Short Communication

High prevalence of pfmdr1 N86Y and Y184F mutations in *Plasmodium falciparum* isolates from Bioko island, Equatorial Guinea

Jian Li[1], Jiangtao Chen[2], Dongde Xie[2], Santiago-m Monte-Nguba[3], Juan Urbano Monsuy Eyi[4], Rocio Apicante Matesa[4], Maximo Miko Ondo Obono[4], Carlos Sala Ehapo[4], Liye Yang[5], Danjie Lu[5], Hui Yang[5], Hui-Tian Yang[5], Min Lin[5]

[1]Department of Parasitology, Hubei University of Medicine, Hubei province, China, [2]The Chinese medical aid team to the Republic of Equatorial Guinea, Guangzhou, Guangdong Province, China, [3]Medical Laboratory, Malabo Regional Hospital, Malabo, Equatorial Guinea, [4]Central Blood Transfusion Service, Malabo Regional Hospital, Malabo, Equatorial Guinea, [5]Central Laboratory, Chaozhou Central Hospital Affiliated to Southern Medical University, Chaozhou, Guangdong province, China

Objective: Drug resistance against *Plasmodium falciparum* has been recognized as the crucial obstacle to curbing mortality and morbidity from malaria. To investigate the distribution and pattern of multidrug resistance 1 (pfmdr1) gene polymorphisms in *P. falciparum*, isolates collected from the malaria high-endemic Bioko Island, Equatorial Guinea.

Methods: Blood samples were collected from 217 patients with *P. falciparum* malaria during rainy season in 2012 on Bioko Island. These samples were extracted using Chelex to obtain parasite DNA. Nest-polymerase chain reaction (PCR) and sequencing were employed to detect mutations (N86Y, E130K, Y184F, S1034C, N1042D, V1109I, and D1246Y) and haplotypes in pfmdr1 gene.

Results: A total of 151 samples were successfully detected for pfmdr1 mutations from the 217 patients. Pfmdr1 mutations were found in 91.39% (138/151) *P. falciparum* isolates. However, no mutation at 130 and 1109 was identified from these samples. Four haplotypes coding 86, 184, 1034, 1 042, and 1 246 were found including NYSND, YYSND, NFSND, and YFSND, which accounted for 8.61% (13/151), 2.65% (4/151), 29.80% (45/151), and 58.94% (89/151), respectively.

Conclusions: Our results exhibited hypersensitivity to lumefantrine (LU) and mefloquine (MQ) and resistance to chloroquine (CQ) and amodiaquine (AQ) in *P. falciparum* isolates from Bioko Island. This information will be useful for anti-malarial drug policy in Equatorial Guinea.

Keywords: *Plasmodium falciparum* multidrug resistance 1, Mutation, Drug resistance, Bioko Island

Introduction

Human malaria kills approximately one million people annually, mainly caused by an intracellular protozoan-*Plasmodium falciparum*. Overall, 109 countries are endemic for malaria, 45 of them in Africa. Although a package of antimalarials treatment and vector control were exceptionally victorious measures for malaria control, the morbidity and mortality associated with malaria have worsened over latest decades in many high-epidemic areas of the tropics and subtropics. At present, the reappearance of malaria is largely related with the increased resistance of the *P. falciparum* parasite to almost all presently used antimalarials including quinine (QN), amodiaquine (AQ), chloroquine (CQ), mefloquine (MQ), Artemether–lumefantrine (AT-LU), sulfadoxine–pyrimethamine (SP), and more recently, artemisinin (ART).[1–5] The antimalarial drug's resistance became a major threat to human health in high-epidemic area of malaria. Multidrug resistance of *P. falciparum* is rapid and widespread in Asia, South America, and even in Africa, and this has made the treatment of *falciparum* malaria gradually more complicated.[6] However, the molecular mechanisms of multidrug-resistant in *P. falciparum* remain unclear.

Single nucleotide polymorphisms (SNPs) of the *P. falciparum* multidrug resistance 1 (pfmdr1) gene that encodes P-glycoprotein transporter (Pgh-1) have been implicated. Notably, recent research has shown that

Correspondence to: Min Lin, Chaozhou Central Hospital Affiliated to Southern Medical University, China. Email: Konfutea@hotmail.com

DOI 10.1179/2047773214Y.0000000158

the drug susceptibility-related interaction mechanisms of Pgh-1 include alteration of food vacuole physiology, or as candidate antimalarial drug targets.[7] The main implicated pfmdr1 SNPs include N86Y, Y184F, S1034C, and N1042D.[8] The SNPs are associated with modulation of parasite tolerance or susceptibility to a number of antimalarials including QN, AQ, CQ, MQ, and LU.[4–6,9,10] Previous study showed that resistance to AQ and its metabolic product has been related to pfmdr1 mutations.[11] The wild type N86 in pfmdr1 has also been associated with increased tolerance to the AT and LU drugs separately.[5,12] Furthermore, it also indicated that amplification of the pfmdr1 gene may cause resistance to ART.[13] In general, the pfmdr1 gene amplification leads to MQ resistance.[14] Even if MQ has been adopted as partner drug to ART, it has been observed to select for N86.[15] In contrast, parasites with the mutant 86Y show increased susceptibility to MQ.[16] Although pfmdr1 link to CQ resistance has been proposed to follow pfcrt, CQ selects for parasites with pfmdr1 86Y mutation shows a converse relationship with MQ.[8]

This study was aimed to investigate the distribution and pattern of multidrug resistance 1 (pfmdr1) gene polymorphisms in *P. falciparum* isolates collected from the malaria high-endemic Bioko Island, Equatorial Guinea, where no such molecular study have been published previously.

Materials and Methods

Study site and population

Clinical samples were collected in 2012 from Bioko (spelled also Bioco, in Europe traditionally called Fernando Pó), which is an island 32 km off the west coast of Africa, specifically Cameroon, in the Gulf of Guinea. It is the northernmost part of Equatorial Guinea with a population of 130 000 and an area of 2 017 km^2. It is volcanic with its highest peak the Pico Basile at 3 012 m. Bioko Island is considered a high-endemic malaria area ($\geq$1/1 000), according to parasitological surveys in children less than 5 years old and number of malaria deaths at local hospitals. Malaria is present throughout the year, with a marked increase after rains peak during April and May. The main drugs for malaria treatment are artemisinin combination therapy (ACT) with AT-LU.

Patients diagnosed with *falciparum* malaria based on Giemsa-stained thick and thin smear were included in this study, after obtaining informed consent. Inclusion criteria were individuals with age $\geq$3 months, uncomplicated malaria, monoinfection with *P. falciparum*, and unaware of first trimester pregnancy. The study was approved by the National Institute of Public Health/the Republic of Equatorial Guinea Ethics Research Committee.

Sample collection

Blood samples for this study were collected from regional hospitals in Bioko, Equatorial Guinea between April 2012 and October 2012. The regional hospitals serve as referral centers for all district hospitals in each of the regions. Five milliliters of blood sample was collected from each participant into tubes containing EDTA-K_2 by trained and licensed medical laboratory technologists from the regional hospitals. All blood samples collected were stored on ice and transported to the parasitological research laboratory of the Malabo Regional Hospital. The samples were detected by microscope recommended from World Health Organization (WHO) and ICT malaria *P.f.* Cassette Test (ICT Diagnostics, South Africa). The *Plasmodium spp.* was confirmed by real-time polymerase chain reaction (PCR) assay. About 1 ml of each blood sample collected was spotted on a 3MM Whatman filter paper. The blood spots were air-dried and stored at -80°C in plastic envelopes containing silica gel.

DNA extraction

Plasmodium falciparum DNA was extracted from all filter paper spots using the described Chelex-100 extraction method[17] with modification. Briefly, about 5 mm $\times$ 5 mm portion of each blood spot was incubated 2 hours at room temperature in 1.5 ml centrifuge tube with 1.0 ml sterile water, followed by centrifugation for 5 minutes at 16 000 rpm and discard the supernatant. Add 160 µl of 5% Chelex-100, vortexed for 30 seconds, and heated at 56°C for 2 hours The mixture was heated at 95°C for 10 minutes, vortexing for 30 seconds every 2 minutes, followed by centrifugation for 5 minutes at 16 000 rpm. About 140 µl of the supernatants was then transferred into new 1.5 ml tube, centrifuged for 10 minutes at 16 000 rpm, and 120 µl of the supernatant was transferred into new 1.5 ml tube, to ensure complete removal of chelex beads. All extracted DNA were analyzed by 2% agar gel electrophoresis, containing 0.1 µg/ml ethidium bromide, and analyzed under UV light to check the quality of eluted DNA. The remaining DNA samples were stored at -20°C.

Amplification and analysis of pfmdr1

Nucleotide and amino-acid sequences used in the present study have been reported in PlasmoDB (http://plasmodb.org) under Gene ID. PF3D7_0523000. In order to analyze the SNPs of Asn86Tyr (N86Y), Glu130Lys (E130K), Tyr184Phe (Y184F), Ser1034Cys (S1034C), Val1109Ile (V1109I), Asn1042Asp (N1042D), and Asp1246Tyr (D1246Y) in Pfmdr1 gene, two fragments (526 and 799 bp) were amplified in a nested PCR process. The primers were shown in Table 1. All PCR reactions were targeted for a final volume of 50 µl

Table 1 Oligonucleotide sequence for *Pfmdr1* mutation single nucleotide polymorphism (SNP)

Primer	Sequence (5′–3′)	Size (bp)	Mutation
Primary amplification			
pf MDR1 (1)-N1F	TTAAATGTTTACCTGCACAACATAGAAAATT	612	
pf MDR1 (1)-N1R	CTCCACAATAACTTGCAACAGTTCTTA		
*pf*MDR1 (2)-N1F	AATTTGATAGAAAAAGCTATTGATTATAA	880	
*pf*MDR1 (2)-N1R	TATTTGGTAATGATTCGATAAATTCATC		
Nested amplification			
*pf*MDR1 (1)-N2F	TGTATGTGCTGTATTATCAGGA	526	N86Y, E130K, Y184F
*pf*MDR1 (1)-N2R	CTCTTCTATAATGGACATGGTA		
*pf*MDR1 (2)-N2F	GAATTATTGTAAATGCAGCTTTA	799	S1034C, N1042D, V1109I, D1246Y
*pf*MDR1 (2)-N2R	GCAGCAAACTTACTAACACG		

N86Y, E130K, Y184F, S1034C, N1042D, V1109I, and D1246Y represent Asn86Tyr, Glu130Lys, Tyr184Phe, Ser1034Cys, Asn1042Asp, Val1109Ile, and Asp1246Tyr, respectively.

containing 1 × PCR buffer, 3 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphospate (dNTP), 0.2 μM of each oligonucleotide, 2 μl DNA template, and 0.05 U/μl of Taq polymerase. Polymerase chain reaction amplification was done using Biorad Mini MJ thermal cycler. The gene target was amplified beginning with initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 93°C for 30 seconds, 52°C for 30 seconds, and 72°C for 1 minute, then a final extension at 72°C for 5 minutes.

Polymerase chain reaction products were electrophoretically analyzed in horizontal plate with 2% agarose gel containing 0.1 μg/ml ethidium bromide and analyzed under UV light. The remaining products were purified through the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA), according to the manufacturer's instructions. The purified PCR products confirmed using the ABI PRISM 310 Genetic Analyzer and a Big Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). The sequence was assembled and analyzed using the DNA star analysis software (DNASTAR Inc., Madison, WI, USA).

Data analysis

Data were collected and analyzed by SPSS for Windows version 18 (SPSS Inc., Chicago, IL, USA). The count of samples with mutant and wild-type alleles was used to generate the prevalence of the alleles. Statistical significance was defined as a P-value <0.05. The 95% confidence intervals were calculated with confidence interval calculator for proportions (https://www.mccallum-layton.co.uk/tools/statistic-calculators/confidence-interval-for-proportions-calculator/).

Results

DNA blood samples from 217 patients with uncomplicated *falciparum* malaria were tested. The samples were composed of individuals with 3 months to 80 years and their median age was 29.18 years, with a median age of 31.91 years (range from 11 months to 62 years) for male and 25.91 years (range from 3 months to 80 years) for female. The statistical significance was observed for age between man and woman ($P = 0.008$, $P < 0.05$). The parasitemia range of *P. falciparum*-infected individuals was 16–1 150 000 parasites/μl with a median parasitemia of 35 126 parasites/μl.

Altogether the 155 PCR-positive samples were successfully detected for pfmdr1 SNPs including pfmdr1-N1 and pfmdr1-N2. The amino acid sequence of pfmdr1-N1 includes the mutation site 86, 130, and 184; and the pfmdr1-N2 contains 1034, 1042, 1109, and 1246. Finally, the 151 PCR products were sequenced and analyzed except the four PCR products with weak band.

The prevalence of the pfmdr1 mutant allele was 91.39% (138/151) and that of the wild type allele was 8.61% (13/151). The SNPs were only found at positions 86 and 184. As for the remaining pfmdr1 130, 1 034, 1 042, 1 109, and 1 246 SNPs, all samples had the wild type allele. Four kinds of haplotypes were found including NEYSNVD, *Y*EYSNVD, NE*F*SNVD and *YEF*SNVD (representing amino acids at positions 86, 130, 184, 1 034, 1 042, 1 109, and 1 246). Of the isolates considered single mutant, *Y*EYSNVD and NE*F*SNVD exhibited 2.62% (4/151) and 29.80% (45/151) in mutation alleles, respectively. The most prevalent haplotype of pfmdr1 was *YEF*SNVD, accounted for 58.94% (89/151) (Table 2).

Discussion

At present, AT–LU represents foremost drug for the uncomplicated malaria treatment in the post-CQ and

Table 2 Prevalence of pfmdr1 haplotypes in Bioko, Equatorial Guinea

Haplotypes *n* (%)		No. of mutated site	95% CI
Wild type ($n = 13$)			
NYSND	13 (8.61%)	0	4.14–13.08%
Single type ($n = 49$)			
*Y*YSND	4 (2.65%)	1	0.09–5.21%
N*F*SND	45 (29.80%)	1	22.5–37.1%
Double type ($n = 89$)			
*YF*SND	89 (58.94%)	2	51.09–66.79%

Italicized alleles represent mutations. The haplotypes were constructed considering at codons 86, 184, 1 034, 1 042, and 1 246.

SP epoch in Africa. Understanding the molecular mechanism of multidrug-resistant in *P. falciparum* is essential for the suitable antimalarials treatment.

In this study, the 69.59% (151/217) samples were amplified by nested PCR. It means there remained 30.41% (66/217) samples for PCR negative. Numerous failures to pfmdr1 gene amplification might be ascribed to primer restrictions. A series of studies supported that pfmdr1 amplification is currently rare in Africa including Uganda, Kenya, and Zanzibar.[18–20] The pfmdr1 amplification has been considered as a contributor to alter *Plasmodium* response to lumefantrine *in vivo*.[21] Although the mechanisms remain largely unclear, it may be related to a parasite fitness cost associated with oppose selection by the widespread CQ treatment in Africa.[20]

The pfmdr1 SNPs were expanded drug tolerance or resistance in Asia, South America, and even in Africa. Of all the pfmdr1 SNPs analyzed, allelic variation was only observed in pfmdr1 positions 86 and 184 in this study. As for the remaining pfmdr1 E130K, S1034C, N1042D, V1109I, and D1246Y SNPs, all parasite isolates had the wild type allele. The polymorphisms in pfmdr1 gene have been related to CQ resistance.[22] The observed predominance of the YFSND haplotype in Bioko could be a result of selective pressure of heavy CQ use.

For 86Y mutation in the pfmdr1, the baseline frequency observed (61.58%) was similar to that recently surveyed in east Africa.[23] The presence of the pfmdr1 N86 allele has also been shown to confer less susceptibility to ART *in vitro*.[8] Although ARTs may not select pfmdr1 N86, LU pressure may provide the main selective force on the allele, and thus, co-drive a decrease in sensitivity to both of them.[23] Previous studies identified the pfmdr1 N86 allele as a potential marker of tolerance to LU.[23,24] In Burkina Faso, the relationship between the pfmdr1 86Y and AQ resistance was investigated. It indicated that pfmdr1 could be useful in monitoring AQ resistance.[25]

Although little association with drug susceptibility has previously been seen with polymorphisms at the pfmdr1 184 locus, one previous study suggests that this allele may also play a role in mediating resistance to some antimalarials.[18] It indicated that the pfmdr1 184 locus in Bioko is also mediated resistance to drug.

Bioko and several other African countries are currently altering to AT–LU as the first-line antimalaria drug. It will be important to monitor the efficacy of AT–LU closely, especially in high-transmission areas where drug selection pressure may be the utmost. Whether the pfmdr1 N86 and 184F SNPs all take part in a development of resistance to LU or rather represent compensating mechanisms to maintain or increase parasite fitness remains unclear.

In conclusion, the study identified independent selection of SNPs in the pfmdr1 gene following administration of AT–LU in a region of Africa where malaria is highly endemic. Although these SNPs in pfmdr1 were not associated with clinical treatment failure, they are evidence for the ability of drug combination to drive selection of parasites toward resistant phenotypes. The possible role of the pfmdr1 N86, 184F, and other alleles with pfmdr1 copy number as molecular markers of antimalarials resistance is supposed to further surveyed.

Disclaimer Statements

Contributors LJ and LM designed the study; CJT, XDD, YLY, LDJ, YH and YHT carried out the study. LDJ, YH and YHT collected and analysed the data. Santiago-m Monte-Nguba, Juan Urbano Monsuy Eyi, Rocio Apicante Matesa, Maximo Miko Ondo Obono and Carlos Sala Ehapo collected and checked the samples.

Funding Medical Research Council

Conflicts of interest All authors declare that there are no conflicts of interest and approved to submit to this journal.

Ethics approval We have received approval from a Research Ethical Commitee in Medical School of Shantou University in China.

References

1 Abdul-Ghani R, Farag HF, Allam AF. Sulfadoxine–pyrimethamine resistance in *Plasmodium falciparum*: a zoomed image at the molecular level within a geographic context. Acta Trop. 2013;125:163–90.
2 Al-Yaman F, Genton B, Mokela D, Narara A, Raiko A, Alpers MP. Resistance of *Plasmodium falciparum* malaria to amodiaquine, chloroquine and quinine in the Madang Province of Papua New Guinea, 1990–1993. Papua and New Guinea Med J. 1996;39:16–22.
3 Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, *et al*. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;505:50–5.
4 Borges S, Cravo P, Creasey A, Fawcett R, Modrzynska K, Rodrigues L, *et al*. Genomewide scan reveals amplification of mdr1 as a common denominator of resistance to mefloquine, lumefantrine, and artemisinin in *Plasmodium chabaudi* malaria parasites. Antimicrob Agents Chemother. 2011;55:4858–65.
5 Lekana-Douki JB, Dinzouna Boutamba SD, Zatra R, Zang Edou SE, Ekomy H, Bisvigou U, *et al*. Increased prevalence of the *Plasmodium falciparum* Pfmdr1 86N genotype among field isolates from Franceville, Gabon after replacement of chloroquine by artemether-lumefantrine and artesunate-mefloquine. Infect Genet Evol. 2011;11:512–7.
6 Preechapornkul P, Imwong M, Chotivanich K, Pongtavornpinyo W, Dondorp AM, Day NP, *et al*. *Plasmodium falciparum* pfmdr1 amplification, mefloquine resistance, and parasite fitness. Antimicrob Agents Chemother. 2009;53:1509–15.
7 Roepe PD. Molecular and physiologic basis of quinoline drug resistance in *Plasmodium falciparum* malaria. Future Microbiol. 2009;4:441–55.
8 Eyase FL, Akala HM, Ingasia L, Cheruiyot A, Omondi A, Okudo C, *et al*. The role of Pfmdr1 and Pfcrt in changing chloroquine, amodiaquine, mefloquine and lumefantrine susceptibility in western-Kenya *P. falciparum* samples during 2008–2011. PloS One. 2013;8:e64299.

9 Povoa MM, Adagu IS, Oliveira SG, Machado RL, Miles MA, Warhurst DC. Pfmdr1 Asn1042Asp and Asp1246Tyr polymorphisms, thought to be associated with chloroquine resistance, are present in chloroquine-resistant and -sensitive Brazilian field isolates of *Plasmodium falciparum*. Exp Parasitol. 1998;88:64–8.
10 Sidhu AB, Valderramos SG, Fidock DA. pfmdr1 mutations contribute to quinine resistance and enhance mefloquine and artemisinin sensitivity in *Plasmodium falciparum*. Mol Microbiol. 2005;57:913–26.
11 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. Infect Genet Evol. 2007;7:562–9.
12 Ngo T, Duraisingh M, Reed M, Hipgrave D, Biggs B, Cowman AF. Analysis of pfcrt, pfmdr1, dhfr, and dhps mutations and drug sensitivities in *Plasmodium falciparum* isolates from patients in Vietnam before and after treatment with artemisinin. Am J Trop Med Hyg. 2003;68:350–6.
13 Alker AP, Lim P, Sem R, Shah NK, Yi P, Bouth DM, *et al*. Pfmdr1 and in vivo resistance to artesunate-mefloquine in *falciparum* malaria on the Cambodian–Thai border. Am J Trop Med Hyg. 2007;76:641–7.
14 Wilson CM, Volkman SK, Thaithong S, Martin RK, Kyle DE, Milhous WK, *et al*. Amplification of pfmdr 1 associated with mefloquine and halofantrine resistance in *Plasmodium falciparum* from Thailand. Mol Biochem Parasitol. 1993;57:151–60.
15 Price R, van Vugt M, Phaipun L, Luxemburger C, Simpson J, McGready R, *et al*. Adverse effects in patients with acute *falciparum* malaria treated with artemisinin derivatives. Am J Trop Med Hyg. 1999;60:547–55.
16 Phompradit P, Wisedpanichkij R, Muhamad P, Chaijaroenkul W, Na-Bangchang K. Molecular analysis of pfatp6 and pfmdr1 polymorphisms and their association with in vitro sensitivity in *Plasmodium falciparum* isolates from the Thai–Myanmar border. Acta Trop. 2011;120:130–5.
17 Bereczky S, Martensson A, Gil JP, Farnert A. Short report: Rapid DNA extraction from archive blood spots on filter paper for genotyping of *Plasmodium falciparum*. Am J Trop Med Hyg. 2005;72:249–51.
18 Dokomajilar C, Nsobya SL, Greenhouse B, Rosenthal PJ, Dorsey G. Selection of *Plasmodium falciparum* pfmdr1 alleles following therapy with artemether-lumefantrine in an area of Uganda where malaria is highly endemic. Antimicrob Agents Chemother. 2006;50:1893–5.
19 Holmgren G, Bjorkman A, Gil JP. Amodiaquine resistance is not related to rare findings of pfmdr1 gene amplifications in Kenya. Trop Med Int Health. 2006;11:1808–12.
20 Sisowath C, Ferreira PE, Bustamante LY, Dahlström S, Mårtensson A, Björkman A, *et al*. The role of pfmdr1 in *Plasmodium falciparum* tolerance to artemether-lumefantrine in Africa. Trop Med Int Health. 2007;12:736–42.
21 Price RN, Uhlemann AC, van VugtM, Brockman A, Hutagalung R, Nair S, *et al*. Molecular and pharmacological determinants of the therapeutic response to artemether-lumefantrine in multidrug-resistant *Plasmodium falciparum* malaria. Clin Infect Dis. 2006;42:1570–7.
22 Picot S, Olliaro P, de Monbrison F, Bienvenu AL, Price RN, Ringwald P. A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in *falciparum* malaria. Malaria J. 2009;8:89.
23 Sisowath C, Stromberg J, Martensson A, Msellem M, Obondo C, Bjorkman A, *et al*. In vivo selection of *Plasmodium falciparum* pfmdr1 86N coding alleles by artemether-lumefantrine (Coartem). J Infect Dis. 2005;191:1014–7.
24 Hastings IM, Ward SA. Coartem (artemether-lumefantrine) in Africa: the beginning of the end? J Infect Dis. 2005;192. author reply 1304–1305.
25 Tinto H, Guekoun L, Zongo I, Guiguemde RT, D'Alessandro U, Ouedraogo JB. Chloroquine-resistance molecular markers (Pfcrt T76 and Pfmdr-1 Y86) and amodiaquine resistance in Burkina Faso. Trop Med Int Health. 2008;13:238–40.