

The Pharmacogenetics of the Antimalarial Amodiaquine

José Pedro Gil^{1,2,3}

¹Department of Physiology and Pharmacology, Drug Resistance Unit, Division of Pharmacogenetics, Karolinska Institutet, Stockholm,

²Department of Biomedical Sciences, University of Algarve IBB-Institute for Biotechnology and Bioengineering, The Centre for Molecular and Structural Biomedicine, Gambelas,

³Department of Biological Sciences, The Harpur College of Arts and Sciences, Binghamton University, Binghamton, the New York State University, NY,

¹Sweden

²Portugal

³USA

1. Introduction

Malaria is globally the most lethal parasitic disease. With an annual number of new cases reaching hundreds of millions and a mortality of *circa* 800.000 (WHO, 2010), this disease represents a worldwide major public health concern.

Malaria generally occurs in tropical and subtropical areas, with most of the lethality focused in the African continent, particularly among children under five. The disease further commands a major economic impact in the Developing World estimated as a Gross Internal Product reduction of more than US\$ 6 billion for the year 2010 alone (Sachs and Malaney, 2002)(WHO, 2010). Such burden has significantly slowed down the social development of these regions in the last decades.

Malaria is caused by an intracellular Protozoan belonging to the *genus Plasmodium*. *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* are the five different species able to infect humans (Levine, 1988)(Cox-Singh *et al.*, 2008). *P. falciparum* is responsible for the most severe forms of the disease, and hence the near totality of the mortality.

The parasites are transmitted through an arthropod vector, the dynamics of the disease being the result of a complex interplay between the human host, the parasite and its mosquito vector.

2. *Plasmodium falciparum* – A brief reference to its life-cycle

As a referential example, the 48-hour life cycle of *P. falciparum*, the major malaria pathogen – and the principal target of complex chemotherapeutic measures – is herein presented.

We assume as an arbitrary starting point the moment when a female *Anopheles* mosquito infected with *P.falciparum* penetrates the human skin to obtain a blood meal. If the mosquito saliva is infected with parasite sporozoites, these will be injected into the bloodstream of the host. These forms travel in the bloodstream to the liver where they invade hepatocytes. In this intracellular environment it rapidly divides asexually, generating the next life cycle stage form, the merozoites. These, following the rupture of the hepatocyte, are released in the bloodstream. Here, they invade erythrocytes. Once inside the erythrocyte, the merozoite develops towards the mature trophozoite stage. After these, the parasite undergoes a series of asexual divisions to produce a large segmented schizont filled with mono-nucleated merozoites. The erythrocyte then ruptures releasing the merozoites, a clinically important event associated to the characteristic malaria peaks of fever and chills. These merozoites swiftly reinvade new red blood cells, reinitiating the intra-erythrocytic cycle. In parallel, a small proportion of merozoites take a new development route towards becoming sexual forms: the male and female gametocytes. These can reinvade the mosquito vector during its blood meal. Inside the mosquito, zygotes will form after meiotic events. Further development will lead to the formation of oocysts. These, after repeated mitotic divisions, produce a large number of sporozoites, which actively migrate to the salivary glands of the mosquito, ready to be injected into the bloodstream of a human. The cycle is hence restarted.

3. Malaria chemotherapy

Chemotherapy has been the mainstay for the clinical control of malaria for hundreds of years. Starting with the introduction of artemisinin rich teas in China 1500 years ago (Hsu, 2009) and the use of barks containing quinine in South America in the XVII Century (Peters, 1970), the XX Century saw the development of several synthetic and semi-synthetic compounds. Quinine prevailed as the major antimalarial drug used worldwide for near 300 years, until the advent of the Second World War. The extension of this conflict drove the search for alternative synthetic variants. Derived from these efforts, chloroquine (CQ), a highly effective 4-aminoquinoline, emerged in the immediate post-War as the global mainstay for the treatment and control of malaria (Coatney *et al.*, 1963). By the end of the 1950s the next major malaria challenge emerged: *P. falciparum* have been able to develop resistance to this drug (Young and Moore, 1961). From two main *foci* in South East Asia and South America, resistance parasites invaded most of the other malaria-affected regions. In the late 1970s reached Africa. By the end of the Century the burden of malaria topped in a calculated multi-million death toll and an uncontrolled situation in large regions, particularly in the African Continent (Marsh, 1998).

The severity of the situation demanded a change in concept. This came from South East Asia. In the late 1980s, Thailand - a region known to be a major cradle for the development of drug resistance - was fighting with a steep decrease of efficacy of their main national control programme drug, mefloquine (Nosten *et al.*, 1991). A strategy of combining this long half-life drug with the fast acting/short half-life antimalarials of the artemisinin class (re-discovered in China during the 1970s) saved the former during the next decade (Nosten *et al.*, 2000).

The success of artemisinin combination therapy (ACT) in the Thai national malaria programme drove the rapid adoption, and progressive worldwide implementation of this strategy for the treatment of uncomplicated malaria.

This relatively new antimalaria strategy is based on the powerful pharmacodynamic action of the artemisinin derivatives (ARTs). These, typically artesunate or artemether, are known to have a Parasite Reduction Ratio of 1:10.000 (i.e., a reduction towards 0.01% of the initial parasitaemia in 48 hours of treatment), orders of magnitude above the typically found with long lasting quinoline drugs (White, 1997). This effect is short-lived, due to the characteristic very short half live of the ARTs, typically 20 minutes to 2-3 hours (Gautam *et al.*, 2009). Upon this first impact, the long-standing partner is expected to handle more efficiently the remaining parasite population. The association of drugs with different expected mechanisms of action and associated modes of parasite resistance has been deemed as a strong deterrent for the development of the latter (Eastman and Fido, 2009).

The two global main ACTs in use are artemether-lumefantrine and artesunate-amodiaquine. (WHO, 2010). The latter represents the main drive for this review.

4. Amodiaquine

Amodiaquine (AQ) emerged in the shadow of CQ success, in the late 1940s (Burckhalter *et al.*, 1948). Similar to CQ, AQ has a core 4-aminoquinoline structure. Contrarily to CQ it also represents a Mannich base.

AQ was never used to the same extent as CQ due to the large prevalence of the latter in the global malaria control programmes until the end of the XX Century. Also, its use was severely curtailed in the beginning of the 1990s, upon its removal from the WHO list of recommended antimalarials for the treatment of uncomplicated malaria (WHO, 1990). This decision followed the emergence of a number of clinical reports on rare (*ca.* 1:2000) but life-threatening secondary events associated to its use in prophylaxis regimens among Caucasian travellers (Larrey *et al.*, 1986)(Rouveix *et al.*, 1989)(Neftel *et al.*, 1986)(Hatton *et al.*, 1986). These included in most cases acute agranulocytosis, but also severe liver damage. During the 1990s, research - mainly based on *in vitro* approaches and the use of animal models - have pointed for the causing agent to be a toxic short lived quinone-imine (QI) metabolite of AQ (Jewell *et al.*, 1995)(Tingle *et al.*, 1995)(Naisbitt *et al.*, 1997). The mode of action of this putative metabolite is still under discussion, but it is generally accepted that it operates by binding covalently to cell structures or/and as a hapten associated to a specific anti-AQ IgG antibody driven immunological response (Clarke *et al.*, 1990).

The described prophylaxis effects were never formally confirmed in circumstances of the regular treatment of uncomplicated malaria. This lack of confirmatory data has raised some criticisms concerning a possible over-cautious decision at WHO (Olliaro *et al.*, 1996). In fact, the actual usefulness of this decision in a time when the efficacy of CQ was collapsing worldwide is still open to discussion. Nevertheless, AQ as a monotherapy kept being used in many African and South American regions for decades, both in the public and in the private sector.

Interestingly, the fact that AQ has not been as intensively used as CQ possibly slowed down the development of parasite resistance against this drug. AQ was recovered for global use in the XXI Century as combination therapy partner, due its low price and capacity to handle CQ resistance parasites. Two combinations are available, artesunate-AQ (an ACT), and AQ-sulfadoxine-pyrimethamine, although the latter has been recently considered to be withdrawn from the WHO list of recommended drugs for the treatment of uncomplicated malaria (WHO,

2010). Artesunate-AQ (AS-AQ) is particularly prevalent in the African continent, the epicentre of malaria mortality and morbidity. The combination represents the first or second line antimalarial treatment for uncomplicated malaria in more than twenty sub-Saharan countries (www.who.int/entity/malaria/am_drug_policies_by_region_afro/en/).

AS-AQ is administrated orally in one daily dose, for three days. The present guideline is: 4mg/Kg/day artesunate + 10mg/Kg/day AQ (WHO, 2010). Contrarily to other antimalarials (e.g. lumefantrine, CQ, piperazine), the age of the patient does not seem to affect the plasma concentrations of AQ. The present dosing has shown to be effective in areas where resistance to AQ monotherapy was not widespread (<20% of the infections). In the event of rising resistance, changes in the formulation might be necessary, namely increasing the dosing in AQ. Such changes in the dosing have been applied in the past, e.g. with CQ (Peters, 1970) and mefloquine (Carrara *et al.*, 2009).

Presently, AS-AQ is available as a fixed formulation in three tablet options: 25 mg AS/67.5 mg AQ, 50 mg AS/135 mg AQ or 100 mg AS/270 mg AQ (Coarsucam®/Winthrop®, Sanofi-Aventis, Paris; DNDi, Geneva).

Both AQ and desethylamodiaquine (DEAQ, AQ main active metabolite) show significant inter-individual variation on their pharmacokinetic parameters. AQ has a relatively short half-life of 4-12 hours (Giao and de Vries., 2000), being readily biotransformed in the liver towards DEAQ. DEAQ is a fully active antimalarial that, albeit less potent than AQ (Gerstner *et al.*, 2003)(Echeverry *et al.*, 2007) is the main responsible for the long pharmacodynamic effect of AQ therapy (Winstanley *et al.*, 1990). DEAQ has a longer half-life of 3-20 days (Hombhanje *et al.*, 2005)(Hietala *et al.*, 2007). Although AQ has been even considered as a pro-drug due to its short half-life, circumstances of extreme AQ exposure have been documented where this drug was detected in the urine of patients, months after its administration (Winstanley *et al.*, 1987).

AQ has been associated with both mild and severe adverse events. Mild events include mainly gastro-intestinal effects, particularly vomiting (Brasseur *et al.*, 1999)(Adjuik *et al.*, 2002)(Cairns *et al.*, 2010)(Nankabirwa *et al.*, 2010) and self-reported abdominal pain (Parikh *et al.*, 2007)(Bojang *et al.*, 2010), both frequently observed in amodiaquine efficacy clinical trials. Most importantly, several studies have noted situations of (clinically asymptomatic) neutropenia upon AQ therapies in subsets of the treated population (Staedke *et al.*, 2001)(Adjuik *et al.*, 2002). Although this relatively common drop in neutrophil count is not an exclusive effect of this drug (Nankabirwa *et al.*, 2010), it is possibly linked with the rare severe adverse events observed in AQ prophylaxis. Accordingly, in this (now abandoned) higher dose regimens of typically 400 mg/week, the most documented serious adverse event was agranulocytosis (~1:2000 prevalence), followed by hepatic toxicity (~1:16000)(Phillips-Howard and West, 1990)(Hatton *et al.*, 1986).

The few data available suggest that the AQ adverse events are drug dose dependent (Hatton *et al.*, 1986)(Cairns *et al.*, 2010). Although drug associated severe and lethal reactions have not been generally observed in AQ regimens for the treatment of uncomplicated malaria (Olliaro *et al.*, 1996)(Olliaro and Mussano, 2003), mild events are relatively frequent, being a threat for full regimen compliance (e.g. Gerstl *et al.*, 2010), leading to incomplete cure and potential selection of resistant parasites. This effect can be decreased through genetic evidence-based adjustment of the dose, at a personalized level.

In addition, the knowledge of the frequencies of rare genetic variants associated with documented AQ-driven adverse events in certain ethnic groups and regions under AQ exposure can be useful as a pharmacovigilance tool. As an example, and taking in account the surpassing of a specific (evidence-based) threshold of allele frequency, the use of alternative first line ACTs (e.g aminoalcohol quinoline based, as artemether-lumefantrine) for those regions could be decided. Such type of measures - although not totally preventing - have the potential of decreasing the occurrence of serious events.

It should be noted that such a population pharmacogenetics approach can be of great importance: the effect of fatal adverse events in the public opinion can be very significant, especially after the 1990s temporary withdrawal of AQ from the WHO list of recommended antimalarials. Such dramatic events - in particular involving the typical children under five - could lead to a further mistrust of the public about a drug that is pivotal in a large number of national malaria control programmes.

5. The main players of AQ disposition

As previously mentioned, upon oral absorption AQ is readily metabolized towards the pharmacologically active DEAQ. This biotransformation occurs mostly in the liver and is almost exclusively performed by the cytochrome P450 (CYP) 2C8 (Li *et al.*, 2002). The high specificity of CYP2C8 for this reaction has even led to the proposal of AQ as a specific probe drug for this P450 isoform (Walsky *et al.*, 2004). Besides this main step, other putative AQ metabolites have been proposed and at least partially confirmed (figure 1). These include 2-hydroxy-DEAQ (Churchill *et al.*, 1985), N-Bis-DEAQ (Mount *et al.*, 1986) and M2, the latter initially detected in the microsome-based seminal studies of Li *et al.* (2002), and recently confirmed through *in vitro* electrochemical approaches (Johansson *et al.*, 2009). A fraction of DEAQ itself is believed to also be transformed in 2-hydroxy-DEAQ through P450 action. Several of these post-CYP2C8 action steps are catalyzed by members of the CYP1 sub-family (Li *et al.*, 2002)(Gil and Gil-Berglund, 2007)(Johansson *et al.*, 2009).

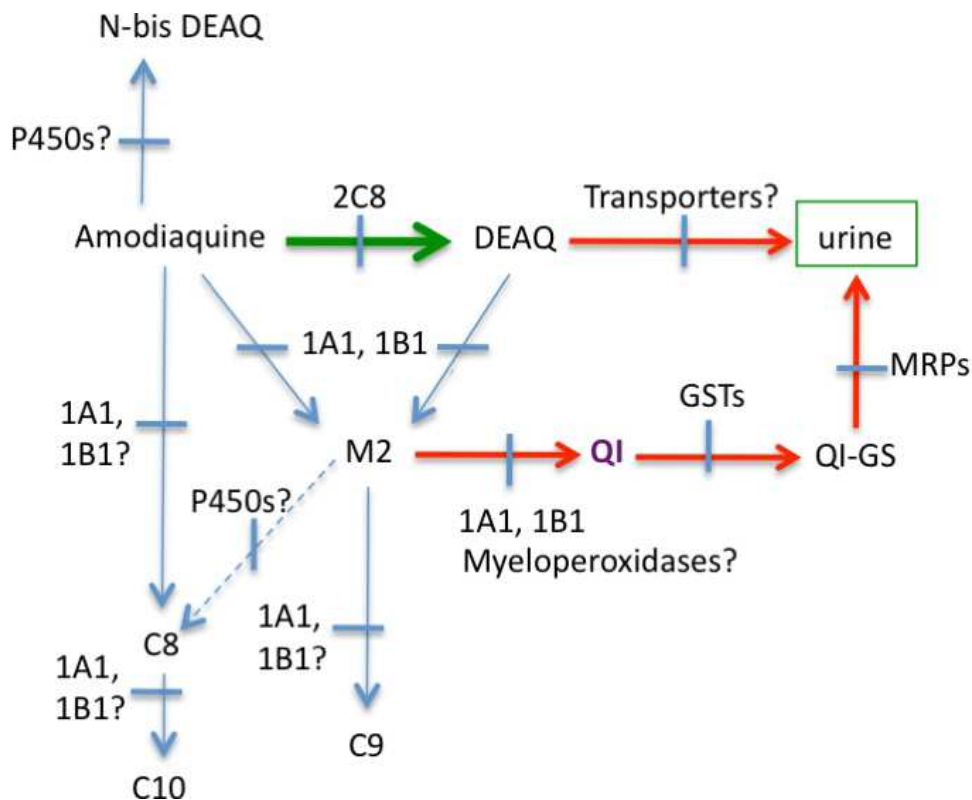
Most importantly, both AQ and DEAQ are able to generate the highly reactive quinone-imine (QI) metabolite responsible for the described serious side effects of AQ therapies. DEAQ is considered less prone to create QIs (Tingle *et al.*, 1995), reinforcing the action of CYP2C8 over AQ as a protective event. The generation of these compounds has been previously proposed to occur spontaneously, but recent investigations have pointed to a fundamental role of CYP1A1 and CYP1B1 as well as the family of myeloperoxidases in the process. In this processes, the M2 metabolite initially identified by Li *et al.* has been proposed to be a central player in the generation of the toxic QIs (Johansson *et al.*, 2009).

Taking in account the fact that these species are most likely short-lived, it is expected that the enzymes involved in its generation will be present in the location of its toxic action. Suggestively, CYP1A1 and CYP1B1 are essentially extra-hepatic P450 isoforms, with confirmed expression in several blood cell types (e.g. leukocytes)(Furukawa *et al.*, 2004), the most frequent location for these fatal toxicities (neutropenia).

Scarce information is available concerning other ADME phases in AQ metabolism. The analysis of QI adducts in animal models have pointed for the presence of glutathione conjugates, although no specific isoform has ever been determined (Masubuchi *et al.*, 2007). The potential production of phase II metabolites further points to the likely involvement of

phase III (transport) systems. The most common phase III glutathione conjugate transporters are the members of the ATP binding cassette (ABC) superfamily of proteins, and more specifically of the ABCC (also referred as MRP) type of transporters.

A summarized proposal of the complex metabolism of AQ is presented in figure 1.



Abbreviations - 1A1: CYP1A1; 1B1: CYP1B1; DEAQ:desethylAQ; QI: quinoneimine;
N-bis DEAQ: bis-desethylAQ; C8: compound 8; C9: compound 9; C10: compound 10;
QI-GS: quinoneimine-glutathione conjugates; GST: glutathione S-transferase;
MRPs: multidrug resistance proteins.

Fig. 1. The metabolism of AQ. Although the main biotransformation in this process is the synthesis of DEAQ, a constellation of minor - but most likely non-negligible - metabolites have been proposed to result in parallel (Churchill *et al.*, 1985)(Mount *et al.*, 1986)(Li *et al.*, 2002). Most importantly, events leading to the formation of the toxic QIs, involving both AQ and DEAQ are included among them. The presented scheme represents a summary of the available data from *in vitro* studies with microsomes, animal models, the few available human *in vivo* data, as well as the most recent information utilizing electrochemical approaches to simulate drug oxidation *in vitro* (Harrison *et al.*, 1992)(Jewell *et al.*, 1995)(Tingle *et al.*, 1995)(Naisbitt *et al.*, 1997)(Li *et al.*, 2002)(Johansson *et al.*, 2009).

The previously identified M2 metabolite (Li *et al.*, 2002) seems to be a central component in the generation of the QIs from both AQ and DEAQ, with CYP1A1 and CYP1B1 being the main enzymes involved in the process (Johansson *et al.*, 2009). The QIs have been proposed to result from the action of myeloperoxidases, an event that is herein tentatively proposed to involve the M2 metabolite, although a direct action of these enzymes in both AQ and DEAQ cannot be disregarded.

In vitro electrochemical approaches have generated a number of putative compounds, the most relevant being indicated here (C8, C9 and C10). This complex of compounds, due to its expected hydroxylated structures has been proposed to most likely represent the previously reported 2-hydroxy-DEAQ (Churchill *et al.*, 1985).

6. The pharmacogenetics of AQ metabolism

As described, based on current knowledge, three main drug metabolizing enzymes are involved in the biotransformation of AQ: CYP2C8 (the main one), CYP1A1 and CYP1B1. All of them harbour significant genetic polymorphisms, some with marked effects in the catalytic activities of the respectively coded proteins.

6.1 CYP2C8

The CYP2C8 comprises nine exons. 14 alleles have been annotated in the gene coding for this 490 amino acid enzyme (<http://www.cypalleles.ki.se/cyp2c8.htm>), most of them rare. Apart from the most prevalent alleles, 2C8*1 (wild type), 2C8*2, 2C8*3, and 2C8*4, the majority of the remaining ones (2C8*5 - 2C8*12, see figure 2) have been identified only in the Japanese population (Soyama *et al.*, 2001)(Nakajima *et al.*, 2003). Few works have evaluated the presence of these minor alleles in other regions (Cavaco *et al.*, 2006)(Suarez-Kurtz *et al.*, 2010), although the output of large consortium projects (e.g. HapMap, www.sanger.ac.uk/resources/downloads/human/hapmap3.html) supports the view of these alleles being specific among the Japanese. On the other hand, 2C8*2, 2C8*3 and 2C8*4 are rare in this population. (Nakajima *et al.*, 2003)(table 1).

African settings represent the major context where AQ therapy is practiced, both in combination with artesunate or sulfadoxine-pyrimethamine. The first CYP2C8 pharmacogenetic study in endemic African populations was performed in East African populations, in the islands of Zanzibar (Cavaco *et al.*, 2005)(table 1). In this population all the most studied mutant alleles, namely 2C8*2, 2C8*3 and 2C8*4, were detected. Further studies in other regions of the continent have confirmed 2C8*2 as the main allele among the native populations (see table 1). Interestingly, outside Zanzibar, 2C8*3 (when found) has been documented at lower frequencies, while 2C8*4 has not been detected at all. These results point to the populations of Zanzibar as somewhat particular, perhaps due to the historical/migration influences from the Arabian Peninsula (Low and Smith, 1976).

The data from native African populations was consistent with previous reports in African-American, where the 2C8*2 allele represents the main mutant allele in Black populations (table 1), with the non-active 2C8*3 mostly present among Caucasians (Cavaco *et al.*, 2006).

A number of *in vitro* studies have been performed to characterize the phenotypic effect of the CYP2C8 polymorphisms.

Region (n*)	2C8*2	2C8*3	2C8*4	Reference
Zanzibar (Unguja and Pemba)(n= 165)	13.9%	2.1%	0.6%	Cavaco <i>et al.</i> , 2005
Ghana (Accra)(n= 204)	17.9%	0%	0%	Kudzi <i>et al.</i> , 2009
Ghana (n= 92)	17.9%	n/ta	n/ta	Adjei <i>et al.</i> , 2008
Ghana (Tamale) (n= 200)	16.8%	0.0%	0.0%	Rower <i>et al.</i> , 2005
Burkina Faso (Bobo-Dioulasso) (n=275)	11.5%	0.4%	n/t	Parikh <i>et al.</i> , 2007
Southern India (n=245)	0.8%	2.9%	n/t	Arun Kumar <i>et al.</i> , 2011
Malaysia ^b (n= 57)	0.8%	1.2%	0%	Muthiah <i>et al.</i> , 2005
South East Asia (n= 20) ^c	0.0%	5.0%	0%	Solus <i>et al.</i> , 2004
Papua-New Guinea (Madang) (n = 305)	0%	0%	0%	Hombhanje <i>et al.</i> , 2005
Brazil (scattered regions)(n= 1034) ^d	6.4%	8.6%	3.4%	Suarez-Kurtz <i>et al.</i> , 2010

*number of subjects analysed; n/t (non tested)

^a Any 2C8*3 or 2C8*4 alleles present confounded in the 2C8*1 group.

^b Indian ethnic group

^c Derived from a commercial repository – no specific origin disclosed.

^d Ethnically mixed populations, originated from several scattered regions of the country. The sample is claimed by the authors to be representative of the present day Brazilian population; when considering self-reporting ethnic origin, the 2C8*3 frequency increased among “White” subjects, while the *2 among “Black” individuals.

(note: this compilation is intended to be a representative sample of the published information and not an exhaustive collection of the available data)

Abbreviation – CYP2C8: 2C8

Table 1. CYP2C8 main allele frequencies in populations from malaria endemic regions (Africa, Asia, Oceania and South America).

The CYP2C8*2 allele is characterized by the presence of an I269F SNP. Quantitative HPLC/UV analysis of the DEAQ resulting from *in vitro* incubations of recombinant CYP2C8*2 enzyme with AQ pointed to a significant decrease of approximately 30% in the V_{max} , (maximum substrate transformation rate) as compared with the wild-type (2C8*1) reference ($0.16 \pm 0.06 \mu\text{mol}/\text{min}/\mu\text{mol}$ P450 *vs* $0.23 \pm 0.09 \mu\text{mol}/\text{min}/\mu\text{mol}$ P450). In parallel, the mutant allele was associated with a three-fold increase in its K_m (substrate concentration at which the reaction reaches half of the V_{max} value) ($2.55 \pm 1.06 \mu\text{M}$ *vs* $0.81 \pm 0.23 \mu\text{M}$) (Parikh *et al.*, 2007). This decreased performance was reflected in a significantly lower intrinsic clearance (V_{max}/K_m) of AQ (2C8*2: $0.05 \text{ l}/\text{min}/\mu\text{mol}$ P450 *vs* 2C8*1: $0.30 \text{ l}/\text{min}/\mu\text{mol}$ P450). As for the CYP2C8*3, the presence of the two linked mutations characterizing this protein (R139K and R399K) had a marked effect on its catalytic capacities, with no AQ metabolism detected at any of the tested substrate concentrations (Parikh *et al.*, 2007). It was concluded that the 2C8*3 protein has very low AQ metabolism activity. These results obtained with AQ are supported by data from previous studies using the CYP2C8 probe drug paclitaxel (Dai *et al.*, 2001).

As for 2C8*4, *in vitro* experiments by Singh *et al.* have reported this protein as having a 10 fold decrease in paclitaxel 6- α hydroxylase activity. The associated SNP (I264M, see figure 2) was proposed to affect heme insertion and the correct folding of the protein (Singh *et al.*, 2008). It is expectable that this also represents a low activity allele as for the biotransformation of amodiaquine.

More recently, Gao and collaborators performed tests based on the heterologous expression of 2C8*2, 2C8*3 and 2C8*4 in *Saccharomyces cerevisiae*. Microsomes prepared from the transfected yeast cells were incubated with different concentrations of AQ. The experiments showed a decrease in the overall activity of the mutant alleles towards ~45-75% of the wild type values (Gao *et al.*, 2010). The reasons behind the observed large differences in the capacities of these alleles between the microsome based systems and the *in vitro* based ones (i.e. reconstituted protein) are not fully clear.

Some of the remaining minor alleles have been studied in some detail in Japan. The 2C8*5 allele carries a 475delA mutation causing a frameshift effect leading to a premature translation termination at residue 177, and hence no functional protein (Soyama *et al.*, 2002). Using microsomes obtained from CYP2C8 transfected COS-1 cells, the 2C8*6, 2C8*9 and 2C8*10 proteins were shown not to significantly differ from the wild type (2C8*1) on paclitaxel 6- α hydroxylase activity. As for 2C8*8, this allele showed more than ten fold decrease in catalytic activity, associated with a less stable protein. As expected, 2C8*7 - involving the introduction of a translational stop codon - had no detectable enzyme activity (Hichiya *et al.*, 2005). Finally, Hanioka and collaborators have recently used the same yeast system for testing the 2C8*13 and 2C8*14 alleles. While 2C8*13 did not show significant differences in V_{max} and K_m for paclitaxel 6- α hydroxylation as compared with 2C8*1, 2C8*14 showed a 3 fold higher K_m , albeit with no changes in the V_{max} .

A summary of relevant CYP2C8 SNPs and the associated phenotype characteristics is presented in figure 2.

In vivo, there is limited information on the impact of CYP2C8 polymorphisms in AQ therapy, both in terms of efficacy and risk of adverse events.

Parikh and collaborators have performed a retrospective analysis of an AQ monotherapy efficacy trial searching for *in vivo* phenotype/2C8 genotype associations (Parikh *et al.*, 2007). The study involved 275 uncomplicated malaria patients from the region of Bobo-Dioulasso, southwest Burkina Faso. No associations were found between the clinical success of AQ therapy and the presence of minor CYP2C8 alleles (2C8*2 and 2C8*3). This is probably due to the fact that DEAQ, the main AQ metabolite, also represents a fully active antimalarial entity, i.e. enhanced AQ metabolism does not lead to an inactive metabolite that would decrease the clinical (pharmacodynamic) success of the therapy. More importantly, Parikh *et al* found a positive correlation between harbouring a 2C8*2 allele and events of mild side effects, mainly abdominal pain (self reported): 52% of occurrences among the 2C8*2 carriers *versus* 30% for the 2C8*1/*1 subjects. This is until now the only reported association between the patient CYP2C8 status and effects of AQ therapy. Another trial, performed in Ghana did not report such an association (Adjei *et al.*, 2008). It also did not find clear associations between the pharmacokinetic parameters of AQ and the presence of less active alleles, although a trend for decreased DEAQ plasma concentrations among the 2C8*2 carriers was noted. It must be noted however, that the smaller size of this study (n=92) might have prevented the detection of such associations.

These are the only two published studies exploring the impact of the *CYP2C8* polymorphism in AQ based therapies, while near 100 million treatments performed in the last five years (WHO, 2010). It is evident that there is an urgent need of further research in this area, in order to guarantee the longest "useful life" for this drug, presently a cornerstone in the global control of malaria.

6.2 CYP1A1

CYP1A1 represents a 512 amino acid cytochrome P450 enzyme mainly expressed in extra-hepatic tissues, including the lungs (Willey *et al.*, 1997), intestine (Paine *et al.*, 1999)(Paine *et al.*, 2006), placenta (Hakkola *et al.*, 1996) and, importantly, lymphocytes (Dey *et al.*, 2001, van Duursen *et al.*, 2005). It is usually present at low constitutive baseline levels. The gene is readily inducible through the AHR (Aryl Hydrocarbon Receptor) regulatory pathway, typically by exposure to PAHs (polycyclic aromatic hydrocarbons, e.g. components of tobacco smoke). Upon induction, CYP1A1 can also be found in the liver, where it usually is at very low (pre-induction) baseline levels. CYP1A1 is able to metabolize a number of clinically relevant drugs, including the calcium blocker flunarizine, the anticancer drug toremifene and the cardiovascular disease drug fluvastatin. Significant inter-individual variability in the elimination of these drugs has been described. This variability is probably of minor clinical concern, due to the non-hepatic patterns of expression of the gene. In the context of this review, the importance of CYP1A1 is mainly focused in its probable involvement in the generation of short lived but toxic AQ metabolites (Johansson *et al.*, 2009).

The *CYP1A1* gene (15q24.1) is organized in 7 exons. It harbours significant polymorphisms, comprising eleven established alleles (figure 2), as well as a number of SNPs with haplotype associations still to be assigned (<http://www.cypalleles.ki.se/cyp1a1.htm>). A significant portion of the non-synonymous polymorphisms is concentrated at exon 7 (figure 2)(table 2).

The *CYP1A1*2* allele has been documented *in vitro* to represent a more drug responsive gene upon exposure to the prototype inducer 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD)(studies with the *1A1*2A* and *1A1*2B* haplotypes)(Spurr *et al.*, 1987)(Landi *et al.*, 1994). Also, at least in the case of *1A1*2B* and *1A1*2C*, this allele was shown to code for more active enzymes (Cosma *et al.*, 1993). The increased transcriptional response is related to the presence of a T3801C transition in the gene's 3' UTR, while the referred enhanced enzymatic activity (reflected specifically in an increased V_{max}) is linked with a non-synonymous I462V SNP at exon 7 (Cosma *et al.*, 1993). This enhanced activity of the proteins harbouring 462V (*1A1*2B* and *1A1*2C*) was also confirmed in terms of estrone and 17 β -estradiol 2-hydroxylation, as a 5-10 fold increase in comparison to the wild type (Kisselev *et al.*, 2005).

The T461N SNP (defining *1A1*4*) was shown not to alter significantly the behaviour of its coded enzyme (Kisselev *et al.*, 2005). As for the 3' UTR T3204C transition defining the *1A1*3* allele (proposed to be specific of African populations) it does not seem to influence the levels of *CYP1A1* expression (Smart and Daly, 2000). *1A1*7* represents a frameshift mutation leading to the generation of a stop codon and hence precluding the production of an active enzyme. Finally, alleles *1A1*8* (T448N), *1A1*9* (R464C) and *1A1*10* (R477W) carry non-synonymous SNPs located close to the protein heme binding protein, and are hence expected to affect the activity of the enzyme (Saito *et al.*, 2003).

Region (n*)	1A1*2	1A1*7	1A1*3	1A1*4	Reference
Namibia ^a (n= 134)	14.9% ^b	n/t	n/t	n/t	Fujihara <i>et al.</i> , 2009
Southwest Libya (El Awaynat and Tahala) ^c (n=129)	6.5% ^d	n/t	0%	18.1%	Martinez-Labarga <i>et al.</i> , 2007
Midwest and Southeast Nigeria (n= 250)	24.2% ^e	n/t	13%	0%	Okobia <i>et al.</i> , 2005
South Africa ^f (n= 96)	0% ^g	n/t	n/t	n/t	Dandara <i>et al.</i> , 2002
Zimbabwe (n= 148)	0% ^g	n/t	n/t	n/t	Dandara <i>et al.</i> , 2002
Tanzania (n= 114)	1.3% ^g	n/t	n/t	n/t	Dandara <i>et al.</i> , 2002
Mali (n=116)	24.0% ^g	n/t	n/t	n/t	Garte <i>et al.</i> , 1998
East India (Chennai) (n=150)	33% ^g	n/t	n/t	n/t	Suneetha <i>et al.</i> , 2011
Southwest India (Kerala) (n = 146)	20.9%	n/t	n/t	n/t	Sreeja <i>et al.</i> , 2005
Northern India (Delhi) (n= 309)	71.4% ^h	n/t	n/t	0%	Kumar <i>et al.</i> , 2010
Northern Thailand (Lampang)(n= 287)	84.9% ^{h,i}	n/t	n/t	n/t	Pisani <i>et al.</i> , 2006
China (Peking)(n= 284) ⁱ	28.2% ^h	n/t	n/t	0%	Zhang <i>et al.</i> , 2010

* number of subjects analyzed; n/t: not tested

^a Bantu (Ocambo) ethnicity

^b T3798C tested, defining the presence of 1A1*2A or 1A1*2B. As the I462V was not determined (1A1*2C contribution not included) it is not possible to distinguish between these two forms.

^c Although these are regions not directly affected by malaria, the nomadic characteristics of the Tuareg populations under study puts them in risk when travelling to sub-Saharan areas (e.g. Northern Mali, Mauritania)

^d 1A1*2A= 4.5%, 1A1*2B= 0.2%, 1A1*2C=1.8%

^e 1A1*2A= 24%, 1A1*2C= 0.2%. Data on 1A1*2B not available.

^f Venda ethnicity

^g Only T3798C tested, so the data should be considered as the result of the 1A1*2A + 1A1*2B composite.

^h T3798C and I462V tested but no information on the composite of the two giving rise to *2B

ⁱ 286 subjects analysed for 1A1*2A

^j Only the I462V analysed so 1A1*2A not included and 1A1*2B contribution not available

(note: this compilation is intended to be a representative sample of the published information and not an exhaustive collection of the available data).

Abbreviation - CYP1A1: 1A1

Table 2. CYP1A1 allele frequencies of population from malaria affected regions of Africa and Asia.

6.3 CYP1B1

Similarly to CYP1A1, the expression of the CYP1B1 isoform is also predominantly extra-hepatic, including peripheral blood cells (Hanaoka *et al.*, 2002)(Furukawa *et al.*, 2004), and it is inducible by exposure to several xenobiotics, namely PAHs (Nebert *et al.*, 2004).

Null alleles are rare in the general population. Most were identified among patients with glaucoma, for which these alleles are an established risk factor (Stoilov *et al.*, 1998), and are not a major concern in the context of this review. The main polymorphic positions in this gene are summarized in figure 2.

The functional consequence of the major *CYP1B1* alleles has been studied *in vitro* upon heterologous expression in *E. coli* and the quantification of the enzyme's capacity of catalyzing the 4- and 2- hydroxylation of estradiol. The introduction of the R48G, A119S and N453S SNPs did not significantly change the V_{max} and K_m of the resulting proteins, as compared with the wild type (1B1*1) (Li *et al.*, 2000). The L432V SNP was on the other hand associated increased of *circa* 3 fold in the K_m . Later works using *Saccharomyces cerevisiae* heterologous expression systems supported the view of L432V carrier proteins as less active ($>K_m$ values $<V_{max}$ values). Interestingly, in these works this effect was only observed in the context of a SNP haplotype including other polymorphic positions (R48G + A119S + L432V)(Aklillu *et al.*, 2002). Finally, several minor allele of this SNP (present in the 1B1*3, 1B1*5, 1B1*6 and 1B1*7 alleles) have been proposed as associated with a transcriptionally less active gene (Helmig *et al.*, 2009, 2010).

Presently there is still scarce information available concerning the *CYP1B1* allele frequencies in populations living in malaria regions (table 3).

Region (n*)	1B1*2	1B1*3	1B1*4	1B1*5	1B1*6	1B1*7	Reference
Ethiopia (Adis Ababa) (n=150)	36.7%	39.0%	2.0%	0.7%	6.3%	7.0%	Aklillu <i>et al.</i> , 2002
North India (Lucknow) (n=200)	33.0%	21.3%	18.3%	n/t	n/t	n/t	Shah <i>et al.</i> , 2008
South Central Chin (Chengdu) (N= 400 ^a)	n/t	57.5% ^b	n/t	n/t	n/t	n/t	Wang <i>et al.</i> , 2011
Northeast China (Nanjing) (N= 227)	n/t	15.1% ^c	n/t	n/t	n/t	n/t	Liang <i>et al.</i> , 2005

* number of subjects analyzed; n/ t: not tested.

^a Only women were included in the study.

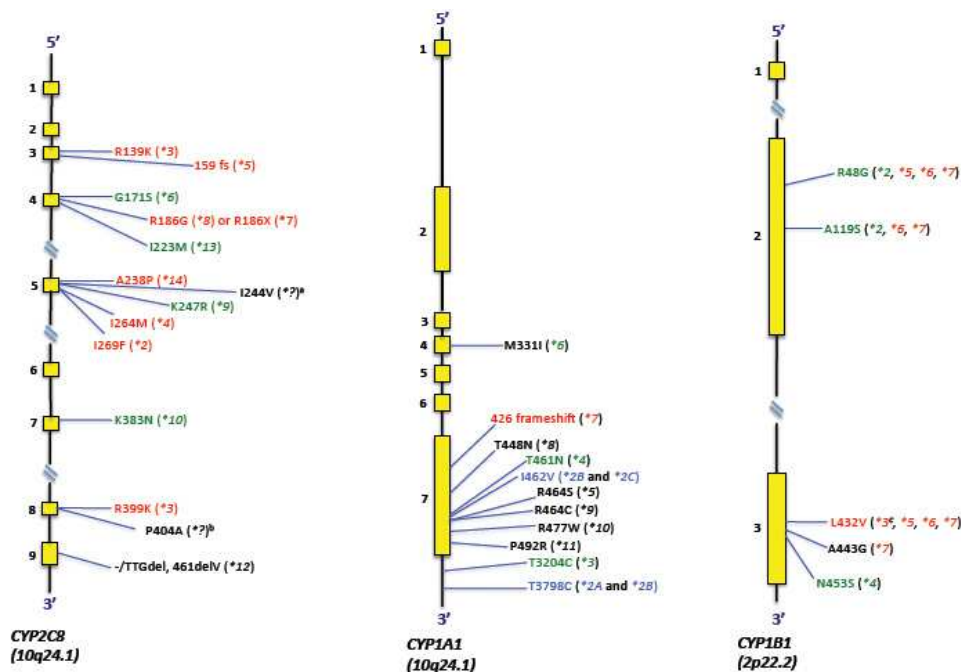
^b Only the L432V SNP was analysed, meaning that the declared frequency for 1B1*3, also is likely to include also the 1B1*5, 1B1*6 and 1B1*7 alleles.

^c Only women included in the study. The sole analysis of the A119S does not distinguish between the 1B1*2, 1B1*6 and 1B1*7.

(note: this compilation is intended to be a representative sample of the published information and not an exhaustive collection of the available data).

Abbreviation - *CYP1B1*: 1B1

Table 3. *CYP1B1* allele frequencies of population from malaria affected regions in Africa and Asia.



^a Haplotype not determined yet (reference SNP: rs11572102, NCBI (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=11572102);

^b Discovered by Soyama *et al.*, 2001 and phenotypically characterized by reduced catalytic activity (Soyama *et al.*, 2002)(Jiang *et al.*, 2011).

^c The colour code was herein applied according to the data of Aklillu *et al.* (2002). It is to note that initial studies by Li *et al* (2000) noticed a significant decrease in the activity of the protein.

Fig. 2. Genetic diversity of *CYP2C8*, *CYP1A1* and *CYP1B1*, the main polymorphic genes involved in AQ phase I metabolism. Although the gene structures are represented, the locations of established polymorphisms are presented using the amino acid nomenclature as this is more relevant for the present review. For the sake of clarity, only the most studied alleles, with established haplotype structures are presented. Alleles shown in red color indicate protein products reported to be less active than the reference wild type (*1). Alleles in green do not have a significant differences as compared with *1, whereas alleles flagged in blue have higher activity than the wild type. For alleles in black there is currently no experimental information available. In the case of the *CYP1B1* the use of the colors is restricted to alleles (and not SNPs) due to the availability of haplotype data (Akillilu *et al.*, 2002).

7. The CYP2C8, CYP1A1, CYP1B1 trio - Potential implications

In terms of therapeutic efficacy, since DEAQ is a powerful and clinically valuable antimalarial, variations in the *CYP2C8* enzymatic capacity are not expected to have significant pharmacodynamic consequences. As mentioned, this expectation is in line with observations by Parikh *et al* in Burkina-Faso, where the presence of *CYP2C8**2 did not influence the treatment outcome of AQ monotherapy. On the other hand, pharmacogenetics might be of particular importance in the identification of individuals in higher risk of

developing AQ-related adverse events. This is supported by the observation in the same study that the presence of *CYP2C8*2* (still an allele associated with significant catalytic capacity, as compared with the much less functional *2C8*3* and *2C8*4*) can influence the individual risk for mild adverse events, *even* in heterozygous form could.

Recently *in vitro* evidence was published supporting the involvement of CYP1A1 and CYP1B1 in the generation of toxic QIs from AQ (and DEAQ) (Johansson *et al.*, 2009). This confirmation of preliminarily reports (Li *et al.*, 2002, I. Cavaco, Universidade do Algarve, unpublished) has opened a new perspective towards individualization of AQ therapy to minimize adverse events. In fact, contrarily to the previous view of a spontaneous formation of QIs (Tingle *et al.*, 1995), the involvement of these polymorphic P450s reinforces a broader genetic basis for the phenomenon, which was until now almost exclusively focused on *CYP2C8* (Cavaco *et al.*, 2005). The now recognized more extensive genetic background of AQ adverse drug events underlines the importance of individualized medicine and the possibility to identify appropriate molecular markers for predicting response to treatment/risk of adverse reactions.

With this new data, the hypothetical characterization of the sub-group of subjects in higher risk is starting to emerge. The first assumption is that such individuals carry a deficient *CYP2C8* gene (*2C8*2*, ideally the very low active *2C8*3* or *2C8*4*), allowing an extended half-life for AQ (Parikh *et al.*, 2007). This will probably represent the group with $T_{1/2}$ values >12 hours (Giao and de Vries, 2001), as well as the observed outliers showing very long exposure to the drug (Winstanley *et al.*, 1987). With this increased pool of circulating AQ (as previously referred, a compound more prone than DEAQ for the formation of the QI reactive species (Tingle *et al.*, 1995)), the drug will have higher chances to be extra-hepatically catalysed by CYP1A1 and CYP1B1 - both present in leucocytes, where AQ tends to accumulate (Naisbitt *et al.*, 1997). The rate of QI formation is expected to be further enhanced if particularly efficient versions of CYP1A1 and CYP1B1 are present, namely the *CYP1A1*2*, and *CYP1B1*1* (wild type) enzymes. The resulting enhanced generation of QIs in the blood, coupled with the referred low *CYP2C8* activity could be the basis of the previously documented AQ induced severe agranulocytosis.

Interestingly, the involvement of CYP1A1 and CYP1B1 has another implication: In adult patients whom are smokers, both genes are likely to be induced by the polycyclic aromatic hydrocarbons (PAHs), which is present in tobacco smoke, through the activation of the aryl hydrocarbon receptor (AhR) based pathway (Nebert *et al.*, 2004). This will also lead to the expression of these genes in the liver, another location for fatal AQ induced toxicity. Adult patients are characteristic of areas of low transmission, where the low exposure during the earlier periods of life does not allow the development of natural immunity to the diseases as an adult (premunity, frequent in the African continent (Struik and Riley, 2004)). Such malaria settings are the norm in South America regions, where populations carry a significant Caucasian genetic background and hence, an expected high frequency of the *CYP2C8*3* allele. This has been confirmed in a pilot screening study conducted in populations of Northern Colombia where the frequency of this allele was *ca.* 7% (I. Cavaco, Universidade do Algarve, unpublished). In such areas it would be worthwhile to conduct trials in order to understand the influence that tobacco habits might have in the incidence of mild and serious adverse events associated to AQ treatments. Its connection with the *CYP2C8/CYP1A1/CYP1B1* polymorphic set would be of interest, e.g. identifying patients that should be advised to decrease their smoking rate in time periods relevant for treatment.

Currently the epicentre of AQ use is in the Africa where the 2C8*3 and 2C8*4 alleles seem to be rare in the native populations. However, this observation is based on a very limited number of small studies, surely not representative of the overall population of the continent, the genetically most diverse on the planet (Lambert and Tishkoff, 2009). In addition, even though these alleles are considered “rare”, one has to take into account the dimensions of the malaria control challenge. A frequency of 1% for the 2C8*3 allele in a universe of at least 20 million AS-AQ treatments performed per year (WHO, 2010 - just the public sector, and not counting with the SP-AQ combination), would translate to ~2,000 homozygotes for 2C8*3 patients per year. Secondly, AQ resistance, although still not globally prevalent, is slowly increasing (Holmgren *et al.*, 2006). Its potential expansion might lead to the need for increased drug dosage, as previously decided in the cases of CQ (Peters, 1970)(Ursing *et al.*, 2009, 2011) and mefloquine (Carrara *et al.*, 2009). By approaching the top of the AQ therapeutic window, the risks of toxicity will increase to a point when serious side effects might be “under the reach” of the less compromised 2C8*2 allele.

8. The CYP2C8, CYP1A1, CYP1B1 trio - Potential applications

Pharmacogenetic markers in AQ therapies are expected to serve mostly as surveillance tools of adverse events of the drug. The identification of individuals with a genetic predisposition to AQ side effects (e.g. a 2C8*2/*2 carrier) would prompt the direct diversion of this patient towards different doses or the available second line treatment (e.g. artemether-lumefantrine or artesunate-mefloquine).

An alternative therapeutic strategy could be the use of a more personalized (i.e. more optimized) AQ dosing, with values below the conventional 10 mg/Kg, or through a different regimen schedule. As the incidence of side effects is dose dependent (Cairns *et al.*, 2010), individualized treatment would be expected to reduce those events. The success of such strategies could boost the patient’s (and specially their guardians) trust on the treatment, leading to increased compliance to the full dosing regimen. This effect has been witnessed with the anti-HIV drug abacavir, where the application of pharmacogenetic testing has increased the use of this drug (Ingelman-Sundberg, 2008)(Chaponda and Pirmohamed, 2011). Unfortunately, clinical studies to support future guidelines concerning the ideal dose for certain pharmacogenetic configurations are clearly missing. Initiatives to address this issue, such as the WANECAM Consortium in East Africa, are presently under way (see: www.edctp.org/annualreport2010/EDCTP_Annual_Report_2010_English.pdf).

The application of pharmacogenetics can contribute to an extended useful life of AQ, through its better use. Chemotherapy represents a central strategy for the long desired global elimination of the disease (malERA Consultative Group on Drugs, 2011), an event fundamental for the social-economical development of the Developing World. AQ is one of its central tools in these efforts (Bhattarai *et al.*, 2007) – its safer and consequently longer use can be pivotal in this process.

But, although promising, are these pharmacogenetic applications possible in the present context of the Developing World health systems? In short, no. The prices of pharmacogenetic testing are still very high, totally eclipsing the costs of the therapy itself. A complete AS-AQ treatment in Africa will cost below US\$5, already taking in

consideration a price of US\$ 0.5-1 for the Coarsucam®/Winthrop® fixed combination supplied by Sanofi-Aventis (Shillcutt *et al.*, 2008). As previously calculated (Ferreira *et al.*, 2008), the analysis of the *CYP2C8*3* allele alone will demand a minimum of US\$50, taking in account the local costs of human resources and reagents. An upgrade towards the determination of the *CYP2C8*2* allele, plus *CYP1B1*2* and *CYP1A1*2*, would increase the financial burden per patients to values well above US\$ 100 per patient, the equivalent of more than one hundred treatments. Besides, the maintenance of a system with the required quality standards for molecular diagnostic analysis would be in the order of many tens of thousands of US\$/year. All this has to be put further in the context of populations with an available national health expenditure of less than US\$25/year/citizen. The pharmacogenetic testing for AQ therapy alone would consume the funds of four years of health care.

So, what is the translational value of pharmacogenetics in such scenario? In fact there are at least two venues of development.

The first is what will be called here as “population pharmacogenetics”. As explained, the application of individual dose adjusted AQ therapies based on pharmacogenetics is essentially not viable. Alternatively, the study of the allele frequencies of pivotal markers in representative samples of specific populations/regions can be of considerable interest for national and regional health programmes, particularly in countries with a rich ethnic diversity, as it is frequent in the African continent. This would define the populations where therapeutics based in AQ would be safe, and the ones where an alternative antimalarial (like artemether-lumefantrine) would be the better choice. In a way, it would be the equivalent of rationally deciding not to introduce AQ in Europe, due to the high prevalence of the *CYP2C8*3* allele (Cavaco *et al.*, 2006). Several malaria endemic countries have more than one first line recommended treatment (e.g. Burkina Faso, Mali, Colombia, Peru (<http://www.who.int/malaria/publications/treatment-policies/en/index.html>), having as such a start up capacity for implementing region-specific policies. Such a programme could be centralized taking advantage of pre-existing molecular technology facilities used e.g. for molecular parasitology. In this context it is worth mentioning that new techniques are surfacing for the extraction of DNA from Rapid Diagnostic Tests (P. Ferreira, Karolinska Institutet, pers. commun)(Alam *et al.*, 2011). These can be further coupled with novel inexpensive amplification-free genotyping approaches (Aw *et al.*, 2011), allowing the ready molecular characterization of the patient in field settings.

Such an application of pharmacogenetics would aid evidence-based decisions for optimized antimalarial use, at a population level. This would allow the use of safer ACT alternatives for the benefit of specific populations.

The second venue has to do with the target country. Malaria is a significant public health concern in three of the “BRIC” emerging economies: Brazil, India and China. These large countries are rapidly developing economic capacity compatible with a first large scale application of personalised anti-infection therapies. In this context, it should be noted that the populations of Brazil and India (incidentally, the ones with larger malaria burden) have been shown to harbour non-negligible *CYP2C8*3* frequencies (Arun Kumar *et al.*, 2011)(Suarez-Kurtz *et al.*, 2010)(table 1). In these countries the resources for personalized medicine as previously described are potentially available.

9. Conclusions

Although tens of millions of AQ doses are prescribed per year, the knowledge of the pharmacogenetics of this drug is still limited. In particular, stronger *in vivo* phenotype/genotype associations are needed for the definition of genetic markers of AQ overexposure risk.

This must be obtained through several venues. A basic need is the performance of clinical trials designed for the detection of adverse events, mild or eventually serious. These are needed to be relatively large (>500 subjects), due to the non-precise nature of the former (e.g. self reported abdominal pain in children under five), and the rarity of the latter. Such studies must include a long follow up for the detection of possible late onset events, as well as the reaction of the subject upon repetitive treatments. Also, the inclusion of full sequencing approaches in such reference studies would be fundamental, especially in cases of particularly relevant phenotypes. As previously mentioned, studies of this type are presently undergoing. Such trials will allow the establishment of better phenotype/genotype associations that can be further explored in the context of health structures integrated in national malaria programmes. In this second step, technologies like the DNA extraction from Rapid Diagnostic Test devices, coupled with novel simple genotyping methods with applications in the field can be used in order to further establish polymorphisms in CYP2C8, CYP1A1, CYP1B1 or other relevant genes (e.g. myeloperoxidases) as risk markers of AQ over-exposure.

With true personalized medicine being presently out of reach in most malaria affected countries, population pharmacogenetics of such markers will supply information for the use of the best available chemotherapy option, at a public health level. An example of such application would be the use of an alternative second line treatment (e.g. artemether-lumefantrine) as first liner for certain regions and/or populations of a country. Such a strategy would optimize the use of the available antimalarial arsenal in the national control programmes. A more personalized analysis could be potentially applied in the much less frequent (< 10%) situations of AS-AQ use as second liner.

A population-based approach, as described, can offer true benefits for the optimization of national malaria treatment, in particular when integrated in malaria elimination efforts, where the maintenance of the useful life of well established and effective therapies is key. Once the incidence of malaria decreases, as for example witnessed in the Zanzibari islands (Bhattarai *et al.*, 2007), countries will be able to start supporting more personalized application of pharmacogenetics. In conclusion, upon the solid establishment of pharmacogenetics markers, the success of their application at a population level can lay the basis for a future more personalized pharmacogenetics, once the countries would be able to finance it. By the same token, the Developing World would not be left behind in this area of translational medicine.

10. Acknowledgments

This work was partially supported by The Karolinska Institutet Fond.

11. References

Adjei GO; Kristensen K; Goka BQ; Hoegberg LC; Alifrangis M; Rodrigues OP & Kurtzhals JA (2008). Effect of concomitant artesunate administration and cytochrome

- P4502C8 polymorphisms on the pharmacokinetics of amodiaquine in Ghanaian children with uncomplicated malaria. *Antimicrob Agents Chemother.*, 52(12):4400-4406.
- Adjuik M, Agnamey P, Babiker A, Borrmann S, Brasseur P, Cisse M, Cobelens F, Diallo S, Faucher JF, Garner P, Gikunda S, Kreamsner PG, Krishna S, Lell B, Loolpapit M, Matsiegui PB, Missinou MA, Mwanza J, Ntoumi F, Olliaro P, Osimbo P, Rezbach P, Some E, Taylor WR (2002). Amodiaquine-artesunate versus amodiaquine for uncomplicated *Plasmodium falciparum* malaria in African children: a randomised, multicentre trial. *Lancet.*, 359(9315):1365-1372.
- Aklillu E; Oscarson M; Hidestrand M; Leidvik B; Otter C; & Ingelman-Sundberg M. Functional analysis of six different polymorphic CYP1B1 enzyme variants found in an Ethiopian population (2002). *Mol Pharmacol.*, 61(3):586-594.
- Alam MS; Mohon AN; Mustafa S; Khan WA; Islam N; Karim MJ; Khanum H; Sullivan DJ Jr & Haque R. (2011). Real-time PCR assay and rapid diagnostic tests for the diagnosis of clinically suspected malaria patients in Bangladesh. *Malar J.*, 10:175.
- Arun Kumar AS; Chakradhara Rao US; Umamaheswaran G; Ramu P; Kesavan R; Shewade DG; Balachandar J & Adithan C (2011). Haplotype structures of common variants of CYP2C8; CYP2C9; and ADRB1 genes in a South Indian population. *Genet Test Mol Biomarkers.*, 15(6):407-413.
- Aw W; Lezhava A; Hayashizaki Y & Ishikawa T (2011). A new trend in personalized medicine: rapid detection of SNPs in drug transporter genes by the SmartAmp method. *Clin Pharmacol Ther.*, 89(4):617-620.
- Bhattarai A, Ali AS, Kachur SP, Mårtensson A, Abbas AK, Khatib R, Al-Mafazy AW, Ramsan M, Rotllant G, Gerstenmaier JF, Molteni F, Abdulla S, Montgomery SM, Kaneko A, Björkman A. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar (2007). *PLoS Med.*, 4(11):e309.
- Bojang K, Akor F, Bittaye O, Conway D, Bottomley C, Milligan P, Greenwood B (2010). A randomised trial to compare the safety, tolerability and efficacy of three drug combinations for intermittent preventive treatment in children. *PLoS One.*, 5(6):e11225.
- Brasseur P, Guiguemde R, Diallo S, Guiyedi V, Kombila M, Ringwald P, Olliaro P (1999). Amodiaquine remains effective for treating uncomplicated malaria in west and central Africa. *Trans R Soc Trop Med Hyg.*, 93(6):645-650.
- Cairns M, Cisse B, Sokhna C, Cames C, Simondon K, Ba EH, Trape JF, Gaye O, Greenwood BM, Milligan PJ (2010). Amodiaquine dosage and tolerability for intermittent preventive treatment to prevent malaria in children. *Antimicrob Agents Chemother.*, 54(3):1265-1274.
- Carrara VI; Zwang J; Ashley EA; Price RN; Stepniewska K; Barends M; Brockman A; Anderson T; McGready R; Phaiphun L; Proux S; van Vugt M; Hutagalung R; Lwin KM; Phyo AP; Preechapornkul P; Imwong M; Pukrittayakamee S; Singhasivanon P; White NJ & Nosten F (2009). Changes in the treatment responses to artesunate-mefloquine on the northwestern border of Thailand during 13 years of continuous deployment. *PLoS One.*, 4(2):e4551.

- Cavaco I; Strömberg-Nörklit J; Kaneko A; Msellem MI; Dahoma M; Ribeiro VL; Bjorkman A & Gil JP (2005). CYP2C8 polymorphism frequencies among malaria patients in Zanzibar. *Eur J Clin Pharmacol.*, 61(1):15-18.
- Cavaco I; Piedade R; Gil JP & Ribeiro V (2006). CYP2C8 polymorphism among the Portuguese. *Clin Chem Lab Med.*, 44(2):168-170.
- Chaponda M & Pirmohamed M (2011). Hypersensitivity reactions to HIV therapy. *Br J Clin Pharmacol.*, 71(5):659-671.
- Churchill FC; Patchen LC; Campbell CC; Schwartz IK; Nguyen-Dinh P & Dickinson CM (1985). Amodiaquine as a prodrug: importance of metabolite(s) in the antimalarial effect of amodiaquine in humans. *Life Sci.*, 36(1):53-62.
- Clarke JB; Maggs JL; Kitteringham NR & Park BK (1990). Immunogenicity of amodiaquine in the rat. *Int Arch Allergy Appl Immunol.*, 91(4):335-342.
- Coatney GR (1963). Pitfalls in a discovery: the chronicle of chloroquine. *Am J Trop Med Hyg.*, 12:121-128.
- Cosma G; Crofts F; Taioli E; Toniolo P & Garte S (1993). Relationship between genotype and function of the human CYP1A1 gene. *J Toxicol Environ Health.*, 40(2-3):309-316.
- Cox-Singh J; Davis TM; Lee KS; Shamsul SS; Matusop A; Ratnam S; Rahman HA; Conway DJ & Singh B (2008). Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. *Clin Infect Dis.*, 46(2):165-171.
- Dai D; Zeldin DC; Blaisdell JA; Chanas B; Coulter SJ; Ghanayem BI & Goldstein JA (2001). Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics.*, 11(7):597-607.
- Dandara C; Sayi J; Masimirembwa CM; Magimba A; Kaaya S; De Sommers K; Snyman JR & Hasler JA (2002). Genetic polymorphism of cytochrome P450 1A1 (Cyp1A1) and glutathione transferases (M1; T1 and P1) among Africans. *Clin Chem Lab Med.*, 40(9):952-957.
- Dey A; Parmar D; Dayal M; Dhawan A; & Seth PK (2001). Cytochrome P450 1A1 (CYP1A1) in blood lymphocytes evidence for catalytic activity and mRNA expression. *Life Sci.*, 69(4):383-393.
- Eastman RT & Fidock DA (2009). Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. *Nat Rev Microbiol.*, 7(12):864-874.
- Echeverry DF; Holmgren G; Murillo C; Higuera JC; Björkman A; Gil JP & Osorio L (2007). Short report: polymorphisms in the pfcrt and pfmdr1 genes of Plasmodium falciparum and *in vitro* susceptibility to amodiaquine and desethylamodiaquine. *Am J Trop Med Hyg.*, 77(6):1034-1038.
- Ferreira PE; Cavaco I & Gil JP (2008). Pharmacogenetic tools for malaria and TB in the Developing World. *Personalized Med.*, 5(6): 627-639.
- Fujihara J; Yasuda T; Iida R; Takatsuka H; Fujii Y & Takeshita H (2009). Cytochrome P450 1A1; glutathione S-transferases M1 and T1 polymorphisms in Ovambos and Mongolians. *Leg Med (Tokyo).*, Suppl 1:S408-410.
- Furukawa M; Nishimura M; Ogino D; Chiba R; Ikai I; Ueda N; Naito S; Kuribayashi S; Moustafa MA; Uchida T; Sawada H; Kamataki T; Funae Y & Fukumoto M. (2004) Cytochrome p450 gene expression levels in peripheral blood mononuclear cells in comparison with the liver. *Cancer Sci.*, 95(6): 520-529.

- Gao Y; Liu D; Wang H; Zhu J & Chen C (2010). Functional characterization of five CYP2C8 variants and prediction of CYP2C8 genotype-dependent effects on *in vitro* and *in vivo* drug-drug interactions. *Xenobiotica.*, 40(7):467-475.
- Garte S (1998). The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1. *Carcinogenesis.*, 19(8):1329-1332.
- Gautam A; Ahmed T; Batra V & Paliwal J (2009). Pharmacokinetics and pharmacodynamics of endoperoxide antimalarials. *Curr Drug Metab.*, 10(3):289-306.
- Gerstl S, Dunkley S, Mukhtar A, Baker S, Maikere J (2010). Successful introduction of artesunate combination therapy is not enough to fight malaria: results from an adherence study in Sierra Leone. *Trans R Soc Trop Med Hyg.*, 104(5):328-35.
- Gerstner U; Prajakwong S; Wiedermann G; Sirichaisinthop J; Wernsdorfer G & Wernsdorfer WH (2003). Comparison of the in-vitro activity of amodiaquine and its main metabolite; monodesethyl-amodiaquine; in *Plasmodium falciparum*. *Wien Klin Wochenschr.*, 115 (Suppl) 3:33-38.
- Giao PT & de Vries PJ (2001). Pharmacokinetic interactions of antimalarial agents. *Clin Pharmacokinet.*, 40(5):343-373.
- Gil JP; & Gil Berglund E (2007). CYP2C8 and antimalaria drug efficacy. *Pharmacogenomics*, 8(2):187-198.
- Hakkola J; Pasanen M; Hukkanen J; Pelkonen O; Mäenpää J; Edwards RJ; Boobis AR & Raunio H. Expression of xenobiotic-metabolizing cytochrome P450 forms in human full-term placenta. *Biochem Pharmacol.*, 51(4):403-411.
- Hanaoka T; Yamano Y; Pan G; Hara K; Ichiba M; Zhang J; Zhang S; Liu T; Li L; Takahashi K; Kagawa J; & Tsugane S (2002). Cytochrome P450 1B1 mRNA levels in peripheral blood cells and exposure to polycyclic aromatic hydrocarbons in Chinese coke oven workers. *Sci Total Environ.*, 296(1-3):27-33.
- Harrison AC, Kitteringham NR, Clarke JB, Park BK (1992). The mechanism of bioactivation and antigen formation of amodiaquine in the rat. *Biochem Pharmacol.*, 43(7):1421-1430.
- Hatton CS; Peto TE; Bunch C; Pasvol G; Russell SJ; Singer CR; Edwards G & Winstanley P (1986). Frequency of severe neutropenia associated with amodiaquine prophylaxis against malaria. *Lancet*, 1(8478):411-414.
- Helmig S; Hadzaad B; Döhrel J & Schneider J (2009). Influence of the Cyp1B1 L432V gene polymorphism and exposure to tobacco smoke on Cyp1B1 mRNA expression in human leukocytes. *Drug Metab Dispos*; 37(7):1490-1495.
- Helmig S; Seelinger JU; Philipp-Gehlhaar M; Döhrel J & Schneider J (2010). Cyp1B1 mRNA expression in correlation to cotinine levels with respect to the Cyp1B1 L432V gene polymorphism. *Eur J Epidemiol.*; 25(12):867-873.
- Hichiya H; Tanaka-Kagawa T; Soyama A; Jinno H; Koyano S; Katori N; Matsushima E; Uchiyama S; Tokunaga H; Kimura H; Minami N; Katoh M; Sugai K; Goto Y; Tamura T; Yamamoto N; Ohe Y; Kunitoh H; Nokihara H; Yoshida T; Minami H; Saijo N; Ando M; Ozawa S; Saito Y & Sawada J (2005). Functional characterization of five novel CYP2C8 variants; G171S; R186X; R186G; K247R; and K383N; found in a Japanese population. *Drug Metab Dispos.*, 33(5):630-636.

- Hietala SF; Bhattarai A; Msellem M; Röshammar D; Ali AS; Strömberg J; Hombhanje FW; Kaneko A; Björkman A & Ashton M (2007). Population pharmacokinetics of amodiaquine and desethylamodiaquine in pediatric patients with uncomplicated falciparum malaria. *J Pharmacokinet Pharmacodyn.*, 34(5):669-686.
- Holmgren G; Gil JP; Ferreira PM; Veiga MI; Obonyo CO & Björkman A (2006). Amodiaquine resistant Plasmodium falciparum malaria *in vivo* is associated with selection of pfprt 76T and pfmdr1 86Y. *Infect Genet Evol.*, 6(4):309-314.
- Hombhanje FW; Hwaihwanje I; Tsukahara T; Saruwatari J; Nakagawa M; Osawa H; Panu MM; Takahashi N; Lum JK; Aumora B; Masta A; Sapuri M; Kobayakawa T; Kaneko A & Ishizaki T (2005). The disposition of oral amodiaquine in Papua New Guinean children with falciparum malaria. *Br J Clin Pharmacol.*, 59(3):298-301 (2005).
- Hsu E (2009). Diverse biologies and experiential continuities: did the ancient Chinese know that qinghao had anti-malarial properties? *Can Bull Med Hist.*, 26(1):203-213.
- Ingelman-Sundberg M (2008). Pharmacogenomic biomarkers for prediction of severe adverse drug reactions. *N Engl J Med.*, 358(6):637-639.
- Jewell H; Maggs JL; Harrison AC; O'Neill PM; Ruscoe JE & Park BK (1995). Role of hepatic metabolism in the bioactivation and detoxication of amodiaquine. *Xenobiotica.*, 25(2):199-217.
- Jiang H; Zhong F; Sun L; Feng W; Huang ZX & Tan X (2011). Structural and functional insights into polymorphic enzymes of cytochrome P450 2C8. *Amino Acids.*, 40(4):1195-1204.
- Johansson T; Jurva U; Grönberg G; Weidolf L & Masimirembwa C (2009). Novel metabolites of amodiaquine formed by CYP1A1 and CYP1B1: structure elucidation using electrochemistry; mass spectrometry; and NMR. *Drug Metab Dispos.*, 37(3):571-579.
- Kisselev P; Schunck WH; Roots & Schwarz D (2005). Association of CYP1A1 polymorphisms with differential metabolic activation of 17beta-estradiol and estrone. *Cancer Res.*, 65(7):2972-2978.
- Kudzi W; Doodoo AN; Mills JJ (2009). Characterisation of CYP2C8; CYP2C9 and CYP2C19 polymorphisms in a Ghanaian population. *BMC Med Genet.*; 10:124.
- Kumar V; Singh S; Yadav CS; Ahmed RS; Gupta S; Pasha ST; Tripathi AK & Banerjee BD (2010). CYP1A1 and CYP3A4 polymorphic variations in Delhi population of Northern India. *Environ Toxicol Pharmacol.*, 29(2):126-130.
- Lambert CA, Tishkoff SA (2009). Genetic structure in African populations: implications for human demographic history. *Cold Spring Harb Symp Quant Biol.* 274:395-402.
- Landi MT; Bertazzi PA; Shields PG; Clark G; Lucier GW; Garte SJ; Cosma G & Caporaso NE (1994). Association between CYP1A1 genotype; mRNA expression and enzymatic activity in humans. *Pharmacogenetics.*, 4(5):242-246.
- Larrey D; Castot A; Pèssayre D; Merigot P; Machayekhy JP; Feldmann G; Lenoir A; Rueff B & Benhamou JP (1986). Amodiaquine-induced hepatitis. A report of seven cases. *Ann Intern Med.*, 104(6):801-803.
- Levine ND (1988). Progress in taxonomy of the Apicomplexan protozoa. *J Protozool.*, (4):518-520.

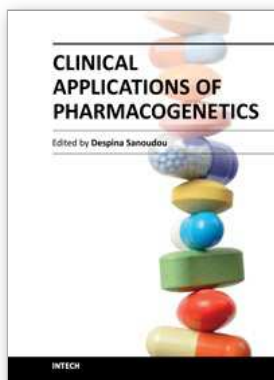
- Li DN; Seidel A; Pritchard MP; Wolf CR & Friedberg T (2000). Polymorphisms in P450 CYP1B1 affect the conversion of estradiol to the potentially carcinogenic metabolite 4-hydroxyestradiol. *Pharmacogenetics.*, 10(4):343-353.
- Li XQ; Bjorkman A; Andersson TB; Ridderstrom & Masimirembwa CM (2002). Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: a new high affinity and turnover enzyme-specific probe substrate. *J Pharmacol Exp Ther.*, 300(2):399-407.
- Liang G; Pu Y & Yin L (2005). Rapid detection of single nucleotide polymorphisms related with lung cancer susceptibility of Chinese population. *Cancer Lett.*, 223(2):265-274.
- Low DA & Smith A (1976). *History of East Africa*. Oxford University Press; Oxford, UK.
- malERA Consultative Group on Drugs (2011). A research agenda for malaria eradication: drugs. *PLoS Med.*, 8(1):e1000402.
- Martinez-Labarga C; Lelli R; Tarsi T; Babalini C; De Angelis F; Ottoni C; Giambra V; Pepe G; Azebi E; Frezza D; Biondi G; & Rickards O (2007). Polymorphisms of the COL1A2; CYP1A1 and HS1;2 Ig enhancer genes in the Tuaregs from Libya. *Ann Hum Biol.*, 34(4):425-436.
- Masubuchi N; Makino C; Murayama N (2007). Prediction of *in vivo* potential for metabolic activation of drugs into chemically reactive intermediate: correlation of *in vitro* and *in vivo* generation of reactive intermediates and *in vitro* glutathione conjugate formation in rats and humans. *Chem Res Toxicol.*, 20(3):455-464.
- Marsh K (1998). Malaria disaster in Africa. *Lancet.*, 352(9132):924.
- Mount DL; Patchen LC; Nguyen-Dinh P; Barber AM; Schwartz IK & Churchill FC (1986). Sensitive analysis of blood for amodiaquine and three metabolites by high-performance liquid chromatography with electrochemical detection. *J Chromatogr.*, 383(2):375-386.
- Muthiah YD; Lee WL; Teh LK; Ong CE & Ismail R (2005). Genetic polymorphism of CYP2C8 in three Malaysian ethnics: CYP2C8*2 and CYP2C8*3 are found in Malaysian Indians. *J Clin Pharm Ther.*, 30(5):487-490.
- Naisbitt DJ; Ruscoe JE; Williams D; O'Neill PM; Pirmohamed M & Park BK (1997). Disposition of amodiaquine and related antimalarial agents in human neutrophils: implications for drug design. *J Pharmacol Exp Ther.*, 280(2):884-893.
- Nakajima M; Fujiki Y; Noda K; Ohtsuka H; Ohkuni H; Kyo S; Inoue M; Kuroiwa Y; & Yokoi T (2003). Genetic polymorphisms of CYP2C8 in Japanese population. *Drug Metab Dispos.*, 31(6):687-690.
- Nankabirwa J, Cundill B, Clarke S, Kabatereine N, Rosenthal PJ, Dorsey G, Brooker S, Staedke SG (2010). Efficacy, safety, and tolerability of three regimens for prevention of malaria: a randomized, placebo-controlled trial in Ugandan schoolchildren. *PLoS One.*, 5(10):e13438.
- Nebert DW; Dalton TP; Okey AB & Gonzalez FJ (2004). Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J Biol Chem.*, 279(23): 23847-23850.
- Neftel KA; Woodtly W; Schmid M; Frick PG & Fehr J (1986). Amodiaquine induced agranulocytosis and liver damage. *Br Med J.*, 292(6522): 721-723.

- Nosten F; ter Kuile F; Chongsuphajaisiddhi T; Luxemburger C; Webster HK; Edstein M; Phaipun L; Thew KL & White NJ (1991). Mefloquine-resistant falciparum malaria on the Thai-Burmese border. *Lancet.*, 337(8750): 1140-1143.
- Nosten F; van Vugt M; Price R; Luxemburger C; Thway KL; Brockman A; McGready R; ter Kuile F; Looareesuwan S & White NJ (2000). Effects of artesunate-mefloquine combination on incidence of Plasmodium falciparum malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet.*, 356(9226):297-302.
- Okobia M; Bunker C; Zmuda J; Kammerer C; Vogel V; Uche E; Anyanwu S; Ezeome E; Ferrell R & Kuller L. (2005) Cytochrome P4501A1 genetic polymorphisms and breast cancer risk in Nigerian women. *Breast Cancer Res Treat.*, 94(3):285-293.
- Olliaro P; Nevill C; LeBras J; Ringwald P; Mussano P; Garner P & Brasseur P (1996). Systematic review of amodiaquine treatment in uncomplicated malaria. *Lancet.*, 348(9036):1196-1201.
- Olliaro P, Mussano P (2003). Amodiaquine for treating malaria. *Cochrane Database Syst Rev.*, (2):CD000016.
- Paine MF; Schmiedlin-Ren P & Watkins PB (1999). Cytochrome P-450 1A1 expression in human small bowel: interindividual variation and inhibition by ketoconazole. *Drug Metab Dispos.*, 27(3):360-364.
- Paine MF; Hart HL; Ludington SS; Haining RL; Rettie AE & Zeldin DC (2006). The human intestinal cytochrome P450 "pie". *Drug Metab Dispos.*, 34(5):880-886.
- Parikh S; Ouedraogo JB; Goldstein JA; Rosenthal PJ & Kroetz DL (2007). Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. *Clin Pharmacol Ther.*, 82(2):197-203.
- Peters W (1970). *Chemotherapy and drug resistance in malaria*. Academic press; London, UK.
- Phillips-Howard PA, West LJ (1990). Serious adverse drug reactions to pyrimethamine-sulphadoxine, pyrimethamine-dapsone and to amodiaquine in Britain. *J R Soc Med.*, 83(2):82-85.
- Pisani P; Srivatanakul P; Randerson-Moor J; Vipasrinimit S; Lalitwongsa S; Unpunyo P; Bashir S & Bishop DT (2006). GSTM1 and CYP1A1 polymorphisms; tobacco; air pollution; and lung cancer: a study in rural Thailand. *Cancer Epidemiol Biomarkers Prev.*, 15(4): 667-674.
- Rouveix B; Coulombel L; Aymard JP; Chau F & Abel L (1989). Amodiaquine-induced immune agranulocytosis. *Br J Haematol.*, 71(1):7-11.
- Röwer S; Bienzle U; Weise A; Lambertz U; Forst T; Otchwemah RN; Pfützner A & Mockenhaupt FP (2005). Short communication: high prevalence of the cytochrome P450 2C8*2 mutation in Northern Ghana. *Trop Med Int Health.*, 10(12):1271-1273.
- Saito T; Egashira M; Kiyotani K; Fujieda M; Yamazaki H; Kiyohara C; Kunitoh H & Kamataki T (2003). Novel nonsynonymous polymorphisms of the CYP1A1 gene in Japanese. *Drug Metab Pharmacokinet.*, 18(3):218-221.
- Shah PP; Singh AP; Singh M; Mathur N; Mishra BN; Pant MC & Parmar D (2008). Association of functionally important polymorphisms in cytochrome P4501B1 with lung cancer. *Mutat Res.*, 643(1-2): 4-10.

- Shillcutt S; Morel C; Goodman C; Coleman P; Bell D; Whitty CJ & Mills A (2008). Cost-effectiveness of malaria diagnostic methods in sub-Saharan Africa in an era of combination therapy. *Bull World Health Organ.*, 86(2): 101-110.
- Singh R; Ting JG; Pan Y; Teh LK; Ismail R & Ong CE (2008). Functional role of Ile264 in CYP2C8: mutations affect haem incorporation and catalytic activity. *Drug Metab Pharmacokinet.*, 23(3): 165-74 (2008).
- Smart J & Daly AK (2000). Variation in induced CYP1A1 levels: relationship to CYP1A1; Ah receptor and GSTM1 polymorphisms. *Pharmacogenetics.*; 10(1):11-24.
- Solus JF; Arietta BJ; Harris JR; Sexton DP; Steward JQ; McMunn C; Ihrie P; Mehall JM; Edwards TL & Dawson EP (2004). Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. *Pharmacogenomics*, 5(7): 895-931.
- Soyama A; Saito Y; Hanioka N; Murayama N; Nakajima O; Katori N; Ishida S; Sai K; Ozawa S & Sawada JI (2001). Non-synonymous single nucleotide alterations found in the CYP2C8 gene result in reduced *in vitro* paclitaxel metabolism. *Biol Pharm Bull.*, 24(12): 1427-1430 (2001).
- Soyama A; Saito Y; Komamura K; Ueno K; Kamakura S; Ozawa S & Sawada J (2002). Five novel single nucleotide polymorphisms in the CYP2C8 gene; one of which induces a frame-shift. *Drug Metab Pharmacokinet.*, 17(4): 374-377.
- Soyama A; Hanioka N; Saito Y; Murayama N; Ando M; Ozawa S & Sawada J (2002). Amiodarone N deethylation by CYP2C8 and its variants; CYP2C8*3 and CYP2C8 P404A. *Pharmacol Toxicol.*, 91(4): 174-178.
- Spurr NK; Gough AC; Stevenson K & Wolf CR (1987). Msp-1 polymorphism detected with a cDNA probe for the P-450 I family on chromosome 15. *Nucleic Acids Res.*, 15(14): 5901.
- Sreeja L; Syamala V; Hariharan S; Madhavan J; Devan SC & Ankathil R (2005). Possible risk modification by CYP1A1; GSTM1 and GSTT1 gene polymorphisms in lung cancer susceptibility in a South Indian population. *J Hum Genet.*, 50(12):618-627.
- Staedke SG, Kanya MR, Dorsey G, Gasasira A, Ndeezi G, Charlebois ED, Rosenthal PJ (2001). Amodiaquine, sulfadoxine/pyrimethamine, and combination therapy for treatment of uncomplicated falciparum malaria in Kampala, Uganda: a randomised trial. *Lancet.*, 358(9279):368-74.
- Stoilov I; Akarsu AN; Alozie I; Child A; Barsoum-Homsy M; Turacli ME; Or M; Lewis RA; Ozdemir N; Brice G; Aktan SG; Chevrette L; Coca-Prados M & Sarfarazi M (1998). Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *Am J Hum Genet.*, 62(3): 573-584.
- Struik SS, Riley EM (2004). Does malaria suffer from lack of memory?, *Immunol Rev.* 201:268-290.
- Suarez-Kurtz G; Genro JP; de Moraes MO; Ojopi EB; Pena SD; Perini JA; Ribeiro-Dos-Santos A; Romano-Silva MA; Santana I & Struchiner CJ (2010). Global pharmacogenomics: Impact of population diversity on the distribution of polymorphisms in the CYP2C cluster among Brazilians. *Pharmacogenomics J.*, 10(6): 1-10.

- Suneetha KJ; Nancy KN; Rajalekshmy KR; Rama R; Sagar TG & Rajkumar T (2011). Role of glutathione-s-transferase and CYP1A1*2A polymorphisms in the therapy outcome of south Indian acute lymphoblastic leukemia patients. *Indian J Med Paediatr Oncol.*, 32(1): 25-29.
- Tingle MD; Jewell H; Maggs JL; O'Neill PM & Park BK (1995). The bioactivation of amodiaquine by human polymorphonuclear leucocytes *in vitro*: chemical mechanisms and the effects of fluorine substitution. *Biochem Pharmacol.*, 50(7): 1113-1139.
- Ursing J, Kofoed PE, Rodrigues A, Bergqvist Y, Rombo L (2009). Chloroquine is grossly overdosed and overused but well tolerated in Guinea-bissau. *Antimicrob Agents Chemother.*, 53(1):180-185.
- Ursing J, Kofoed PE, Rodrigues A, Blessborn D, Thoft-Nielsen R, Björkman A, Rombo L (2011). Similar efficacy and tolerability of double-dose chloroquine and artemether-lumefantrine for treatment of Plasmodium falciparum infection in Guinea-Bissau: a randomized trial. *J Infect Dis.*, 203(1):109-116
- Van Duursen MB; Sanderson JT & van den Berg M (2005). Cytochrome P450 1A1 and 1B1 in human blood lymphocytes are not suitable as biomarkers of exposure to dioxin-like compounds: polymorphisms and interindividual variation in expression and inducibility. *Toxicol Sci.*, 85(1): 703-712.
- Walsky RL & Obach RS (2004). Validated assays for human cytochrome P450 activities. *Drug Metab Dispos.*, 32(6): 647-660.
- Wang Q; Li H; Tao P; Wang YP; Yuan P; Yang CX; Li JY; Yang F; Lee H; & Huang Y (2011). Soy Isoflavones; CYP1A1; CYP1B1; and COMT Polymorphisms; and Breast Cancer: A Case-Control Study in Southwestern China. *DNA Cell Biol.*, 30(8): 585-595.
- White NJ (1997). Assessment of the pharmacodynamic properties of antimalarial drugs *in vivo*. *Antimicrob Agents Chemother*, 41(7): 1413-1422.
- Wiley JC; Coy EL; Frampton MW; Torres A; Apostolakos MJ; Hoehn G; Schuermann WH; Thilly WG; Olson DE; Hammersley JR; Crespi CL & Utell MJ (1997). Quantitative RT-PCR measurement of cytochromes p450 1A1; 1B1; and 2B7; microsomal epoxide hydrolase; and NADPH oxidoreductase expression in lung cells of smokers and nonsmokers. *Am J Respir Cell Mol Biol.*, 17(1): 114-124.
- Winstanley P; Edwards G; Orme M & Breckenridge A (1987). The disposition of amodiaquine in man after oral administration. *Br J Clin Pharmacol.*, 23(1): 1-7.
- Winstanley PA; Simooya O; Kofi-Ekue JM; Walker O; Salako LA; Edwards G; Orme ML & Breckenridge AM (1990). The disposition of amodiaquine in Zambians and Nigerians with malaria. *Br J Clin Pharmacol.*, 29(6): 695-701.
- WHO (1990). Practical chemotherapy of malaria. Report of a WHO Scientific Group; - Technical Report Series n0 805, Geneva, Switzerland.
- WHO (2010); World Malaria Report 2010; WHO; Geneva, Switzerland
- Young MD; & Moore DV (1961). Chloroquine resistance in Plasmodium falciparum. *Am J Trop Med Hyg.*, 10: 317-320.

Zhang J; Deng J; Zhang C; Lu Y; Liu L; Wu Q; Shao Y; Zhang J; Yang H; Yu B; & Wan J (2010). Association of GSTT1; GSTM1 and CYP1A1 polymorphisms with susceptibility to systemic lupus erythematosus in the Chinese population. *Clin Chim Acta.*, 411(11-12): 878-881.



Clinical Applications of Pharmacogenetics

Edited by Dr. Despina Sanoudou

ISBN 978-953-51-0389-9

Hard cover, 292 pages

Publisher InTech

Published online 21, March, 2012

Published in print edition March, 2012

The rapidly evolving field of Pharmacogenetics aims at identifying the genetic factors implicated in the inter-individual variation of drug response. These factors could enable patient sub-classification based on their treatment needs thus expediting drug development and promoting personalized, safer and more effective treatments. This book presents Pharmacogenetic examples from a broad spectrum of different drugs, for different diseases, which are representative of different stages of evaluation or application. It has been designed so as to serve both the unfamiliar reader through explanations of basic Pharmacogenetic concepts, the clinician with presentation of the latest developments and international guidelines, and the research scientist with examples of Pharmacogenetic applications, discussions on the limitations and an outlook on the new scientific trends in this field.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

José Pedro Gil (2012). The Pharmacogenetics of the Antimalarial Amodiaquine, Clinical Applications of Pharmacogenetics, Dr. Despina Sanoudou (Ed.), ISBN: 978-953-51-0389-9, InTech, Available from: <http://www.intechopen.com/books/clinical-applications-of-pharmacogenetics/the-pharmacogenetics-of-the-antimalarial-amodiaquine>

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.