Influence of Cytochrome P450 (CYP) 2C8 Polymorphisms on the Efficacy and Tolerability of Artesunate-Amodiaquine Treatment of Uncomplicated Plasmodium Falciparum Malaria in Zanzibar

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Abstract

Background The antimalarial drug amodiaquine, a commonly used long acting partner drug in artemisinin-based combination therapy, is metabolized to active desethyl-amodiaquine (DEAQ) by cytochrome P450 2C8 (CYP2C8). The CYP2C8 gene carries several polymorphisms including the more frequent minor alleles CYP2C8*2 and CYP2C8*3. These minor alleles have been associated with decreased enzymatic activity, slowing the amodiaquine biotransformation towards DEAQ. This study aimed to assess the influence of CYP2C8 polymorphisms on the efficacy and tolerability of artesunate-amodiaquine treatment for uncomplicated Plasmodium falciparum malaria in Zanzibar.

Methods We analysed data from 618 children <5 years with uncomplicated P. falciparum malaria enrolled in two randomized clinical trials comparing artesunate-amodiaquine and artemether-lumefantrine in 2002-2005 in Zanzibar. CYP2C8*2 and CYP2C8*3 genotypes were determined by PCR-restriction fragment length polymorphism and assessed in relation to clinical data on treatment outcome and tolerance.

Results The allele frequencies of CYP2C8*2 and CYP2C8*3 were 17.5% (95% CI 15.4-19.7%) and 2.7% (95% CI 1.8-3.7%), respectively. There was no significant difference in the proportion of subjects carrying either CYP2C8*2 or CYP2C8*3 alleles amongst those with reinfections (44.1 %; 95% CI 33.8-54.8) or those with recrudescent infections (48.3%; 95% CI 29.4-67.5), compared to those with adequate clinical and parasitological response (36.7 %; 95% CI 30.0-43.9) (P = 0.25 and P = 0.31, respectively). However, patients carrying either the CYP2C8*2 or CYP2C8*3 allele were significantly associated with increased occurrence of non-serious adverse events compared with CYP2C8*1/*1 wildtype homozygotes (44.9%; 95% CI 36.1-54.0 versus 28.1%; 95% CI 21.9-35.0, respectively; P = 0.003).

Conclusions CYP2C8 genotypes did not influence treatment efficacy directly, but the tolerability to ASAQ may be reduced in subjects carrying the CYP2C8*3 and CYP2C8*2 alleles. The importance of this non-negligible association with regards to amodiaquine-based malaria chemotherapy warrants further investigation.

Background

In the mid-1980s, amodiaquine was recommended as a malaria prophylaxis for travellers but several reports pointed high level of toxicity, mainly agranulocytosis and hepatotoxicity [1, 2], leading to the removal of amodiaquine monotherapy from the Essential Drug List of WHO in 1990 [3]. Some years later, an updated appraisal of available data suggested that amodiaquine toxicity related with severe liver damages and agranulocytosis was primarily seen in non-Africans and only after several weeks of regular chemoprophylaxis, reinstating this drug as an option for treatment of malaria [4, 5]. Amodiaquine was reintroduced as an important, slowly eliminated partner drug in artemisinin-based combination therapy (ACT), the current global mainstay for the treatment of uncomplicated Plasmodium falciparum malaria. Nowadays, artesunate-amodiaquine (ASAQ), a first-generation ACT, is used as first- or second-line
treatment in many countries in Africa [6]. Amodiaquine is also increasingly used in combination with sulfadoxine-pyrimethamine (SP-AQ) in seasonal malaria chemoprevention, i.e., monthly distribution of intermittent preventative treatment in young children during peak malaria transmission, in several countries of the Sahel sub-region [7, 8]. In numerous clinical trials ASAQ efficacy has been high, with an estimated mean of 95.1% cure rate in a large meta-analysis of studies in Africa [9]. Furthermore, treatment (as opposed to prophylaxis) of malaria with amodiaquine has been associated with mild adverse events including gastrointestinal effects, abdominal pain, neutropenia, nausea, dizziness, and pruritus, but typically not with serious adverse events [4, 10–12].

Amodiaquine is short lived (half-life 2–8 hours) and is primarily metabolized by cytochrome P450 2C8 (CYP2C8) to its main, biologically active metabolite desethyl-amodiaquine (DEAQ) [13] which has a long terminal elimination half-life (9–18 days) [14]. The main antimalarial action of amodiaquine is thus carried out by DEAQ, including an initial immediate treatment effect (parasite clearance), as well as a temporary post-treatment protective effect during the elimination phase of the metabolite. The CYP2C8 gene carries several polymorphisms including the most frequent minor alleles CYP2C8*2 and CYP2C8*3, coding for enzymes with altered activity in comparison with the CYP2C8*1 wildtype [15]. The CYP2C8*2 variant has been associated in vitro with a six-fold lower amodiaquine metabolism activity than the CYP2C8*1 wildtype enzyme [3]. The effect was even greater in the CYP2C8*3 variant, suggesting that any impact of reduced CYP2C8 metabolism would be more pronounced in CYP2C8*3 carriers. CYP2C8*2 is most prevalent in those of African descent, whereas CYP2C8*3 is highly frequent among Caucasians [14, 16–18].

It has been postulated that the impaired conversion of amodiaquine to DEAQ among low activity CYP2C8*2 and CYP2C8*3 carriers is not likely to impact treatment efficacy as both amodiaquine and DEAQ have antimalarial activity, the latter considered the major active component [3]. However, the prolonged pharmacokinetic profile in poor-metabolizers may lead to a non-negligible increased risk of amodiaquine-related adverse events among populations with these specific genotypes [14, 19, 20]. Albeit of interest, only a few studies have investigated the potential association between slow amodiaquine metabolizers and reduced treatment efficacy and/or increased risk of adverse events [3, 20–22]. In vivo data on the impact of the low activity CYP2C8*2 allele are sparse, and almost non-existent among CYP2C8*3 carriers due to the very low CYP2C8*3 allele frequency in the generality of African populations, where ASAQ is primarily used [6, 14].

Zanzibar, where ASAQ has been the first line treatment for uncomplicated malaria since 2003, has similar CYP2C8*2 (13.9%) frequencies but higher CYP2C8*3 (2.1%) allele frequency than in other places in sub-Saharan Africa [3, 17]. This latter particular characteristic sets the opportunity to a more complete investigation the effect of CYP2C8 polymorphism on amodiaquine-based antimalarial treatment. We therefore retrospectively assessed the impact of these CYP2C8 polymorphisms on treatment outcome and tolerability in two ASAQ malaria efficacy trials conducted in Zanzibar in 2002–2005, when malaria in these islands was still characterized by high incidence (Bhattarai et al., 2007). More specifically, we assessed if CYP2C8*2 and CYP2C8*3 carriers were at increased risk of new and/or recrudescent
infections during the 42-day follow-up period, and if CYP2C8*2 and CYP2C8*3 carriers were at increased risk of experiencing adverse events after ASAQ treatment.

**Material And Methods**

**Study setting and participants**

Two randomized clinical trials (ClinicalTrials.gov identifiers: NCT03764527 and NCT03768908) comparing ASAQ with artemether-lumefantrine (AL) [23–25] were conducted in Zanzibar, Tanzania during 2002–2005 when malaria transmission was still high [26, 27] in these islands. Both trials were conducted at Kivunge Hospital, Unguja Island, and Micheweni Hospital, Pemba Island and included standard weight-based, three-day supervised treatment courses, with a post-treatment follow up of 42 days. The ASAQ PCR corrected cure rates during the WHO recommended 28-day follow up period were 94% and 96% in the two trials respectively [25].

CYP2C8*2 and CYP2C8*3 alleles were successfully analysed in 618 malaria affected children under 5 years of age (Fig. 1). Among these, 329 patients were enrolled in the two ASAQ clinical trial arms, of which 133 subjects had recurrent infections during post-treatment follow up, and 196 were selected among the remaining subjects with an adequate clinical and parasitological response (ACPR). 289 subjects were available for analysis among 380 patients enrolled in the two AL treatment arms. For the AL treated subjects no influence of the CYP2C8 polymorphisms were expected as CYP2C8 is not involved in the metabolism of either artemether or lumefantrine. These patients were therefore not included in the analyses for treatment outcome but were included as a control in the analysis of adverse events.

**Defining treatment outcome**

“Recurrent infection” refers to any infection occurring after initial parasite clearance (from day 14) during follow up. Recurrent infections were defined as either a recrudescent or newly acquired infection, by pairwise molecular analyses of the *P. falciparum* merozoite surface protein 2 (pfmsp2) gene in accordance to the WHO guidelines available when the clinical trials were conducted [24]. The size of the pfmsp2 PCR amplicon for the originally treated infections on day 0 and the day of recurrent parasitaemia were compared by gel electrophoresis [23–25].

**Reporting of adverse events**

Non-serious adverse events were defined as any undesirable medical occurrence in a subject during the follow-up and were reported according to perceived severity (mild, moderate and severe) in a case report form for each case. A serious adverse event was defined as an adverse event that resulted in death or was life threatening, an event that required hospitalization, and/or resulted in persistent or significant disability or incapacity. Serious adverse events resulting in withdrawal from the clinical trial were excluded from this study.

**Molecular analysis of CYP2C8*2 and CYP2C8*3**
Genomic DNA was extracted by incubation of 3–5 ø 3 mm punches of peripheral blood samples preserved on Whatman 3MM filter papers at 95 °C in 200 µl of PBS. PCR-restriction fragment length polymorphism (RFLP) was used for the analysis of the CYP2C8*2 I269F (805A > T) and CYP2C8*3 R139K (416A > G) single nucleotide polymorphisms (SNPs). The CYP2C8*3 K399R SNP was not included in the analyses due to the absolute linkage with CYP2C8*3 R139K (R² = 1) [17]. The forward (Fwd) and reverse (Rev) PCR oligonucleotide primers were for (a) CYP2C8*2 I269F Fwd 5'-ATGTTGCTCTTTACACGAAGTTACA-3' and Rev 5'-ATCTTACCTGCTCCATTTGA-3', and for (b) CYP2C8*3 R139K Fwd 5'-CTTCCGTGCTACATGATGACG-3' and Rev 5'-CTGCTGAGAAAGGCATGAAG-3'. The PCR thermal cycles were: 94 °C for 1 min, followed by 40 cycles at 91 °C for 30 s, 62 °C for 30 s, 72 °C for 20 s and 4 °C for 10 min. PCR amplifications were followed by discriminative restriction with BclI (CYP2C8*2 I269F) and XmnI (CYP2C8*3 R139K).

**Defining CYP2C8*2 and CYP2C8*3 genotypes**

CYP2C8*1 was defined as the absence of CYP2C8*2 and CYP2C8*3 alleles, i.e., homozygous *1/*1 "wild type" genotypes. CYP2C8*2 carriers included *1/*2 and *2/*2 genotypes and CYP2C8*3 carriers included *1/*3, *3/*3, and *2/*3 genotypes.

**Statistical analysis**

Linkage disequilibrium between CYP2C8*3 SNPs was calculated with the LDlink 4.1.0 LDassoc Tool [28]. Allele frequencies and Hardy Weinberg equilibrium were analyzed through the Fisher’s Exact test. Statistical associations between CYP2C8*2 and/or CYP2C8*3 allele carriers and treatment outcome or adverse events were assessed by Fisher’s Exact test. All analyses were performed in STATA/SE version 16.0; statistical significance was defined as P < 0.05.

**Results**

**CYP2C8*2 and CYP2C8*3 genotype and allele frequencies in Zanzibar**

The CYP2C8*2 and CYP2C8*3 allele frequencies in the studied population were 17.5% (95% CI 15.4–19.7%) and 2.7% (95% CI 1.8–3.7%), respectively (Table 1). The proportion of subjects carrying at least one copy of the CYP2C8*2 or CYP2C8*3 allele were 32.5% (95% CI 28.8–36.4%) and 4.9% (95% CI 3.3–6.6%), with 2.9% (95% CI 1.7–4.6%) of the subjects being homozygous for either the CYP2C8*2 or CYP2C8*3 slow metabolizer alleles. Both alleles were found in Hardy-Weinberg equilibrium with CYP2C8*1 (P = 0.79).
Table 1

**CYP2C8 genotype and allele frequencies in Zanzibar.** Relative and absolute (n) frequencies among 618 children under five years with uncomplicated *P. falciparum* malaria. The 2C8*2/2C8*3 genotype are individuals (n = 5) that were heterozygous carriers for both *CYP2C8*2 and *CYP2C8*3. For these, 5 alleles were attributed each to 2C8*2 and 2C8*3.

| Relative and (absolute) CYP2C8 genotype frequencies | Relative and (absolute) CYP2C8 allele frequencies |
|-----------------------------------------------------|--------------------------------------------------|
| 2C8*1/2C8*1 0.634 (392)                              | 2C8*1 0.798 (987)                                |
| 2C8*2/2C8*2 0.024 (15)                              | 2C8*2 0.175 (216)                                |
| 2C8*3/2C8*3 0.005 (3)                               | 2C8*3 0.027 (33)                                |
| 2C8*1/2C8*2 0.293 (181)                             |                                                 |
| 2C8*1/2C8*3 0.036 (22)                              |                                                 |
| 2C8*2/2C8*3 0.008 (5)                               |                                                 |

**Association of CYP2C8*2 and CYP2C8*3 genotype frequencies with treatment outcome**

ASAQ PCR corrected cure rates during the WHO recommended 28-day follow up period were 94% and 96% in the two trials respectively [25]. There was no significant difference in the proportion of subjects carrying the *CYP2C8*2 allele among subjects with recurrent infection within the 42 day follow up in the ASAQ arms (38.3%; 95% CI 30.1–47.2%) compared to those with adequate clinical and parasitological response (ACPR) (31.1%; 95% CI 24.7–38.1%); P = 0.19 (Table 2). There was also no significant difference in the proportion of subjects carrying the *CYP2C8*3 allele in those with recurrent infections (5.3%; 95% CI 2.1–10.5%) and those with ACPR (5.6%; 95% CI 2.8–9.8); P = 1.00.

Table 2

**CYP2C8 genotype frequencies by treatment outcome after treatment with artesunate-amodiaquine.** Relative (%) and absolute (n) genotype frequencies by treatment outcome among children under five years with uncomplicated *P. falciparum* malaria in Zanzibar. ACPR = adequate clinical and parasitological response; IA = Inconclusive analysis

| Treatment outcome                  | *1/*1 carriers | *2 carriers | *3 carriers | Total |
|------------------------------------|----------------|-------------|-------------|-------|
| ACPR; % (n)                        | 63.3 (124)     | 31.1 (61)   | 5.6 (11)    | 100 (196) |
| Recurrent infections; % (n)        | 56.4 (75)      | 38.4 (51)   | 5.3 (7)     | 100 (133) |
| Reinfections; % (n)                | 55.9 (52)      | 37.6 (35)   | 6.5 (6)     | 100 (93)  |
| Recrudescences; % (n)              | 51.7 (15)      | 44.8 (13)   | 3.5 (1)     | 100 (29)  |
| Recurrent infections IA; % (n)     | 72.7 (8)       | 27.3 (3)    | 0.0 (0)     | 100 (11)  |

Among the 133 recurrent infections in the ASAQ arm, 122 were successfully PCR corrected, with 29 recrudescences (clinical failures) and 93 reinfections identified during the 42-day follow-up (Table 2).
There was no significant difference in the proportion of subjects carrying either $CYP2C8^*2$ or $CYP2C8^*3$ alleles amongst those with reinfections (44.1%; 95% CI 33.8–54.8) or those with recrudescent infections (48.3%; 95% CI 29.4–67.5), compared to those with ACPR (36.7%; 95% CI 30.0-43.9) (P = 0.25 and P = 0.31, respectively).

**CYP2C8*2 and CYP2C8*3 genotype frequencies in association to occurrence of adverse events**

Overall the ASAQ treatment was well tolerated. Among all patients 33% reported a non-serious adverse event of which 95% were perceived as mild or moderate and 5% were perceived as severe. The incidence of adverse events after treatment with ASAQ was higher in subjects carrying either the $CYP2C8^*2$ or $CYP2C8^*3$ alleles (44.9%; 95% CI 36.1–54.0) compared to the incidence in the $CYP2C8^*1/*1$ wildtype homozygotes (28.1%; 95% CI 21.9–35.0) (P = 0.003) (Table 3). No significant difference was observed in the incidence of adverse events after treatment with AL in $CYP2C8^*2$ or $CYP2C8^*3$ carriers (22.1%; 95% CI 14.2–31.8) compared to the incidence in the $CYP2C8^*1/*1$ wildtype homozygotes (23.4%; 95% CI 17.6–30.1) (P = 0.88).

|                  | *1/*1   | *2 carriers | *3 carriers |
|------------------|---------|-------------|-------------|
| Adverse Events; % (n) | 28.1 (55) | 45.9 (50)   | 38.9 (7)    |
| No adverse events; % (n) | 71.9 (141) | 54.1 (59)   | 61.1 (11)   |
| Total; % (n)    | 100 (196) | 100 (109)   | 100 (18)    |

**Table 3** Incidence of adverse events reported in the ASAQ treatment arm according to $CYP2C8$ genotype. Out of the 329 subjects analysed for $CYP2C8$, 6 had incomplete data regarding adverse events and were therefore excluded from these calculations.

**Discussion**

We assessed the $CYP2C8^*2$ and $CYP2C8^*3$ minor allele frequencies in association to treatment outcome and occurrence of adverse events after antimalaria treatment in Zanzibar. The observed $CYP2C8^*3$ (2.7%) allele frequency was consistent with our previous study [17], suggesting that Zanzibar is a region in Africa with relatively high $CYP2C8^*3$ prevalence, as compared with other African regions [3, 16, 19]. The $CYP2C8^*2$ allele frequency (17.5%) is more in line with most previous reports from the African continent [3, 18–20, 29, 30], but not as high as was recently reported in the Brazzaville, Republic of Congo (37%) [21]. Our results show that $CYP2C8^*2$ and $CYP2C8^*3$ carriers were at increased risk of presenting with adverse events after ASAQ treatment, but without increased risk of experiencing newly acquired or recrudescent *P. falciparum* infections during a 42-day follow-up.

Similar to our findings, no association between $CPY2C8^*2$ heterozygotes and treatment outcome was observed in a study conducted in Burkina Faso, whilst there was an increase in self-reported abdominal pain in $CPY2C8^*2$ heterozygotes but no significant association with other specific adverse events.
including nausea, vomiting, fatigue, and jaundice [3]. The observed CYP2C8*3 allele frequency (0.3%) was too low for any association analyses in that study. Another report, in Ghana, observed a slight but non-significant (P = 0.58) reduction in plasma DEAQ concentrations among subjects with mutant CYP2C8*2 genotypes compared to those with wild-type alleles or heterozygotes [20]. This reduction was however, not associated with treatment outcome or occurrence of adverse events, although the small sample size (N = 81) was lifted as a limiting factor in these analyses. Finally, despite no direct assessments of the association between CYP2C8*2 genotypes and occurrence of adverse events in Congo, the high CYP2C8*2 allele frequency (37%) reported in Brazzaville has been suggested to have had implications on the choice of first line treatment in the country [21]. ASAQ and AL were the first- and second-line treatments respectively, when ACTs where first introduced into the national treatment guidelines in 2006. Interestingly, in 2014 the guidelines were updated with AL as first line, after ASAQ had been associated with a higher number of drug-related adverse events than AL, possibly due to the high frequency of the CYP2C8*2 allele in the population.

Overall, the evidence for the association between CYP2C8*2 and CYP2C8*3 genotypes with amodiaquine and ASAQ treatment outcome and treatment associated adverse events is still largely inconclusive, and much hampered by the small sample sizes of previous studies. However, especially the latter association warrants further investigation. The impact on treatment tolerability may be of particular importance in populations where the CYP2C8*3 allele is more frequent, potentially affecting treatment compliance. The CYP2C8*3 allele has primarily been reported in Caucasian populations [16, 18], which could partly explain the high level of toxicity reported in Western travellers after repeated intake of amodiaquine as a malaria prophylaxis. Our larger sample size together with the relatively high frequency of CYP2C8*3 in Zanzibar may explain why we were able to detect a significant association between CYP2C8*2 and CYP2C8*3 carriers and occurrence of adverse events in this current study. Indeed, more than 40% of the CYP2C8*2 and CYP2C8*3 carriers presented with at least one adverse event. This might be considered in the pharmacovigilance of treatment with ASAQ in Zanzibar, seeing that these alleles are present in more than one third of the population.

The relatively high frequency of CYP2C8*3 in Zanzibar may be related with historical links of these islands with Caucasian populations from the Arabian Peninsula. Similar CYP2C8*3 prevalence may be significant in countries along the Sahel region in the northern part of sub-Saharan Africa, bordering the Maghreb countries. Several of these countries (e.g. Chad, Eritrea and Mauritania) use ASAQ as the first line antimalarial treatment, and many of them use SP-AQ for seasonal malaria chemoprophylaxis [7]. CYP2C8*3 status may significantly influence treatment tolerability with implications on the uptake of malaria control efforts in these countries.

**Limitations**

A relative limitation of this retrospective work is the absence of detailed description of adverse events including the reporting of causality, and concomitant intake of drugs that may act as CYP2C8 inhibitors and further inhibit enzyme activity [3, 6]. Better pharmacovigilance reporting should be considered for
ASAQ treatment in this specific target population. In addition, individual day 7 DEAQ concentration data could shed better light on this association were not available for these 2002–2005 trials.

**Conclusions**

*CYP2C8* genotypes did not appear to influence treatment efficacy directly but the tolerability of ASAQ may be reduced in subjects carrying the *CYP2C8*<sup>3</sup> and *CYP2C8*<sup>2</sup> alleles. The importance of this non-negligible association with regards to amodiaquine-based malaria chemotherapy, particularly in terms of treatment adherence, warrants further investigation.

**List Of Abreviations**

WHO: World Health Organization

ACT: artemisinin-based combination therapy

ASAQ: artesunate-amodiaquine

SP-AQ: sulfadoxine-pyrimethamine

CYP2C8: cytochrome P450 2C8

DEAQ: desethyl-amodiaquine

AL: artemether-lumefantrine

ACPR: adequate clinical and parasitological response

IA: inconclusive analysis

ZHRC: Zanzibar Health Research Council

pfmsp2: *P. falciparum* merozoite surface protein 2

PCR: polymerase chain reaction

DNA: deoxyribonucleic acid

RFLP: PCR-restriction fragment length polymorphism

SNP: single nucleotide polymorphism

CI: confidence interval

**Declarations**
Ethics approval and consent to participate: Both clinical trials were implemented in accordance with the Helsinki Declaration and approved by the Zanzibar Health Research Council (ZHRC) and Regional Research Ethics Board in Stockholm, Sweden.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors’ contributions: MM, AM and AB conducted the collection of samples in the field. LPL and PG designed the analysis and LPL conducted the molecular analysis. LPL and UM analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

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References

1. Hatton CS, Peto TE, Bunch C, Pasvol G, Russell SJ, Singer CR, Edwards G, Winstanley P: Frequency of severe neutropenia associated with amodiaquine prophylaxis against malaria. *Lancet* 1986, 1:411-414.

2. Neftel KA, Woodtly W, Schmid M, Frick PG, Fehr J: Amodiaquine induced agranulocytosis and liver damage. *Br Med J (Clin Res Ed)* 1986, 292:721-723.

3. Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ, Kroetz DL: Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. *Clin Pharmacol Ther* 2007, 82:197-203.

4. Olliaro P, Nevill C, LeBras J, Ringwald P, Mussano P, Garner P, Brassequ P: Systematic review of amodiaquine treatment in uncomplicated malaria. *Lancet* 1996, 348:1196-1201.

5. CDC: Agranulocytosis associated with the use of amodiaquine for malaria prophylaxis. *MMWR Morb Mortal Wkly Rep* 1986, 35:165-166.

6. Country antimalarial drug policies: by region [https://www.who.int/malaria/am_drug_policies_by_region_afro/en/]

7. Cairns M, Roca-Felttrer A, Garske T, Wilson AL, Diallo D, Milligan PJ, Ghani AC, Greenwood BM: Estimating the potential public health impact of seasonal malaria chemoprevention in African
children. Nature Communications 2012, 3.
8. WHO: Seasonal malaria chemoprevention with sulfadoxine-pyrimethamine plus amodiaquine in children: A field guide. 2013.
9. Venkatesan M, Gadalla NB, Stepniewska K, Dahal P, Nsanzabana C, Moriera C, Price RN, Mårtensson A, Rosenthal PJ, Dorsey G, et al: Polymorphisms in Plasmodium falciparum chloroquine resistance transporter and multidrug resistance 1 genes: parasite risk factors that affect treatment outcomes for P. falciparum malaria after artemether-lumefantrine and artesunate-amodiaquine. Am J Trop Med Hyg 2014, 91:833-843.
10. MacLehose HG, Klaes D, Garner P: Amodiaquine: A systematic review of adverse events. 2003.
11. Olliaro P, Mussano P: Amodiaquine for treating malaria. Cochrane Database Syst Rev 2003:CD000016.
12. WHO: Guidelines for the treatment of malaria. Third edition. 2015.
13. Li XQ, Björkman A, Andersson TB, Ridderström M, Masimirembwa CM: 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 2002, 300:399-407.
14. Gil JP, Gil Berglund E: CYP2C8 and antimalaria drug efficacy. Pharmacogenomics 2007, 8:187-198.
15. Hiratsuka M: Genetic Polymorphisms and in Vitro Functional Characterization of CYP2C8, CYP2C9, and CYP2C19 Allelic Variants. Biol Pharm Bull 2016, 39:1748-1759.
16. Daily EB, Aquilante CL: Cytochrome P450 2C8 pharmacogenetics: a review of clinical studies. Pharmacogenomics 2009, 10:1489-1510.
17. Cavaco I, Stromberg-Norklit J, Kaneko A, Msellem MI, Dahoma M, Ribeiro VL, Bjorkman A, Gil JP: CYP2C8 polymorphism frequencies among malaria patients in Zanzibar. European Journal of Clinical Pharmacology 2005, 61:15-18.
18. Cavaco I, Piedade R, Gil JP, Ribeiro V: CYP2C8 polymorphism among the Portuguese. Clinical Chemistry and Laboratory Medicine 2006, 44:168-170.
19. Röwer S, Bienzle U, Weise A, Lambertz U, Forst T, Otchwendah RN, Pfützner A, Mockenhaupt FP: Short communication: high prevalence of the cytochrome P450 2C8*2 mutation in Northern Ghana. Trop Med Int Health 2005, 10:1271-1273.
20. Adjei GO, Kristensen K, Goka BQ, Hoegberg LC, Alifrangis M, Rodrigues OP, Kurtzhals JA: Effect of concomitant artesunate administration and cytochrome P4502C8 polymorphisms on the pharmacokinetics of amodiaquine in Ghanaian children with uncomplicated malaria. Antimicrob Agents Chemother 2008, 52:4400-4406.
21. Peko SM, Ntoumi F, Vouvoungui C, Nderu D, Kobawila SC, Velavan TP, Koukouikila-Koussounda F: Distribution of the cytochrome P450 CYP2C8*2 allele in Brazzaville, Republic of Congo. Int J Infect Dis 2019, 85:49-53.
22. Somé FA, Bazíé T, Ehrlich HY, Goodwin J, Lehane A, Neya C, Zachari K, Wade M, Ouattara JM, Foy BD, et al: Investigating selected host and parasite factors potentially impacting upon seasonal malaria
chemoprevention in Bama, Burkina Faso. *Malar J* 2020, 19:238.

23. Holmgren G, Hamrin J, Svard J, Martensson A, Gil JP, Bjorkman A: Selection of *pfmdr1* mutations after amodiaquine monotherapy and amodiaquine plus artemisinin combination therapy in East Africa. *Infection Genetics and Evolution* 2007, 7:562-569.

24. Martensson A, Stromberg J, Sisowath C, Msellem MI, Gil JP, Montgomery SM, Olliaro P, Ali AS, Björkman A: Efficacy of artesunate plus amodiaquine versus that of artemether-lumefantrine for the treatment of uncomplicated childhood *Plasmodium falciparum* malaria in Zanzibar, Tanzania. *Clinical Infectious Diseases* 2005, 41:1079-1086.

25. Msellem M, Morris U, Soe A, Abbas FB, Ali AW, Barnes R, Frumento P, Ali AS, Mårtensson A, Björkman A: Increased Sensitivity of *Plasmodium falciparum* to Artesunate/Amodiaquine Despite 14 Years as First-Line Malaria Treatment, Zanzibar. *Emerg Infect Dis* 2020, 26:1767-1777.

26. Bhattarai A, Ali AS, Kachur SP, Martensson A, Abbas AK, Khatib R, Al-Mafazy AW, Ramsan M, Rotllant G, Gerstenmaier JF, et al: Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Med* 2007, 4:e309.

27. Bjorkman A, Shakely D, Ali AS, Morris U, Mkali H, Abbas AK, Al-Mafazy AW, Haji KA, Mcha J, Omar R, et al: From high to low malaria transmission in Zanzibar-challenges and opportunities to achieve elimination. *BMC Med* 2019, 17:14.

28. Machiela MJ, Chanock SJ: LDassoc: an online tool for interactively exploring genome-wide association study results and prioritizing variants for functional investigation. *Bioinformatics* 2018, 34:887-889.

29. Paganotti GM, Gramolelli S, Tabacchi F, Russo G, Modiano D, Coluzzi M, Romano R: Distribution of human CYP2C8*2 allele in three different African populations. *Malar J* 2012, 11:125.

30. Hodoameda P, Duah-Quashie NO, Hagan CO, Matrevi S, Abuaku B, Koram K, Quashie NB: Plasmodium falciparum genetic factors rather than host factors are likely to drive resistance to ACT in Ghana. *Malar J* 2020, 19:255.