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Application of pharmacogenomics to malaria: a holistic approach for successful chemotherapy

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Abstract

Drug resistance in malaria jeopardizes the most elementary objectives of malaria control – reducing suffering and eliminating mortality. An important, and so far the only known, mechanism of drug resistance appears to be polymorphisms in the malaria parasite genes. Efforts to circumvent antimalarial drug resistance now range from the use of combination therapies with existing agents to genomics-based studies directed toward discovering novel targets and agents. However, the potential contribution of host genetic/molecular factors, particularly those associated with antimalarial drug metabolism, remains largely unexplored. Our knowledge concerning the basic mechanisms involved in the pharmacokinetics of antimalarial drugs is fragmentary. In addition, the link between antimalarial drug pharmacokinetics and treatment outcomes is generally unclear. The purpose of this article is to provide general background information on antimalarial drug resistance and associated parasite genetic factors, and subsequently highlight the aforementioned unexplored and unclear areas, with a view to stimulate much needed further research.

Keywords

antimalarial drug metabolism; antimalarial drug resistance; antimalarial drugs; artemisinin drugs; CYP2B6; malaria; UGT2B7

Malaria

Malaria, one of the most notorious parasitic infectious diseases, occurs in over 100 countries, where an estimated 3 billion people are at risk for the disease, 200–400 million people experience at least one infection per year, and 1–2 million malaria-attributable deaths occur annually [1–3,201]. Over the past 40 years, the upsurge in this ‘global killer’ has come from a coincidence of drug-resistant parasites [4], insecticide-resistant mosquito vectors [5], perhaps global climate change [6] and a number of socioeconomic and political factors [7–9]. Population projections indicate that approximately 400 million births will occur in malarious regions by 2010. By this date, the Roll Back Malaria initiative has set itself the challenge of halving the world’s malaria burden [2].

Four species of the protozoan parasite *Plasmodium* cause the disease in humans: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. They differ greatly with respect to their biology and clinical manifestations [10]. Sympatric combinations of these species occur in human populations and within infected individuals [11–13]. Of these species, *P. falciparum* causes the most severe form of the disease and the highest mortality owing to its prevalence, virulence and multidrug

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resistance. Approximately 80% of all *P. falciparum* malaria cases occur in sub-Saharan Africa, where most people become infected during childhood, and most of the morbidity and mortality is seen among children aged under 5 years [14]. High morbidity and mortality associated with falciparum malaria is also seen among young pregnant women [15]. Due to resistance to the most commonly used drugs, chloroquine (CQ) and sulfadoxine-pyrimethamine (S-P; Fansidar®), *P. falciparum* has also become the predominant malaria species in many parts of the world outside of Africa [1], and continues to be a major threat to travelers to tropical regions [16].

The estimated global burden of *P. vivax* malaria is 100–300 million cases annually [17,18]. Outside of Africa, the continent where *P. falciparum* predominates, *P. vivax* accounts for 30–70% of all malaria cases, mainly in Asia, including the Middle East and the western Pacific, and in Central and South America [2,17]. The repeated attacks of *P. vivax* through childhood and adult life can cause clinical and severe disease leading to substantial morbidity and mortality [18–20].

The other two species, *P. malariae* and *P. ovale*, though less prevalent than *P. falciparum* and *P. vivax*, are widespread but exhibit patchy distribution [21]. The incidences of clinical *P. malariae* and *P. ovale* episodes are low, and when they occur, are relatively mild [21]. In addition to these four species, humans naturally infected with the simian parasite, *P. knowlesi*, have recently been identified in Southeast Asia [22–24], suggesting the 'advent' of the fifth malaria species in humans.

Antimalarial chemotherapy & drug resistance

In the situation where mosquito vectors are insecticide-resistant [5,25], and an effective vaccine is not yet available [25,26], chemotherapy and chemoprophylaxis remains the principal means of combating malarial infections. Over the past 60–70 years, since the introduction of synthetic antimalarials, only a small number of compounds have been found suitable for clinical use; this limited armament is now severely compromised because of the parasite's remarkable ability to develop resistance against these compounds [4,27]. Only a few new drugs are in clinical use or development, and many of these belong to long-used classes of antimalarial drugs [28,29].

Overview of common classes of antimalarial drugs

Quinine & related drugs—Quinine, originally extracted from *Cinchona* bark in the early 1800s, along with its dextroisomer quinidine, is still one of the most important drugs for the treatment of uncomplicated malaria, and often the drug of last resort for the treatment of severe malaria. CQ, a 4-aminoquinoline derivative of quinine, has been the most successful, and therefore the most widely used, antimalarial drug since the 1940s. However, its usefulness has rapidly declined in those parts of the world where CQ-resistant strains of *P. falciparum* and *P. vivax* have emerged and are now widespread. Amodiaquine is an analog of CQ, and is effective against low-level CQ-resistant falciparum malaria. Other quinine-related drugs commonly used include mefloquine, a 4-quinoline-methanol derivative of quinine, and the 8-aminoquinoline derivative, primaquine, specifically used for eliminating relapse-causing, latent hepatic forms (hypnozoites) of *P. vivax* [4,28,29].

Antifolate combination drugs—Antifolate drugs include various combinations of dihydrofolate reductase (DHFR) enzyme inhibitors, such as pyrimethamine, proguanil, chlorproguanil and cycloguanil, and dihydropteroate synthase (DHPS) enzyme inhibitors, such as sulfadoxine, sulfalene and dapsone. With rapidly growing S-P resistance, a new combination drug, Lapdap® (chlorproguanil-dapsone), is being tested in Africa [30,31], and some safety concerns have been noticed [32].

Artemisinin & its derivatives—Artemisinin drugs, which originated from the Chinese herb qinghao (*Artemisia annua*), belong to a unique class of compounds, sesquiterpene lactone endoperoxide, and are the new generation of potent antimalarials [33]. The parent compound is artemisinin (quinghaosu), whereas dihydroartemisinin (DHA), artesunate, artemether and β-arteether are the most common derivatives of artemisinin; DHA is the main bioactive metabolite of all artemisinin derivatives (artesunate, artemether, β-arteether and so on), and is also available as a drug in itself [28,29,33].

Artemisinin-based combination therapies, such as artemether-lumefantrine (Coartem®), artesunate-mefloquine and artesunate-amodiaquine, are widely used in China, Southeast Asia and many parts of Africa [34]. Introduction of artemisinin-based combination therapies has initiated a noticeable reduction in malaria prevalence in these, as well as other endemic regions of the world [33]. The current high-level of interest in these drugs is due to their following pharmacological advantages: these drugs act rapidly upon asexual blood stages of CQ sensitive as well as CQ-resistant strains of both *P. falciparum* and *P. vivax*, and reduce the parasite biomass very quickly, by approximately 4-logs for each asexual cycle. Also, these drugs are gametocytocidal [33,35]. Through rapid killing of asexual blood stages and developing gametocytes, artemisinin drugs significantly limit the transmission potential of the treated infections [36]. These drugs have large therapeutic windows. Although there have been some safety concerns in animal models, however, on the basis of extensive human use, these drugs appear to be safe, even in children and mid/late pregnant women [33]. Furthermore, there is no reported 'added toxicity' when these drugs are used in combination with other types of antimalarial compounds [33].

Antibiotics & other compounds in combination therapies—Tetracycline and its derivatives, such as doxy-cycline, are consistently active against all species of malaria, and in combination with quinine, are particularly useful for the treatment of severe falciparum malaria [28]. Halofantrine and lumefantrine, structurally related to quinine, are effective against multidrug-resistant falciparum malaria. A combination of lumefantrine and artemether (Coartem) has proved to be effective in treating uncomplicated acute falciparum malaria [28, 29,33]. Until recently, a combination of atovaquone, a hydroxynaphthoquinone, and proguanil (Malarone®) was considered to be effective against CQ-and multidrug-resistant falciparum malaria; atovaquone resistance has now been reported in Africa [37,38]. Piperaquine, another member of the 4-aminoquinoline group, in combination with DHA (Artekin®), holds the promise of being successful in CQ-resistant endemic areas of Southeast Asia [28,29,33].

Overview of geographic distribution of antimalarial drug resistance

In vivo resistance to antimalarial drugs has been described for three of the four species of malaria parasites: *P. falciparum*, *P. vivax*, and *P. malariae*. *P. falciparum* has developed resistance to nearly all antimalarial drugs in current use [4,39], although the geographic distribution of resistance to any one particular drug varies greatly [4,40]. Southeast Asia has a highly variable distribution of falciparum drug resistance; while some areas have a high prevalence of complete resistance to multiple drugs, else-where there is a spectrum of sensitivity to various drugs [40]. CQ-resistant falciparum malaria has been described from almost every region where *P. falciparum* is transmitted, except from malarious areas of Central America and limited areas of the Middle East and central Asia [4,40]. Due to widespread CQ resistance (CQR) in Africa, many countries shifted to S-P treatment as the first-line alternative to CQ. Unfortunately, S-P resistance, already reported from Southeast Asia and South America, is becoming increasingly prevalent in Africa [41].

Chloroquine- and primaquine-resistant vivax malaria has been reported from a number of countries in Southeast Asia and the western Pacific [42,43], and focal resistance to S-P has

also been noticed in those regions [18]. CQ-resistant *P. vivax* infections are prevalent in South America [44]. Finally, CQ-resistant *P. malariae* has been reported from southern Sumatra, Indonesia [45].

Parasite genetic polymorphisms in antimalarial drug resistance: an overview

It is believed that the selection of parasites harboring polymorphisms, particularly point mutations, which are associated with reduced drug sensitivity, is the primary basis of drug resistance in malaria parasites. Drug-resistant parasites are more likely to be selected if parasite populations are exposed to subtherapeutic drug concentrations through unregulated drug use, the use of inadequate drug regimens and the use of long half-life drugs [27]. In recent years, significant progress has been made to understand genetic/molecular mechanisms underlying drug resistance in malaria parasites [37,46,47].

Genes/polymorphisms associated with drug resistance in *P. falciparum*—

Chloroquine resistance in *P. falciparum* is now linked to point mutations in the CQ resistance transporter gene (*pfcrt*, chromosome 7) [48]. *Pfcrt*-K76T mutation confers resistance *in vitro*, and is the most reliable molecular marker for CQR. CQ-sensitive strains from all geographic regions maintain an invariable wild-type amino acid allele, *CVMNK*, 72–76, while there are a number of predominant CQR-associated alleles: *CVIET* (Southeast Asia, Africa), S_{agt}*VMNT* (Asia, South America, Tanzania), S_{tct}*VMNT* (South America), *CVMET* (Colombia), and *CVMNT* (South America, Philippines) [48]. Polymorphisms, including copy-number variation and point mutations, in another parasite transporter gene, multidrug resistance gene (*pfmdr1*, chromosome 5), contribute to the parasite's susceptibility to a variety of anti-malarial drugs [48]. Point mutations in this gene play a modulatory role in CQR, which appears to be a parasite strain-dependent phenomenon [48]. Two *pfmdr1* mutant alleles occur in CQ-resistant strains from different geographic regions: 86Y_184Y_1034S_1042N_1246D (predominant in Asia and Africa) and 86N_184F_1034C_1042D_1246Y (predominant in South America) [48]. Although a number of field studies have observed a significant nonrandom association between *pfcrt*-76T and *pfmdr1*–86Y mutations, suggesting a joint contribution of these two genes to the CQR phenotype, other studies have suggested that additional parasite genes are likely to be involved [49].

It has been shown that point mutations in the *P. falciparum* DHPS enzyme (*Pf-dhps*, chromosome 8) (Table 1) are involved in the mechanism of resistance to the sulfa class of antimalarials, and accumulation of mutations in the *P. falciparum* DHFR domain (*Pf-dhfr*, chromosome 4) (Table 1) defines the major mechanism of high-level pyrimethamine resistance [37,49]. In field studies, a *Pf-dhps* double mutant (437G with either 540E or 581G), combined with the *Pf-dhfr* triple mutant (108N_51I_59R), is found to be frequently associated with S-P treatment failure [37,49].

Genes/polymorphisms associated with drug resistance in other malaria species

—Point mutations in the *P. vivax* ortholog of *pfcrt* (*pvcg10*) were not found to be associated with the clinical resistance to CQ, suggesting that the genetic mechanisms of CQR in *P. vivax* are different from those in *P. falciparum* [49]. Recently, the *P. vivax* ortholog of *pfmdr1* (*pvmdr1*) has been identified. Both point mutations and copynumber variations occur in *pvmdr1* [49,50], and a recent study documented a degree of association between the Y976F mutation of *pvmdr1* and reduced susceptibility to CQ [51]. Point mutations in the *pv-dhps* and *pv-dhfr* genes have been identified, and may be involved in the clinical resistance to S-P [49]. To our knowledge, no information is available regarding the genetic basis of CQR in *P. malariae*.

From this overview, it is clear that point mutations in parasite genes play an important role in antimalarial drug resistance, specifically in the case of *P. falciparum* CQ and S-P resistance. However, the contribution of host genetic factors, particularly those associated with the metabolism of antimalarial drugs, has not yet been explored. Substantial interindividual variability in the pharmacokinetics of antimalarial drugs has been noticed, but the relationship between *in vivo* antimalarial drug concentrations and treatment outcome is far from clear (see the antimalarial drug metabolism in the human host section below). Hence, it is possible that a parasite infection, sensitive to a chosen drug, may not be cleared due to therapeutically inadequate levels of the drug or its active metabolite. Furthermore, subtherapeutic levels of an anti-malarial drug/metabolite may increase the risk of developing resistance by creating a predisposing environment ('selection pressure') [52,53].

Antimalarial drug metabolism in the human host

Based upon the *in vitro* evaluation of antimalarial drugs against a variety of *P. falciparum* isolates, antimalarial activity could be attributed mainly to the drug itself (e.g., sulfadoxine and pyrimethamine), to the principal metabolite of a drug (e.g., cycloguanil, the active metabolite of proguanil), or to both the drug and its principal metabolite (e.g., CQ and desethylchloroquine [DCQ], amodiaquine and desethylamodiaquine, and artemisinin drugs and DHA).

Although antimalarial drugs have been in use since the 1930s, until recently, no data was available on the absorption–distribution–metabolism–excretion parameters of these drugs in humans. Over the past 10–15 years, there have been many methodological developments in the area of antimalarial pharmacokinetics. Through these advancements, pharmacokinetics and the major metabolic pathways of a number of antimalarial drugs have been elucidated [54–56]. However, little emphasis has been placed on understanding the basic mechanisms responsible for the pharmacokinetic and pharmacodynamic behaviors of the major classes of antimalarial drugs in various populations. Since drug combinations are increasingly being considered to successfully treat malaria worldwide, this information is essential to optimize antimalarial drug regimens in order to achieve high treatment efficiencies across all malaria-endemic regions.

Overview of antimalarial drug-metabolizing enzymes

Using various heterologous expression systems, human liver microsomes and recombinant enzymes, a wealth of information is now available regarding the role of the cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT) enzyme superfamilies in the metabolism of antimalarial drugs. This information is summarized in Table 2.

Quinine & related drugs—Quinine undergoes 3-hydroxylation mainly via CYP3A4; CYP1A1 may also be involved in the quinine metabolism [57,58]. Similar to quinine metabolism, CYP3A4 metabolizes quinidine predominantly to 3-hydroxyquinidine, although quinidine-*N*-oxide is also formed [59]. CQ undergoes *N*-dealkylation mainly to DCQ, and to a lesser extent to bisdesethyl-CQ, via CYP2C8 and CYP3A4/3A5, and also via CYP2D6 [60,61]. Amodiaquine's metabolism to *N*-desethylamodiaquine is the principal route of disposition mainly via CYP2C8 [62]. Mefloquine and primaquine are metabolized to carboxymefloquine and carboxyprimaquine, by human liver microsomes, respectively [63, 64]. In human hepatocytes and microsomes, another metabolite of mefloquine, hydroxylmefloquine, was also detected [65]. The involvement of CYP3A4 in mefloquine biotransformation was suggested by several lines of evidence, and CYP1A2 and CYP3A4 were identified as the hepatic enzymes responsible for the primaquine metabolism [60,65]. Both halofantrine and lumefantrine are metabolized to desbutyl derivatives predominantly via CYP3A4; CYP3A5 may also be involved in the halofantrine metabolism [55,66].

Antifolate combination drugs—Dapsone undergoes *N*-hydroxylation mainly via CYP2C9 [67] and CYP3A4 [60,68]. Proguanil is metabolized to cycloguanil, and *in vivo*, CYP2C19 is the major isoform responsible for the formation of cycloguanil [69,70]. Apart from dapsone and proguanil, not much is known about the metabolism of other antimalarial DHPS (sulfa drugs) and DHFR enzyme inhibitors. Recombinant CYP1B1 (11%) and CYP2C19 (13%) contributed very little to the metabolism of pyrimethamine when used at high concentrations (20 pmol/incubation) [60]. There is indirect evidence that atovaquone, used in combination with proguanil (Malarone®), may undergo limited metabolism via CYP3A4 [71] and CYP2C9 [72]; however, a specific metabolite has not been identified.

Artemisinin & its derivatives—Artemisinin and its derivatives are metabolized in the liver, mainly through CYP-catalyzed phase I oxidative dealkylation reaction [73] (Table 2). Artemisinin is metabolized primarily via CYP2B6, with probable secondary contributions from CYP3A4 and CYP2A6 [60,74]. CYP2A6 is primarily responsible for artesunate metabolism, with minor involvement of CYP2B6 [60]. In the case of artemether metabolism, the role of CYP3A4 is suggested, but is not clear [55,73]. However, CYP3A4 is the primary enzyme involved in the β-arteether metabolism, with secondary contributions CYP2B6 and CYP3A5 [75]. The enzymes CYP3A4 and CYP3A5 have been shown to be involved in the metabolism of artelinic acid, a water-soluble artemisinin analog [76]. Recent *in vitro* studies revealed that CYP-catalyzed oxidation did not play a significant role in the metabolism of DHA, the major bioactive metabolite of artemisinin derivatives. Instead, DHA is converted to DHA-glucuronide via phase II enzymes UGT1A9 and UGT2B7 [77,78].

Possibility of drug–drug interactions in artemisinin-based combination therapies—Artemisinin drugs, in combination with other antimalarial drugs, are viewed as the most promising treatment regimens for falciparum malaria. However, the clinical pharmacology of artemisinin-based combination therapies is highly complex, and there is a potential for drug–drug interactions [35,55]. Artemisinin and its derivatives induce CYP2B6 and CYP2C19 [79], CYP3A (3A4/3A5) [80] and CYP2A6 [81], and inhibit CYP1A2 [80]. To our knowledge, the effect of artemisinin and its derivatives on CYP2C8 (amodiaquine-metabolizing enzyme) has not been reported. The molecular mechanism of CYP induction by artemisinin may involve transcriptional gene activation mediated by the nuclear receptors, constitutive androstane receptor and pregnane X receptor [82,83]. Despite these results [79–81], which reflect phase I metabolic reactions only, clinical studies with the most commonly-used artemisinin-based combination therapies, artemether-lumefantrine, artesunate-mefloquine and artesunate-amodiaquine, have so far not shown any significant interactions.

Prevalence of antimalarial drug-metabolizing enzyme polymorphisms & their link to antimalarial drug concentrations & treatment outcome

Both the CYP [202] and UGT [203] enzyme super-families in humans are highly polymorphic. Many of these polymorphisms, either singly or in combination, are functionally significant from the therapeutic outcome or drug-safety standpoint in the therapy of a number of medical conditions [84]. However, only a few reports have described the prevalence of such polymorphisms [85], and/or significance of these polymorphisms in affecting antimalarial drug concentrations and treatment outcome in the populations from malarious regions.

Interindividual variability in CQ & DCQ concentrations—In a review of CQ treatment/prophylaxis studies, a 2.5- to 5.6-fold interindividual variability in CQ concentrations was noticed [86]. In African children with acute falciparum malaria, peak plasma CQ and DCQ concentrations ranged between 65–263 and 9–62 ng/ml, respectively [87]. However, effective clinical and parasitological responses to the treatment were observed in 2–4 days in all patients. In Tanzanian schoolchildren, marked interindividual variability in whole-blood CQ (3.3- to

5.1-fold) and DCQ (3.5- to 6.3-fold) concentrations was observed during and after the treatment [88]. The mean highest CQ concentration was significantly lower in the children in whom parasites were detected on day 7 (1473 nmol/l), than that in the children with no detectable parasites (1799 nmol/l). In a subsequent study, whole-blood CQ and DCQ concentrations in Tanzanian schoolchildren were between 6–950 nmol/l and 10–299 nmol/l, respectively [89]. Only 9% of the children had CQ concentrations above 100 nmol/l (CQ concentrations between 100–500 nmol/l considered as 'inhibitory', to such an extent that the host remains asymptomatic [88,90]). In these children, *P. falciparum* trophozoites were significantly less common compared with those children who had CQ concentrations <100 nmol/l. A number of other studies also noted that young children were more likely to fail CQ treatment than older malaria patients, possibly due to lower plasma CQ concentrations [90–92].

In some studies, plasma CQ and DCQ concentrations in sensitive and resistant patients have been compared. In patients from Sudan, large interindividual variability (20–35%) in the mean plasma CQ concentrations was observed on day 3 [93]. The mean plasma CQ concentration was higher in the sensitive group (0.275 μmol/l), than that in the resistant group (0.225 μmol/l). Similarly, the mean plasma CQ:DCQ ratio was higher in the sensitive group (3.14), than that in the resistant group (3.05). However, these differences were not significant [93]. In sensitive and resistant cases from India, plasma and blood-cell concentrations of CQ and DCQ were determined on day 2 and day 7 [94]. On day 2, the mean CQ concentrations were significantly higher in the sensitive cases (plasma, 0.47 μg/ml; blood-cell, 1.51 μg/ml), than those in the resistant patients (plasma, 0.32 μg/ml; blood-cell, 0.46 μg/ml). The mean CQ:DCQ ratio was also significantly higher in the blood cells from the sensitive group (3.07), than that in the blood cells from the resistant cases (1.77) [94].

Although limited in their interpretations, overall, the studies described above suggest that noticeable interindividual variability can occur in the blood concentrations of CQ/DCQ, and this variability may affect the parasitological treatment outcome. Lower blood/plasma CQ concentrations could be attributable to patient noncompliance with drug intake. However, in a number of studies, patients received the anti-malarial treatment under supervision [86–88, 92,94]. Therefore, noncompliance may not be the only factor. The question whether genetic variability in the CQ metabolism also contributed to the variability in CQ/DCQ concentrations, was not addressed in these studies.

Interethnic variability in primaquine & carboxyprimaquine concentrations— Primaquine is used for relapses caused by vivax malaria hypnozoites. In Korean patients treated with a standard CQ-primaquine regimen, the mean peak plasma concentration of primaquine was fivefold higher and that of carboxyprimaquine (the active metabolite of the drug) was 2.6- to 5.2-fold lower compared with the drug/metabolite concentrations in Indian and Thai patients [95]. The pharmacokinetic difference was also observed between Caucasian and Thai male volunteers; the peak plasma concentration of primaquine in Thais was higher (0.233 ± 0.047 μg/ml) than that in Caucasians (0.162 ± 0.02 μg/ml) [96]. Regional differences in the response of vivax malaria to primaquine have been reported [97], and it has been suggested that subtherapeutic doses or concentrations may be among the risk factors for primaquine treatment failures [53]. However, no attempt has yet been made to link the primaquine pharmacokinetics and treatment outcome to genetic variation in the primaquine metabolism.

Interallelic variability in the metabolism of amodiaquine—Amodiaquine is metabolized to its primary metabolite, *N*-desethylamodiaquine, via CYP2C8 [62]. The question remains whether the efficacy of amodiaquine is affected by *CYP2C8* polymorphisms. Among the main variants, CYP2C8*2, *3 and *4 [98], the *2 and *3 variants have been documented to be associated with significant *in vitro* differences in the kinetic parameters of amodiaquine as compared with the wild-type enzyme. The intrinsic clearance (defined as V_{max}/K_m) of

amodiaquine for the CYP2C8*2 variant was sixfold lower than that for the wild-type enzyme [99]. The amodiaquine metabolic activity of the *3 variant was lower than that of the *2 variant [99]. To our knowledge, there is no published data on the amodiaquine metabolism by the *4 variant. *CYP2C8*2* is highly prevalent (>10%) in those of African descent, whereas the **3* (2%) and **4* (<1%) alleles have been detected in East Africa [98,99]. Efficacy outcomes and time to therapeutic failure with amodiaquine monotherapy did not vary between **1/*1* and **1/*2* individuals, although a significant increase in self-reported rate of abdominal pain was observed among the **1/*2* carriers [99]. Although genotype-inferred slow metabolizers are found in 1–4% of African populations [98], *in vivo* pharmacokinetic data on amodiaquine is very limited. Unfortunately, in the study by Parikh *et al.*, amodiaquine/desethylamodiaquine concentrations in patients were not reported [99]. Thus, in order to better understand the impact of *CYP2C8* polymorphisms on the treatment efficacy and adverse events of amodiaquine-based therapies, particularly in Africa, studies combining *in vivo* exposure to the drug with the *CYP2C8* genotype status are needed.

Interindividual variability in the metabolism/efficacy of proguanil—Proguanil metabolism is mediated via CYP2C19 [69,70]. By measuring the proguanil: cycloguanil metabolic ratio and/or the ability of CYP2C19 to metabolize a probe drug, (*S*)-mephenytoin, the poor metabolism (PM) and extensive metabolism (EM) phenotypes are identified [70]. The prevalence of the PM phenotype varies substantially among ethnically different populations (Table 3) [100,101], and two variant alleles, *CYP2C19*2* and *CYP2C19*3*, are largely associated with the PM phenotype [102].

A malaria prophylaxis study with proguanil in Tanzanians found a significant but modest correlation between proguanil:cycloguanil ratio and the number of breakthrough parasitemia episodes (a measure of treatment failure), and suggested that the impaired cycloguanil formation may be a contributing risk factor [103]. However, in Japanese volunteers in Kenya, the prophylactic effect of proguanil was similar between PM and EM subjects, suggesting that poor metabolism of proguanil, and thus the resulting lower concentrations of cycloguanil, may not be associated with proguanil prophylaxis failure [104]. In malaria patients from Vanuatu, proguanil concentrations and proguanil:cycloguanil ratios differed significantly between PM and EM genotype groups [105,106]. However, the therapeutic efficacy of proguanil treatment in PM patients was found to be similar to that in EM patients [107]. There was even a trend toward lower efficacy in EM patients (the mean therapeutic efficacy for falciparum and vivax infections, PM: 75 and 91%; EM: 64 and 88%, respectively) [107]. Based upon these observations, the authors suggested that the parent compound proguanil may have a significant intrinsic efficacy independent of its main metabolite cycloguanil. Another plausible explanation may be that an as yet undefined metabolite, through another metabolic pathway, is responsible for the proguanil efficacy in PM subjects [108]. Therefore, further studies are needed to clarify the clinical significance of *CYP2C19* genotypes in the antimalarial efficacy of proguanil/cycloguanil.

Interindividual variability in the pharmacokinetics of artemisinin drugs: a concern about their effectiveness?—Artemisinin drugs, the most promising antimalarial drugs at present, are available for oral, rectal (suppository) and intramuscular/intravenous administrations [73,109]. These drugs have unique and highly variable pharmacokinetic properties. They are rapidly absorbed and eliminated. Time-to-peak plasma levels for artemisinin drugs vary from minutes to hours, depending on the drug formulation and its route of administration [109,110]. Bioavailability of artemisinin drugs is also highly variable (<25% to >85%), depending on the drug formulation, its route of administration, health status of the individual and the nature of malaria infection [73]. Biotransformation into the active metabolite DHA occurs almost immediately for artesunate (within 15 min of administration). DHA has an elimination half-life of approximately 45 min [109]. The

elimination half-life of artemisinin is 2–5 h [110,111]. Evidence also suggests rapid elimination of artesunate (in minutes) and artemether (1–11 h) [109,110].

Although it could be argued that artemisinin drugs are metabolized so rapidly that any pharmacokinetic variability does not affect the outcome of treatment with these drugs, a number of observations might suggest otherwise. In malaria patients, given a single oral dose (25–250 mg) of artesunate, plasma DHA concentrations at 2 h varied from twofold to at least 17-fold, depending on the dose of artesunate given [112]. Data suggested that doses of artesunate up to 2 mg/kg were generally correlated with both plasma DHA concentrations and parasite and fever clearance [113]. However, taking into account the considerable interindividual variability in pharmacokinetics and pharmaco-dynamics, use of a larger dose of artesunate was suggested [112].

Despite substantial interindividual variability in the pharmacokinetic properties of artemisinin [113], artesunate/DHA [112,114–117] and artemether/DHA [118], good parasite and fever clearance was observed in Southeast Asian and African malaria patients. This observation is difficult to interpret because of the fact that other antimalarial drugs, such as mefloquine [112–114], S-P [115,116], CQ [116], atovaquone-proguanil [117] or quinine [118], were also given to the patients in these studies. Therefore, one cannot conclude that potentially subtherapeutic plasma concentrations of artemisinin drugs did not occur in some of these study patients. It is possible that in such patients, the observed parasite and fever clearance was primarily due to the other antimalarial drug(s) given. If subtherapeutic plasma concentrations of artemisinin drugs occur in patients in the areas where resistance to other antimalarial drugs occurs or starts to occur, it causes a worrisome situation for treatment of malaria.

None of the aforementioned studies addressed the basis of such a large interindividual pharmacokinetic variability. The question remains, once again, do polymorphisms in CYP and UGT enzymes affect the metabolism and, consequently, the effective plasma concentrations of artemisinin drugs? Although phenotypic consequences of polymorphisms in CYP2B6 and UGT2B7 enzymes (Table 2) on the pharmacokinetics and effectiveness of artemisinin drugs are yet to be determined, it is important to consider that *CYP2B6* functional polymorphisms are highly prevalent in many African [119–123] and Asian countries, including Papua New Guinea [121,124–126]. *UGT2B7* functional polymorphisms are also highly prevalent in West Africa and Papua New Guinea [127]. In all of these countries, except Zimbabwe, artemisinin-based combination therapies have become either the first- or second-line of defense against malaria [204]. Although true parasite resistance does not yet seem to be an issue, failure of treatment with artemisinin-based combination therapies has been reported [33,128]. Given the recent important impact of artemisinin-based combination therapies, it is particularly worrisome that in some areas approximately 10% of patients fail treatment [33]. In order to promote rational use of artemisinin-based combination therapies, therapeutic monitoring and pharmacovigilance of these drugs are now strongly suggested [128–131], in addition to molecular monitoring of parasite resistance [132]. Such an integrated approach is urgently needed to maintain the effectiveness of these drugs, before antimalarial resistance becomes an unmitigated disaster.

Conclusion

Malaria control has traditionally relied upon two approaches: killing the mosquito vector and employing effective chemotherapy and chemoprophylaxis. While not discussed here, an effective malaria vaccine is not yet available, although substantial promising advancements have been made. It is becoming clear that an extensive and unregulated deployment of anti-malarial drugs has provided a significant selection pressure for malaria parasites to develop resistance. Since malaria is a blood infection, the response to an antimalarial treatment is

supposedly determined by the drug and/or active metabolite concentrations in the blood. Although polymorphisms in parasite genes play an important role in antimalarial drug resistance, the role of host genetic/molecular factors has not been explored in this regard. In addition to an extensive and unregulated usage of antimalarial drugs, genetic variability in the host antimalarial drug metabolism, which could also result in altered blood concentrations of the drug, may be another important factor causing treatment failures and contributing to the selection of resistant parasites.

Future perspective: post-genomic promises, concerns & questions

The complete genomes of the malaria parasites *P. falciparum* [133], *P. vivax* [134] and *P. knowlesi* [135], the African mosquito vector *Anopheles gambiae* [136], and the human host are now known. The *P. falciparum* genome sequence comprises of 14 chromosomes containing 5300 identified genes [133]. The completion of these *Plasmodium* spp. genomes provides the opportunity to discover specific molecular targets for malaria therapy and prevention. For example, the identification of potential *P. falciparum* antigens through genomic analysis greatly aids widely applicable, prophylactic vaccine development [137,138]. Gene expression and key regulatory components of metabolism in *P. falciparum* are currently being investigated [139–141]. Linking this increase in advancing new genetic information with the expression of drug resistance will greatly assist in identifying sites of drug resistance as well as improved treatment strategies [138,141]. One such example of a new drug target using a genomics-based approach is the development of AT-specific alkylating anti-malarial drugs targeting the uniquely AT-rich *P. falciparum* genome [142].

The sequencing of *A. gambiae*, the most important vector of *P. falciparum* in Africa, is helpful, however, in a limited manner due to its geographic restriction. Nevertheless, using *A. gambiae* as a starting point for malariavector genomics has led to investigations linking specific genes to insecticide resistance phenotypes, as well as providing the ability to monitor early warning signs of insecticide resistance [5,143,144]. Of considerable interest, the vector genome has opened the door for transgenic mosquito research. Investigations centered on utilizing transgenic mosquito vectors to suppress *Plasmodium* development, and most importantly, to stifle the mosquito vector population as a whole, are being given considerable attention [145, 146]. Does a transgenic mosquito species (really) offer hope to limit malaria despite the ecological and ethical concerns?

With the completion of the human genome, it is estimated that host genetics contributes approximately 24% to the total risk of acquiring malaria infection [147]. Some of the genetic factors, which confer reduced susceptibility/resistance to malaria, are: the sickle cell trait, the Duffy blood group antigen, α^+ thalassemia, glucose-6-phosphate dehydrogenase deficiency and hemoglobin C/E variants [147,148]. It is believed that the genetic basis of resistance to malaria is complex, and there are many undefined host genetic determinants that may affect malaria infection and disease outcome [147].

We are just now starting to chip away at the human genes that are associated with response to drugs. Thus in the post-genomic era, it is crucial to learn how the efficacy of malaria therapy is affected by the human genome at interindividual as well as interethnic levels. In addition, it is no coincidence that HIV/AIDS, TB and malaria, which when combined kill approximately 6 million people per year, occur most prevalently in the same regions of the world [205].

One might ask, what do we know about these three ‘big killers’ with respect to their drug–gene interactions, drug–drug interactions and overall outcomes after combined treatments? Do antiretroviral, anti-TB and antimalarial drugs interact at any level, which may suppress the desired effect of one or all of the treatments? Specifically, assuming that a very large number of people who receive antimalarial treatment also have HIV/AIDS and/or TB, how are

treatments for these latter diseases affecting the overall success of antimalarial treatment? In order to begin to answer some of these questions, pharmacological studies focused on human genetics and antimalarial treatment outcomes are urgently needed.

Executive summary

Malaria

- Human malaria disease is caused by an infection with one or more of four species of the protozoan parasite, *Plasmodium*.
- Malaria is found in over 100 countries, where 3 billion people are at risk of contracting the potentially fatal disease and an estimated 1–2 million deaths per year are attributed to the disease.

Antimalarial chemotherapy and drug resistance

- Chemotherapy and chemoprophylaxis are the main forms of attack against malaria. Each class of antimalarial drugs employs different approaches to attack the parasite.
- A very limited number of successful antimalarial drugs exist. Because of the parasite's ability to quickly develop resistance, this available arsenal is rapidly becoming leaner.
- The most devastating of the four malaria parasites, *Plasmodium falciparum*, has developed resistance to almost every antimalarial drug in use. Geographic distribution and prevalence of resistance phenotypes vary for three of the four *Plasmodium* species.
- Parasite-specific genetic point mutations play a key role in antimalarial drug resistance. However, it is likely that host drug-metabolism genetic characteristics play an equally important role in antimalarial drug resistance, possibly by facilitating a subtherapeutic, resistance-promoting environment for the parasite.

Antimalarial drug metabolism in the human host

- Cytochrome P450 and uridine diphosphate glucuronosyltransferase polymorphic enzyme superfamilies are known to play a role in the metabolism of antimalarial drugs.
- To date, only a few studies have attempted to associate SNPs in the two enzyme superfamilies with the metabolism and blood/plasma levels of antimalarials and consequent treatment outcomes.

Conclusion

- Human host genetic and molecular factors, which may play an important role in antimalarial treatment outcome, have not been fully explored.
- It is conceivable that a flagrant misuse of antimalarials coupled with genetic variability in host drug metabolism could contribute to treatment failure and the selection of resistant parasites.

Future perspective: post-genomic promises, concerns and questions

- In the post-genomic era, a plethora of questions arise in regard to the relationship between human genetics and malaria drug therapy. More questions arise when considering the possible drug–gene and drug–drug interactions among malaria, HIV/AIDS and TB, as well as overall treatment outcome after combined therapy.

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Table 1

Enzyme mutations associated with S-P resistance in *Plasmodium falciparum*.

Enzyme	Codon	Mutation(s)
dhps*	436	S>A
		S>F
		S>C
	437	A>G
	540	K>E
	581	A>G
	613	A>S
		A>T
dhfr‡	16	A>V
	51	N>I
	59	C>R
	108	S>N
		S>T
	164	I>L

*Target for sulfadoxine.

‡Target for pyrimethamine.

In practice, the triple mutant *dhfr* allele, 108N/51I/59R, combined with a double mutant *dhps* allele (437G with either 540E or 581G), is more frequently associated with S-P treatment failure.

S-P: Sulfadoxine-pyrimethamine.

Table 2

Primary isoforms of human CYP and UGT enzymes involved, and the major metabolites identified, in the metabolism of antimalarial drugs.

Antimalarial drug(s)	Primary isoform(s)	Major metabolite
Quinine, quinidine	CYP3A4	3-hydroxyl derivatives
Chloroquine	CYP2C8, CYP3A4/3A5, CYP2D6	Desethyl derivative
Amodiaquine	CYP2C8	Desethyl derivative
Mefloquine, primaquine	CYP3A4, CYP1A2	Carboxy derivatives
Halofantrine, lumefantrine	CYP3A4/3A5	Desbutyl derivatives
Dapsone	CYP2C9, CYP3A4	Hydroxyl/hydroxylamine derivative
Proguanil	CYP2C19	Cycloguanil
Artemisinin	CYP2B6, CYP3A4, CYP2A6	*
Dihydroartemisinin	UGT1A9, UGT2B7	DHA-glucuronide
Artesunate	CYP2A6, CYP2B6	DHA
Artemether	CYP3A4‡	DHA
Arteether	CYP3A4/3A5, CYP2B6	DHA
Artelinic acid	CYP3A4/3A5	DHA

* Not well characterized

‡ Suggested but not conclusive.

CYP: Cytochrome P450; DHA: Dihydroartemisinin; UGT: Uridine diphosphate glucuronosyltransferase.

Table 3

CYP2C19 poor metabolism phenotype frequencies in various populations.

Population(s)	Frequency (%)
Kenyan	35
Zimbabwean, Tanzanian, Ethiopian and Nigerian	4–5.2
Asian	12–23
Caucasian	2–5
New Zealander (Maori)	7
Australian (Aboriginal)	25.6
South Pacific Polynesian	13.6
Vanuatuan	70.6
Papua New Guinean (Sepik)	36

The CYP2C19 poor metabolism phenotype in these populations was determined by measuring the proguanil:cycloguanil metabolic ratio in the urine or plasma, by the ability to metabolize a probe drug (S)-mephenytoin, and/or by determining the *CYP2C19* genotypes (*CYP2C19*2* and *CYP2C19*3*).