahmani, “Role of cytochrome P450 3A in the metabolism of mefloquine in human and animal hepatocytes,” Life Sciences, vol. 66, no. 22, pp. 2193–2212, 2000.

[59] Y. T. Pham, A. Régina, R. Farinotti et al., “Interactions of racemic mefloquine and its enantiomers with P-glycoprotein in an immortalised rat brain capillary endothelial cell line,” GPN. Biochimica et Biophysica Acta (BBA)-General Subjects, vol. 1524, no. 2-3, pp. 212–219, 2000.

[60] S. B. De Laggerie, E. Comets, C. Gautrand et al., “Cerebral uptake of mefloquine enantiomers with and without the P-gp inhibitor elacridar (GF1210918) in mice,” British Journal of Pharmacology, vol. 141, no. 7, pp. 1214–1222, 2004.

[61] A. L. Aarnoudse, R. H. van Schaik, J. Dieleman et al., “MDR1 gene polymorphisms are associated with neuropsychiatric adverse effects of mefloquine,” Clinical Pharmacology & Therapeutics, vol. 80, no. 4, pp. 367–374, 2006.

[62] C. Sisowath, P. E. Ferreira, L. Y. Bustamante et al., “The role of pfmdr1 in Plasmodium falciparum tolerance to artemether-lumefantrine in Africa,” Tropical Medicine and International Health, vol. 12, no. 6, pp. 736–742, 2007.

[63] M. Mungthin, R. Khositnithikul, N. Sitthichot et al., “Avitaminosis and CYP 3A5,” Malaria Journal, vol. 8, no. 1, pp. 1–5, 2009.

[64] G. Lefevre and M. S. Thomsen, “Clinical pharmacokinetics of artemether and lumefantrine (Riamet®),” Clinical Drug Investigation, vol. 18, no. 6, pp. 467–480, 1999.

[65] K. Kiao, A. S. Rodrigues, V. do Rosário, J. P. Gil, and D. Lopes, “The drug transporter ABCR1 c. 3435C>T SNP influences artemether–lumefantrine treatment outcome,” Malaria Journal, vol. 16, no. 1, pp. 1–6, 2017.

[66] A. Djimdé, O. K. Dombbo, J. F. Cortese et al., “A molecular marker for chloroquine-resistant falciparum malaria,” New England Journal of Medicine, vol. 344, no. 4, pp. 257–263, 2001.

[67] X. Q. Li, A. Björkman, T. B. Andersson, L. L. Gustafsson, and C. M. Masimirembwa, “Identification of human cytochrome P450s that metabolise anti-parasitic drugs and predictions of in vivo drug hepatic clearance from in vitro data,” European Journal of Clinical Pharmacology, vol. 59, no. 5, pp. 429–442, 2003.

[68] M. Vezmar and E. Georges, “Direct binding of chloroquine to the multidrug resistance protein (MRP): possible role for MRP in chloroquine drug transport and resistance in tumor cells,” Biochemical Pharmacology, vol. 56, no. 6, pp. 733–742, 1998.

[69] O. A. Farolin, C. Bustamante, G. O. Gbotoho et al., “In vitro amodiaquine resistance and its association with mutations in pfcr7 and pfmdr1 genes of Plasmodium falciparum isolates from Nigeria,” Acta Tropica, vol. 120, no. 3, pp. 224–230, 2011.

[70] G. Holmgren, J. P. Gil, P. M. Ferreira, M. I. Veiga, C. O. Obonyo, and A. Björkman, “Amodiaquine resistant Plasmodium falciparum malaria in vivo is associated with selection of pfcr7 76T and pfmdr1 86Y,” Infection, Genetics and Evolution, vol. 6, no. 4, pp. 309–314, 2006.

[71] X. Q. Li, A. Björkman, T. B. Andersson, M. Riddervord, and C. M. Masimirembwa, “Amodiaquine clearance and its metabolism ton-desethylamodiaquine is mediated by CYP2C8: a new high affinity and turnover enzyme-specific probe substrate,” Journal of Pharmacology and Experimental Therapeutics, vol. 300, no. 2, pp. 399–407, 2002.

[72] B. Witkowski, V. Duru, N. Khim et al., “A surrogate marker of piperazine-resistant Plasmodium falciparum malaria: a phenotype–genotype association study,” The Lancet Infectious Diseases, vol. 17, no. 2, pp. 174–183, 2017.

[73] T. M. Lee, L. Huang, M. K. Johnson et al., “In vitro metabolism of piperazine is primarily mediated by CYP3A4,” Xenobiotica, vol. 42, no. 11, pp. 1088–1095, 2012.

[74] K. Mahotorn, P. Tan-Ariya, T. R. rgba et al., “In vitro sensitivity of pyronaridine in Thai isolates of Plasmodium falciparum,” The American Journal of Tropical Medicine and Hygiene, vol. 98, no. 1, p. 51, 2018.

[75] M. Madimat, S. Briolant, R. A. Almavidet al., “The Plasmodium falciparum chloroquine resistance transporter is associated with the ex vivo P. falciparum African parasite response to pyronaridine,” Parasites & Vectors, vol. 9, no. 1, pp. 1–5, 2016.

[76] S. L. Croft, S. Duparc, S. J. Arbe-Barnes et al., “Review of pyronaridine anti-malarial properties and product characteristics,” Malaria Journal, vol. 11, no. 1, pp. 1–28, 2012.

[77] L. Constantino, P. Paixao, R. Moreira, M. J. Portela, V. E. Du Rosario, and J. Iley, “Metabolism of primaquine by liver homogenate fractions: evidence for monoamine oxidase and cytochrome P450 that metabolise organic cation transporter drugs and natural products on P-glycoprotein mediated drug efflux,” European Journal of Pharmacological Sciences, vol. 29, no. 1, pp. 70–81, 2006.

[78] R. Hayeshi, C. Masimirembwa, S. Mukanganyama, and A. L. Ungell, “The potential inhibitory effect of antiparasitic drugs and natural products on P-glycoprotein mediated drug efflux,” European Journal of Pharmacological Sciences, vol. 56, no. 6, pp. 733–742, 1999.

[79] R. Hayeshi, C. Masimirembwa, S. Mukanganyama, and A. L. Ungell, “The potential inhibitory effect of antiparasitic drugs and natural products on P-glycoprotein mediated drug efflux,” European Journal of Pharmacological Sciences, vol. 56, no. 6, pp. 733–742, 1999.

[80] R. Hayeshi, C. Masimirembwa, S. Mukanganyama, and A. L. Ungell, “The potential inhibitory effect of antiparasitic drugs and natural products on P-glycoprotein mediated drug efflux,” European Journal of Pharmacological Sciences, vol. 56, no. 6, pp. 733–742, 1999.
[81] F. Ariey, B. Witkowski, C. Amaratunga et al., "A molecular marker of artemisinin-resistant Plasmodium falciparum malaria," *Nature*, vol. 505, no. 7481, pp. 50–55, 2014.

[82] D. R. Pillai, R. Lau, K. Khairnar et al., "Artemether resistance in vitro is linked to mutations in PfATP6 that also interact with mutations in PfMDR1 in travellers returning with Plasmodium falciparum infections," *Malaria Journal*, vol. 11, no. 1, pp. 1–9, 2012.

[83] U. S. Svensson and M. Ashton, "Identification of the human cytochrome P450 enzymes involved in the in vitro metabolism of artemisinin," *British Journal of Clinical Pharmacology*, vol. 48, no. 4, p. 528, 1999.

[84] S. G. Senarathna, M. Page-Sharp, and A. Crowe, "MV_he interactions of P-glycoprotein with antimalarial drugs, including substrate affinity, inhibition and regulation," *PLoS One*, vol. 11, no. 4, Article ID e0152677, 2016.

[85] C. Severini and M. Menegon, "Resistance to antimalarial drugs: an endless world war against Plasmodium that we risk losing," *Journal of global antimicrobial resistance*, vol. 3, no. 2, pp. 58–63, 2015.

[86] P. Olliaro, "Mode of action and mechanisms of resistance for antimalarial drugs," *Pharmacology & Therapeutics*, vol. 89, no. 2, pp. 207–219, 2001.

[87] J. P. Gil and E. G. Berglund, "CYP2C8 and antimalaria drug efficacy," *Pharmacogenomics*, vol. 8, pp. 187–198, 2007.

[88] S. Parikh, J. B. Ouedraogo, J. A. Goldstein, P. J. Rosenthal, and D. L. Kroetz, "Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa," *Clinical Pharmacology & Therapeutics*, vol. 82, no. 2, pp. 197–203, 2007.

[89] L. G. Lervolino, P. E. Baldin, S. M. Picado, K. B. Calil, A. A. Viel, and L. A. Campos, "Prevalence of sickle cell disease and sickle cell trait in national neonatal screening studies," *Revista Brasileira de Hematologia e Hemoterapia*, vol. 33, no. 1, pp. 49–54, 2011.

[90] G. R. Serjeant and C. M. Ndugwa, "Sickle cell disease in Uganda: a time for action," *East African Medical Journal*, vol. 80, no. 7, pp. 384–387, 2003.

[91] A. I. Juwah, E. U. Nlemadim, and W. Kaine, "Types of anaemic crises in paediatric patients with sickle cell anaemia seen in Enugu, Nigeria," *Archives of Disease in Childhood*, vol. 89, no. 6, pp. 572–576, 2004.

[92] V. Nakibukwa, G. Ndezi, D. Nakiboneka, C. M. Ndugwa, and J. K. Tumwine, "Prevalence of sickle cell disease and sickle cell trait in national neonatal screening studies," *Revista Brasileira de Hematologia e Hemoterapia*, vol. 33, no. 1, pp. 49–54, 2011.

[93] A. I. Juwah, E. U. Nlemadim, and W. Kaine, "Types of anaemic crises in paediatric patients with sickle cell anaemia seen in Enugu, Nigeria," *Archives of Disease in Childhood*, vol. 89, no. 6, pp. 572–576, 2004.

[94] V. Nakibukwa, G. Ndezi, D. Nakiboneka, C. M. Ndugwa, and J. K. Tumwine, "Prevalence of sickle cell disease and sickle cell trait in national neonatal screening studies," *Revista Brasileira de Hematologia e Hemoterapia*, vol. 33, no. 1, pp. 49–54, 2011.

[95] S. Pelleau, E. L. Moss, S. K. Dhingra et al., "Adaptive evolution of malaria parasites in French Guiana: reversal of chloroquine resistance by acquisition of a mutation in pfcr*, *Proceedings of the National Academy of Sciences*, vol. 112, no. 37, pp. 11672–11677, 2015.

[96] N. A. Helsby, G. Edwards, A. M. Breckenridge, and S. A. Ward, "The multiple dose pharmacokinetics of proguanil," *British Journal of Clinical Pharmacology*, vol. 35, no. 6, pp. 653–656, 1993.

[97] K. Herrlin, A. Y. Massele, G. Rimoy et al., "Slow chloroquine metabolism in Tanzanians compared with white subjects and Asian subjects confirms a decreased CYP2C19 activity in relation to genotype," *Clinical Pharmacology & Therapeutics*, vol. 68, no. 2, pp. 189–198, 2000.

[98] R. N. Price, G. Dorsey, E. A. Ashley et al., "World Antimalarial Resistance Network I: clinical efficacy of antimalarial drugs," *Malaria Journal*, vol. 6, no. 1, pp. 1–9, 2007.

[99] P. Hodoameda, N. O. Duah-Quashie, C. O. Hagan et al., "Plasmodium falciparum genetic factors rather than host factors are likely to drive resistance to ACT in Ghana," *Malaria Journal*, vol. 19, no. 1, pp. 1–8, 2020.

[100] A. L. Pickard, C. Wongarichanalai, A. Purfield et al., "Resistance to antimalarials in Southeast Asia and genetic polymorphisms in pfmdr1," *Antimicrobial Agents and Chemotherapy*, vol. 47, no. 8, pp. 2418–2423, 2003.

[101] S. Pelleau, E. L. Moss, S. K. Dhingra et al., "Adaptive evolution of malaria parasites in French Guiana: reversal of chloroquine resistance by acquisition of a mutation in pfcr*, *Proceedings of the National Academy of Sciences*, vol. 112, no. 37, pp. 11672–11677, 2015.