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Veronika Rae Laird Date

Exploring *Plasmodium falciparum multidrug resistance 1* gene polymorphisms and clinical outcomes after treatment with artemether-lumefantrine, artesunate-amodiaquine, or dihydroartemisinin-piperaquine

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Abstract

Exploring *Plasmodium falciparum multidrug resistance 1* gene polymorphisms and clinical outcomes after treatment with artemether-lumefantrine, artesunate-amodiaquine, or dihydroartemisinin-piperaquine

By Veronika R. Laird

The *Plasmodium falciparum multidrug resistance transporter 1* gene (*pfmdr1*) is associated with altered response to artemisinin-based combination therapies (ACTs), particularly those containing the partner drugs lumefantrine and amodiaquine (i.e., artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ)). Past studies of *pfmdr1* single nucleotide polymorphisms (SNPs) at codons 86, 184, and 1246 have shown different treatment responses to AL and ASAQ. To determine whether patients infected with parasites carrying specific *pfmdr1* SNPs are at increased risk of recurrent parasitemia or treatment failure, patient data on molecular markers of *P. falciparum* from 16 therapeutic efficacy studies in 13 African countries from 2013 to 2019 were analyzed. Conditional logistic regression was used to estimate the exposure odds ratio by treatment arm when the data from all studies were compiled. This exposure odds ratio represented the odds of recurrent infections or recrudescent infections with a specific allele found at baseline compared to the odds of successfully treated infections with that same allele found at baseline. After controlling for study site, the presence of parasites in the initial infection that carried *pfmdr1* N86 (alone or a mixed infection with 86Y) was strongly and negatively associated with recurrent infection occurring between days 14 and 28 after AL treatment (adjusted odds ratio [OR]=0.22, 95% CI=0.05, 0.94, $P < 0.001$). For those treated with ASAQ, the presence of parasites in the initial infection that carried *pfmdr1* N86 (alone or a mixed infection with 86Y) was strongly and negatively associated with recrudescence occurring between days 14 and 28 after ASAQ treatment ([OR]=0.09; 95% CI=0.01, 0.72; $P = 0.02$). There were no statistically significant associations detected between recurrent or recrudescent infections and *pfmdr1* codons 184 and 1246. These findings suggest that administering AL and *pfmdr1* genotype may influence treatment outcome after *P. falciparum* infection.

Exploring *Plasmodium falciparum multidrug resistance 1* gene polymorphisms and clinical outcomes after treatment with artemether-lumefantrine, artesunate-amodiaquine, or dihydroartemisinin-piperaquine

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Table of Contents

1. Introduction

The emergence of *Plasmodium falciparum* resistance to antimalarial drugs poses a challenge to global efforts of controlling malaria. World Health Organization (WHO) Guidelines for Malaria recommend the use of artemisinin combination therapy (ACT) to treat uncomplicated *P. falciparum* infection [1]. The most commonly recommended ACTs vary by region, but those used most regularly in Africa are artemether-lumefantrine (AL), artesunate-amodiaquine (ASAQ), and dihydroartemisinin-piperaquine (DP) [2]. Polymerase chain reaction (PCR)-adjusted efficacy for each ACT remains high throughout most regions [3,4]. However, decreased efficacy to DP and AL have been reported in Asia [5–8] and Africa [9–11] Artemisinin-resistant *P. falciparum* parasites have been found in the Greater Mekong Region over the last decade and recently in Africa [12–14].

Defining the role of the *P. falciparum multidrug resistance transporter 1* gene (*Pfmdr1)* is becoming increasingly important. Although ACT resistance has not been demonstrated with any particular *Pfmdr1* single nucleotide polymorphism (SNP) or haplotype, certain SNPs may alter response to ACT partner drugs such as amodiaquine and lumefantrine [8,15,16]. Five unique SNPs, with amino acid changes in codons 86, 184, 1034, 1042, and 1246 of *pfmdr*1, have been implicated in various regions of the world [16,17]. Several *in vivo* studies in sub-Saharan Africa have found evidence of selection for particular *Pfmdr1* alleles after treatment with AL at codons 86, 184, and 1246 [18,19]. Increased copy number of *Pfmdr1* due to gene duplications has also been associated with reduced susceptibility to lumefantrine [16,20].

In vitro studies of SNPs in *Pfmdr1* have suggested that the 86Y and 1246Y mutations may be associated with decreased response to chloroquine and amodiaquine. These SNPS have also been found in patients who experienced recurrent parasitemia after being treated with

ASAQ [21–23]. Selection of 86Y in recurrent parasites after treatment with amodiaquine or combined with artesunate have been observed in several studies [21–23]. Conversely, N86, 184F, and D1246 alleles have been found in recurrent infections after AL treatment [16,23,24].

Individual therapeutic efficacy studies (TES) often have too small a sample size to statistically power an assessment of the association between parasite genotypes and treatment outcomes. Understanding this association is vital for detecting molecular shifts in parasite populations as indicators of changing parasite susceptibility to lumefantrine or amodiaquine. To examine this, patient data on *in vivo* antimalarial efficacy and molecular markers of *P. falciparum* from 13 countries were analyzed individually and collectively. This study has two main objectives to untangle the relationship between parasite genotypes and treatment outcomes. First, to determine whether patients infected with parasites carrying specific *Pfmdr1* SNPs are at an increased risk of recrudescence. Second, to assess whether patients infected with parasites carrying specific *Pfmdr1* SNPs have increased risk of recurrent infection.

2. Methods

2.1 Selection and inclusion of data

Data for this meta-analysis were compiled from all sixteen individual therapeutic efficacy studies from thirteen countries from 2013-2019 in sub-Saharan Africa, that were funded by the President's Malaria Initiative (PMI) with the Centers for Disease Control and Prevention (CDC) and the United States Agency for International Development (USAID). These studies were chosen to be included because they followed PMI or the World Health Organization's (WHO) molecular analyses guidelines and the data was verified by the PMI CDC team. Children who had symptomatic, clinically diagnosed uncomplicated *P. falciparum* malaria infection that presented at the health facilities in each country and met standard inclusion criteria were welcomed to participate in the study [25–36]. The target age range varied from 6 months to 14 years (168 months) to meet the necessary sample size to power each study. Due to varying transmission intensity, the acceptable initial parasite density ranged from 1000-100,000 parasites/μL with transmission classified as mesoendemic and 2000-200,000 parasites/μL with transmission classified as hyperendemic [25,28]. In addition to parasite density, hemoglobin was measured at enrollment and children having hemoglobin >5 g/dL were eligible for treatment [25].

Study participants were administered either AL (Coartem®; Novartis, Basel, Switzerland), ASAQ (Winthrop; Sanofi Aventis, Paris, France), or DP (Duo-cotecxin®; Holley-Cotec, Beijing, China). Patients given AL or ASAQ were followed for at least 28 days and those treated with DP were followed for 42 days. After follow-up was complete, parasite genotyping of *pfmdr1* was conducted either by PMI with the CDC and USAID, or it was done in-country and the data was shared and verified. Thirteen of these therapeutic efficacy study results were

published in a journal article, two were unpublished country reports, and one is currently under review for journal publication [10,11,13,26–36] (Table 1). Kenya has published the molecular markers found during the therapeutic efficacy study, but the results from the therapeutic efficacy study are currently under review [37]. Anonymized patient data including country, study site, treatment outcome, and parasite genotype data were pulled from these original studies and corresponding datasets. Many of these studies only analyzed randomly selected baseline samples that experienced an adequate and clinical parasitological response (ACPR) while all recurrent infections were analyzed. This under sampling of subjects based on outcome is like a case-control study. Thus, the data has been analyzed by estimating measures of association with the exposure odds ratio which is interpretable as an estimator of the risk odds ratio, as each case-control study is nested within the fixed study cohort [38,39].

2.2 Classification of recrudescent, and recurrent infections

Parasites that recurred (either as a new or recrudescent infection) within the follow-up period were classified as recurrent using the World Health Organization guidelines: microscopically detected infections during follow-up were classified as recurrent; recurrent infections sharing with blood samples taken at day 0 PCR bands in polymorphic merozoite antigens or microsatellite fragment sizes were classified as recrudescent, and recurrent infections not sharing PCR bands or microsatellite fragment sizes with blood samples taken at day 0 were classified as re-infections (new infections) [40]. This was done using either seven neutral microsatellite markers (TA1, Poly-α, PfPK2, TA109, TA2490, C2M34 and C3M69) over six chromosomes [41,42] or using merozoite surface proteins 1 and 2 (*msp1* and *msp2*), and glutamine-rich protein (*glurp*) [40].

Pfmdr1 codons 86, 184, and 1246 were sequenced in parasites collected from enrolled participants and the allele at each codon was recorded. Molecular markers were coded as either single or mixed parasite (infected by more than one parasite) genotypes regarding SNPs. For participants with mixed parasite, there were sequences that showed the presence of N and Y at codon 86, Y and F at codon 184, and D and Y at codon 1246. They were then classified as a mixed infection with N/Y at codon 86, Y/F at codon 184, or D/Y at 1246. For this analysis, mixed infections were reclassified to one allele per codon for each treatment arm. This baseline allele served as the exposure. For participants treated with AL, mixed infections at each codon were classified as the following: 1) N86 2) 184F 3) D1246. Participants treated with ASAQ or DP had mixed infections classified as the following: 1) 86Y 2) 184F 3) 1246Y.

2.3 Statistical analysis

All statistical analyses were conducted using R version 2021.09.1+372 (R Foundation for Statistical Computing, Vienna, Austria). To determine whether patients infected with parasites carrying specific *pfmdr1* SNPs are at increased risk of recurrent (new infections and recrudescent infections) parasitemia, conditional logistic regression was used to estimate the adjusted odds ratio after controlling for study site. A previous meta-analysis assessing this relationship suggested that age and baseline parasitemia were not confounders [16]. They do not meet the definition of confounders by the a priori criteria, either. This adjusted exposure odds ratio represented the odds that a parasite carrying a specific allele was present at baseline in a recurrent infection compared to those odds in an infection successfully cleared by antimalarial treatment. Similarly, conditional logistic regression was also used to investigate whether patients infected parasites carrying specific *pfmdr1* SNPs are at an increased risk of recrudescence after controlling for study site. This adjusted exposure odds ratio represented the odds that a parasite

carrying a specific allele at codon 86, 184, or 1246 was found at baseline in a person with a recrudescent infection compared to those odds in a person whose infection was successfully cleared by antimalarial treatment.

3. Results

3.1 Recurrent infection

After controlling for study site, the presence of parasites in the initial infection that carried *pfmdr1* N86 (alone or a mixed infection with 86Y) was strongly and negatively associated with recurrent infection occurring between days 14 and 28 after AL treatment (adjusted odds ratio [OR]=0.22, 95% CI=0.05, 0.94, $P < 0.001$) (Table 2). Conversely, after controlling for study site, the presence of parasites in the initial infection that carried *pfmdr1* 86Y (alone or mixed with N86) was positively associated with recurrent parasitemia between days 14 to 28 after ASAQ treatment ([OR]=1.98, 95% CI=0.64, 6.16, $P >0.05$) (Table 2). The adjusted odds ratio for the presence of parasites in the initial infection that carried *pfmdr1* 86Y (alone or a mixed infection with N86) had a nearly null association with recurrent infection occurring between days 14 and 28 after DP treatment ([OR]=0.92, 95% CI=0.23, 2.70, $P > 0.05$) (Table 2). The remaining molecular markers, Y184F and D1246Y, were weakly or not associated with recurrence and confidence intervals were wide after controlling for site.

3.2 Recrudescence

After controlling for study site, the presence of parasites in the initial infection that carried *pfmdr1* N86 (alone or a mixed infection with 86Y) was strongly and negatively associated with recrudescence occurring between days 14 and 28 after AL treatment ([OR]=0.09; 95% CI=0.01, 0.72; $P = 0.02$) (Table 3). For those treated with ASAQ, after controlling for study site, the presence of parasites in the initial infection that carried *pfmdr1* 86Y (alone or a mixed infection with N86) was positively associated with recrudescence occurring between days 14 and 28 after ASAQ treatment ([OR]=1.54; 95% CI=0.13, 18.20; $P = 0.73$) (Table 3). The adjusted odds ratio for the DP treatment arm examining the relationship between recrudescence and 86Y

yielded an unstable estimate. The *mdr1* codon 184 was negatively associated with recurrent infection for all treatment types, but confidence intervals included the null. The adjusted odds ratios examining the relationship between the *mdr1* codon 1246 and recrudescenes yielded unstable estimates, likely because recrudescence is a rare outcome.

4. Discussion

This pooled analysis of 16 therapeutic efficacy studies strongly suggests that genotypes of infecting parasites is associated with and may influence the outcome of AL treatment. Patients infected with parasites that carried the N86 allele at baseline and were treated with AL had substantially greater odds of clearing the infection than having a recurrent infection, compared to those who had the 86Y allele at baseline. Similarly, patients infected with parasites that carried the N86 at baseline and were treated with AL had significantly greater odds of clearing the infection than having a recrudescent infection. We found little or no association between parasite alleles at codons 86, 184, 1246 at baseline and recurrent or recrudescent infection for those treated with ASAQ and DP, but confidence intervals were wide – a reflection of relatively the little comparative information after controlling for study site for many of the combinations of alleles and treatments that we studied.

A previous meta-analysis pooled data from 31 clinical trials from East Africa, West Africa, and Asia/Oceania and did not include any of the studies used in this analysis. They discovered that the presence of parasites at baseline that carried *pfmdr1* N86 (alone or with a mixed infection with 86Y) was a significant risk factor for recrudescent infection for those treated with AL (n = 2.543; adjusted hazards ratio [AHR] = 4.74; 95% CI = 2.29, 9.78; $P < 0.001$) [16]. That finding conflicts with what was found during this pooled analysis likely because their sample size was larger, and they had more occurrences of recrudescence. They utilized Cox proportional hazards regression models that controlled for age, baseline parasite density, and total lumefantrine dose. Information on these variables were not available in the data used for our analysis which could explain why our findings suggest the opposite, even though age and baseline parasitemia were not identified as confounders [16].

Similar to this analysis, the previous study did not find a significant association between baseline alleles at *pfmdr1* codons 86, 184, and 1246 and recurrent or recrudescent infections for those treated with ASAQ [16]. They did not present the adjusted hazard ratios of that analysis, but showed that 86Y was significantly associated with recurrent and recrudescent infections after treatment with ASAQ [16]. Our analyses support the conclusion that the presence of parasites at baseline that carried *pfmdr1* 86Y (alone or with a mixed infection) was a risk factor for reinfection and recrudescent infection for those treated with ASAQ, but confidence intervals included the null.

At present, no studies have been conducted to investigate the association between the baseline genotype at *pfmdr1* codons 86, 184, and 1246 and treatment outcome for those treated with DP. However, some studies have suggested that DP exerts a similar selection pressure on *pfmdr1* codons in the same direction as ASAQ [43,44]. From our findings, the adjusted odds ratios for DP are in the same direction as ASAQ for carrying *pfmdr1* 1246Y. Such results would suggest that, if a parasite is carrying 1246Y at baseline, then they are more likely to experience a recurrent or recrudescent infection when treated with DP or ASAQ.

This analysis included a compilation of multiple TESs funded by the President's Malaria Initiative with the Centers for Disease Control and Prevention and followed the WHO guidelines meaning that each study had very similar inclusion and exclusion criteria and consistency regarding how samples were genotyped. This analysis included study site(s) within each country as a confounder to achieve more precise, unbiased estimates. Additionally, DP had not been included in prior studies that investigated the association between *pfmdr1* alleles found at baseline and treatment outcome. DP was only in included in studies that looked at selection of *pfmdr1* codons 86, 184, and 1246 between pre- and post-treatment and found no evidence of

selection for treatment failures (codon 86: *P*=0.85, codon 184: *P*=0.54, codon 1246: *P*=0.50) [45] . This is still important information, if confirmed, because it would suggest that DP could be used in areas where there is concern about resistance to AL.

Unfortunately, there was unequal genotyping for almost every TES included in this analysis. While all recurrent infections were analyzed for molecular markers, only a handful of randomly selected successful treatments were genotyped. This sampling bias should not have impacted the validity of the odds ratio estimates as the study was analyzed as a case-control study. Had all people with a successful outcome been genotyped (and included) in this study, sample sizes would have be larger and the estimates more stable. Additionally, prior studies have shown that *pfmdr1* copy number is associated with treatment outcome, but that data was not reported by every country included in the analysis [16,46,47]. Including data on *pfmdr1* copy number could have provided further evidence that the gene *pfmdr1* is associated with recurrent and recrudescent infections.

These results suggest that *pfmdr1* N86 may be negatively associated with recurrent and recrudescent infections for those treated with AL even though prior studies found the opposite. While this analysis had limitations, this study is one of only two pooled-data analyses that have examined the relationship between *pfmdr1* alleles and clinical outcome by treatment arm through compiling studies rather than only on the country level. Our findings contrast with a previous meta-analysis, suggesting that further studies are needed to clarify and, perhaps, fully account for the case-control nature of some of the studies involved. This is possible as therapeutic efficacy studies are conducted worldwide and published each year. However, it is important that future therapeutic efficacy studies genotype the samples from all treatment success, treatment failures, and detail a plan to account for mixed infections at baseline. This would reduce the potential for

sampling bias and help provide a full picture when conducting analyses like the one done in this study.

Finally, there is a fear that resistance to ACTs will arise in sub-Saharan Africa, the continent most affected by malaria, as resistance to ACTs have been confirmed in Southeast Asia [47–51]. To monitor resistance to *pfmdr1* alleles, further surveillance of the prevalence of the alleles of at least *pfmdr1* codon 86 during therapeutic efficacy study sites could be done routinely which would facilitate additional meta-analyses of therapeutic efficacy studies.

5. References

1. World Health Organization. WHO Guidelines for malaria [Internet]. 2021 [cited 2021 Dec 14]. Available from: https://www.who.int/publications-detail-redirect/guidelines-for-malaria

2. World Health Organization. World malaria report 2020 [Internet]. 2020 [cited 2021 Dec 14]. Available from: https://www.who.int/publications-detail-redirect/9789240015791

3. Derbie A, Mekonnen D, Adugna M, Yeshitela B, Woldeamanuel Y, Abebe T. Therapeutic Efficacy of Artemether-Lumefantrine (Coartem®) for the Treatment of Uncomplicated Falciparum Malaria in Africa: A Systematic Review. J Parasitol Res. 2020;2020:7371681.

4. Shibeshi W, Alemkere G, Mulu A, Engidawork E. Efficacy and safety of artemisinin-based combination therapies for the treatment of uncomplicated malaria in pediatrics: a systematic review and meta-analysis. BMC Infect Dis. 2021;21:326.

5. Veiga MI, Dhingra SK, Henrich PP, Straimer J, Gnädig N, Uhlemann A-C, et al. Globally prevalent PfMDR1 mutations modulate Plasmodium falciparum susceptibility to artemisinin-based combination therapies. Nat Commun. 2016;7:11553.

6. Song J, Socheat D, Tan B, Seila S, Xu Y, Ou F, et al. Randomized trials of artemisinin-piperaquine, dihydroartemisinin-piperaquine phosphate and artemether-lumefantrine for the treatment of multi-drug resistant falciparum malaria in Cambodia-Thailand border area. Malar J. 2011;10:231.

7. Pluijm RW van der, Imwong M, Chau NH, Hoa NT, Thuy-Nhien NT, Thanh NV, et al. Determinants of dihydroartemisinin-piperaquine treatment failure in Plasmodium falciparum malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. Lancet Infect Dis. Elsevier; 2019;19:952–61.

8. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of Artemisinin Resistance in Plasmodium falciparum Malaria. N Engl J Med. 2014;371:411–23.

9. Gansané A, Moriarty LF, Ménard D, Yerbanga I, Ouedraogo E, Sondo P, et al. Anti-malarial efficacy and resistance monitoring of artemether-lumefantrine and dihydroartemisinin-piperaquine shows inadequate efficacy in children in Burkina Faso, 2017–2018. Malar J. 2021;20:48.

10. Dimbu PR, Horth R, Cândido ALM, Ferreira CM, Caquece F, Garcia LEA, et al. Continued Low Efficacy of Artemether-Lumefantrine in Angola in 2019. Antimicrob Agents Chemother [Internet]. American Society for Microbiology; 2020 [cited 2021 Dec 14]; Available from: https://journals.asm.org/doi/abs/10.1128/AAC.01949-20

11. Moriarty LF, Nkoli PM, Likwela JL, Mulopo PM, Sompwe EM, Rika JM, et al. Therapeutic Efficacy of Artemisinin-Based Combination Therapies in Democratic Republic of the Congo and Investigation of Molecular Markers of Antimalarial Resistance. Am J Trop Med Hyg. 2021;tpmd210214.

12. Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana S-I, Yamauchi M, et al. Evidence of Artemisinin-Resistant Malaria in Africa. N Engl J Med. 2021;385:1163–71.

13. Uwimana A, Umulisa N, Venkatesan M, Svigel SS, Zhou Z, Munyaneza T, et al. Association of Plasmodium falciparum kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. Lancet Infect Dis. Elsevier; 2021;21:1120–8.

14. World Health Organization. Report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010-2019) [Internet]. [cited 2021 Dec 14]. Available from: https://www.who.int/publications-detail-redirect/9789240012813

15. Malmberg M, Ferreira PE, Tarning J, Ursing J, Ngasala B, Björkman A, et al. Plasmodium falciparum Drug Resistance Phenotype as Assessed by Patient Antimalarial Drug Levels and Its Association With pfmdr1 Polymorphisms. J Infect Dis. 2013;207:842–7.

16. Venkatesan M, Gadalla NB, Stepniewska K, Dahal P, Nsanzabana C, Moriera C, 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;91:833–43.

17. Humphreys GS, Merinopoulos I, Ahmed J, Whitty CJM, Mutabingwa TK, Sutherland CJ, et al. Amodiaquine and Artemether-Lumefantrine Select Distinct Alleles of the Plasmodium falciparum mdr1 Gene in Tanzanian Children Treated for Uncomplicated Malaria. Antimicrob Agents Chemother [Internet]. American Society for Microbiology; 2007 [cited 2021 Dec 14]; Available from: https://journals.asm.org/doi/abs/10.1128/AAC.00875-06

18. Baliraine FN, Rosenthal PJ. Prolonged Selection of pfmdr1 Polymorphisms After Treatment of Falciparum Malaria With Artemether-Lumefantrine in Uganda. J Infect Dis. 2011;204:1120–4.

19. Maiga H, Grivoyannis A, Sagara I, Traore K, Traore OB, Tolo Y, et al. Selection of pfcrt K76 and pfmdr1 N86 Coding Alleles after Uncomplicated Malaria Treatment by Artemether-Lumefantrine in Mali. Int J Mol Sci. Multidisciplinary Digital Publishing Institute; 2021;22:6057.

20. Price RN, Uhlemann A-C, van Vugt M, 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.

21. Happi CT, Gbotosho GO, Folarin OA, Bolaji OM, Sowunmi A, Kyle DE, et al. Association between mutations in Plasmodium falciparum chloroquine resistance transporter and P. falciparum multidrug resistance 1 genes and in vivo amodiaquine resistance in P. falciparum malaria-infected children in Nigeria. Am J Trop Med Hyg. 2006;75:155–61.

22. Sondo P, Derra K, Nakanabo SD, Tarnagda Z, Kazienga A, Zampa O, et al. Artesunate-Amodiaquine and Artemether-Lumefantrine Therapies and Selection of Pfcrt and Pfmdr1 Alleles in Nanoro, Burkina Faso. PLOS ONE. Public Library of Science; 2016;11:e0151565.

23. Otienoburu SD, Maïga-Ascofaré O, Schramm B, Jullien V, Jones JJ, Zolia YM, et al. Selection of Plasmodium falciparum pfcrt and pfmdr1 polymorphisms after treatment with artesunate–amodiaquine fixed dose combination or artemether–lumefantrine in Liberia. Malar J. 2016;15:452.

24. 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. John Wiley & Sons, Ltd; 2007;12:736–42.

25. World Health Organization. Methods for surveillance of antimalarial drug efficacy [Internet]. World Health Organization; 2009. Available from: https://apps.who.int/iris/handle/10665/44048

26. Davlantes E, Dimbu PR, Ferreira CM, Florinda Joao M, Pode D, Félix J, et al. Efficacy and safety of artemether–lumefantrine, artesunate–amodiaquine, and dihydroartemisinin–piperaquine for the treatment of uncomplicated Plasmodium falciparum malaria in three provinces in Angola, 2017. Malar J. 2018;17:144.

27. Plucinski MM, Dimbu PR, Macaia AP, Ferreira CM, Samutondo C, Quivinja J, et al. Efficacy of artemether–lumefantrine, artesunate–amodiaquine, and dihydroartemisinin–piperaquine for treatment of uncomplicated Plasmodium falciparum malaria in Angola, 2015. Malar J. 2017;16:62.

28. Plucinski MM, Talundzic E, Morton L, Dimbu PR, Macaia AP, Fortes F, et al. Efficacy of Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine for Treatment of Uncomplicated Malaria in Children in Zaire and Uíge Provinces, Angola. Antimicrob Agents Chemother [Internet]. American Society for Microbiology; 2014 [cited 2021 Dec 14]; Available from: https://journals.asm.org/doi/abs/10.1128/AAC.04181-14

29. Beavogui AH, Camara A, Delamou A, Diallo MS, Doumbouya A, Kourouma K, et al. Efficacy and safety of artesunate–amodiaquine and artemether–lumefantrine and prevalence of molecular markers associated with resistance, Guinea: an open-label two-arm randomised controlled trial. Malar J. 2020;19:223.

30. Dentinger CM, Rakotomanga TA, Rakotondrandriana A, Rakotoarisoa A, Rason MA, Moriarty LF, et al. Efficacy of artesunate-amodiaquine and artemether-lumefantrine for uncomplicated Plasmodium falciparum malaria in Madagascar, 2018. Malar J. 2021;20:432.

31. Diarra Y, Koné O, Sangaré L, Doumbia L, Haidara DBB, Diallo M, et al. Therapeutic efficacy of artemether-lumefantrine and artesunate-amodiaquine for the treatment of uncomplicated Plasmodium falciparum malaria in Mali, 2015-2016. RTI International. P.O. Box 12194, Research Triangle Park, NC 27709-2194. Tel: 919-541-6000; e-mail: publications@rit.org; Web site: http://www.rti.org; 2021 [cited 2021 Dec 14]; Available from: https://www.rti.org/publication/therapeutic-efficacy-artemether-lumefantrine-and-artesunate-amodiaquine-treatment

32. Ishengoma DS, Mandara CI, Francis F, Talundzic E, Lucchi NW, Ngasala B, et al. Efficacy and safety of artemether-lumefantrine for the treatment of uncomplicated malaria and prevalence of Pfk13 and Pfmdr1 polymorphisms after a decade of using artemisinin-based combination therapy in mainland Tanzania. Malar J. 2019;18:88.

33. Nhama A, Nhamússua L, Macete E, Bassat Q, Salvador C, Enosse S, et al. In vivo efficacy and safety of artemether–lumefantrine and amodiaquine–artesunate for uncomplicated Plasmodium falciparum malaria in Mozambique, 2018. Malar J. 2021;20:390.

34. Ljolje D, Dimbu PR, Kelley J, Goldman I, Nace D, Macaia A, et al. Prevalence of molecular markers of artemisinin and lumefantrine resistance among patients with uncomplicated Plasmodium falciparum malaria in three provinces in Angola, 2015. Malar J. 2018;17:84.

35. Chidimatembue A, Svigel SS, Mayor A, Aíde P, Nhama A, Nhamussua L, et al. Molecular surveillance for polymorphisms associated with artemisinin-based combination therapy resistance in Plasmodium falciparum isolates collected in Mozambique, 2018. Malar J. 2021;20:398.

36. Ebong C, Sserwanga A, Namuganga JF, Kapisi J, Mpimbaza A, Gonahasa S, et al. Efficacy and safety of artemether-lumefantrine and dihydroartemisinin-piperaquine for the treatment of uncomplicated Plasmodium falciparum malaria and prevalence of molecular markers associated with artemisinin and partner drug resistance in Uganda. Malar J. 2021;20:484.

37. Chebore W, Zhou Z, Westercamp N, Otieno K, Shi YP, Sergent SB, et al. Assessment of molecular markers of anti-malarial drug resistance among children participating in a therapeutic efficacy study in western Kenya. Malar J. 2020;19:291.

38. Flanders WD, Louv WC. The exposure odds ratio in nested case-control studies with competing risks. Am J Epidemiol. 1986;124:684–92.

39. Miettinen O. Design options in epidemiologic research. An update. Scand J Work Environ Health. 1982;8 Suppl 1:7–14.

40. Organization WH. Methods and techniques for clinical trials on antimalarial drug efficacy : genotyping to identify parasite populations : informal consultation organized by the Medicines for Malaria Venture and cosponsored by the World Health Organization, 29-31 May 2007, Amsterdam, The Netherlands [Internet]. World Health Organization; 2008. Available from: https://apps.who.int/iris/handle/10665/43824

41. GREENHOUSE B, MYRICK A, DOKOMAJILAR C, WOO JM, CARLSON EJ, ROSENTHAL PJ, et al. VALIDATION OF MICROSATELLITE MARKERS FOR USE IN GENOTYPING POLYCLONAL PLASMODIUM FALCIPARUM INFECTIONS. Am J Trop Med Hyg. 2006;75:836–42.

42. Nyachieo A, VAN Overmeir C, Laurent T, Dujardin J-C, D'Alessandro U. Plasmodium falciparum genotyping by microsatellites as a method to distinguish between recrudescent and new infections. Am J Trop Med Hyg. 2005;73:210–3.

43. Taylor SM, Juliano JJ. Artemisinin Combination Therapies and Malaria Parasite Drug Resistance: The Game Is Afoot. J Infect Dis. 2014;210:335–7.

44. Conrad MD, LeClair N, Arinaitwe E, Wanzira H, Kakuru A, Bigira V, et al. Comparative impacts over 5 years of artemisinin-based combination therapies on Plasmodium falciparum polymorphisms that modulate drug sensitivity in Ugandan children. J Infect Dis. 2014;210:344–53.

45. Somé AF, Séré YY, Dokomajilar C, Zongo I, Rouamba N, Greenhouse B, et al. Selection of Known Plasmodium falciparum Resistance-Mediating Polymorphisms by Artemether-Lumefantrine and Amodiaquine- Sulfadoxine-Pyrimethamine but Not Dihydroartemisinin-Piperaquine in Burkina Faso. Antimicrob Agents Chemother. 2010;54:1949–54.

46. Lim P, Alker AP, Khim N, Shah NK, Incardona S, Doung S, et al. Pfmdr1 copy number and arteminisin derivatives combination therapy failure in falciparum malaria in Cambodia. Malar J. 2009;8:11.

47. Price RN, Uhlemann A-C, Brockman A, McGready R, Ashley E, Phaipun L, et al. Mefloquine resistance in Plasmodium falciparum and increased pfmdr1 gene copy number. Lancet Lond Engl. 2004;364:438–47.

48. Noedl H, Se Y, Sriwichai S, Schaecher K, Teja-Isavadharm P, Smith B, et al. Artemisinin Resistance in Cambodia: A Clinical Trial Designed to Address an Emerging Problem in Southeast Asia. Clin Infect Dis. 2010;51:e82–9.

49. Rosenthal PJ. Artemisinin Resistance Outside of Southeast Asia. Am J Trop Med Hyg. 2018;99:1357–9.

50. Ouji M, Augereau J-M, Paloque L, Benoit-Vical F. Plasmodium falciparum resistance to artemisinin-based combination therapies: A sword of Damocles in the path toward malaria elimination. Parasite. 25:24.

51. 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. American Society of Tropical Medicine and Hygiene; 2007;76:641–7.

6. Tables

Table 1. Summary of antimalarial therapeutic efficacy studies, 13 countries in Africa, 2013-2019*

Country	Sites	ACT(s) studied	Year(s) of study	Age of patients enrolled	Total numer of samples	Genotyping methodology	Publication
Angola	Uige, Zaire	AL, ASAQ, DP	2013	6 mo-9 y	16	Microsatellites	[10]
	Benguela	AL, ASAQ	2015	6 mo-12 y	157	Microsatellites	[25]
	Lunda Sul, Zaire	AL, ASAQ, DP	2015	6 mo-59 mo	239	Microsatellites	[25]
	Benguela	AL, ASAQ	2017	6 mo-12 y	6	Microsatellites	[26]
	Lunda Sul, Zaire	AL, ASAQ, DP	2017	6 mo-59 mo	21	Microsatellites	[26]
	Benguela	AL, ASAQ	2019	6 mo-12 y	9	Microsatellites	[27]
	Lunda Sul, Zaire	AL, ASAQ, DP	2019	6 mo-59 mo	20	Microsatellites	[27]
Benin	Klouékanmey, Djougou	AL	2017	6 mo-59 mo	117	Microsatellites	Country report
DRC	Kabondo, Kapolowe, Kimpese, Mikalayi, Rutshuru	AL, ASAQ, DP	2017–2018	6 mo-59 mo	206	Microsatellites	[11]
Ethiopia	Arbaminch, Pawe	AL, DP	2017	>6 mo	48	Microsatellites	Pre-publication
Guinea	Labé, Maferinyah	AL, ASAQ	2016	6 mo-59 mo	405	Microsatellites	[28]
Kenya	Siaya	AL, DP	2016–2017	6 mo-59 mo	284	*msp1*, *msp2*, *glurp*	Pre-publication, [29]
Madagascar	Ankazomborona, Antensenavolo, Kianjavato, Matanga, Vohitromby	AL, ASAQ	2018	6 mo-14 y	151	Microsatellites	[30]
Mali	Sélingué	AL, ASAQ	2015–2016	6 mo-59 mo	66	*msp1*, *msp2*, *glurp*	[31]
Mozambique	Massinga, Moatize, Montepuez Mopeia	AL, ASAQ	2018	6 mo-59 mo	101	Microsatellites	[33]
Rwanda	Bugarama, Masaka, Rukara	AL	2018	6 mo-59 mo	208	Microsatellites	[13]
Tanzania	Kibaha, Mkuzi, Mlimba, Ujiji	AL	2016	6 mo-10 y	57	Microsatellites	[32]
Uganda	Aduku, Arua, Masafu	AL, DP	2018–2019	6 mo-10 y	145	Microsatellites	[36]
Zambia	Gwembe, Katete, Mansa	AL, ASAQ, DP	2016	>6 mo	228	*msp1*, *msp2*, *glurp*	Country report

*ACTs, artemisinin-based combination therapies; AL, artemether-lumefantrine; ASAQ, artesunate-amodiaquine; DP, dihydroartemisinin-piperaquine; *msp1*, merozoite surface protein-1; *msp2*, merozoite surface protein-2; *glurp*, glutamate-rich protein; DRC, Democratic Republic of the Congo

Table 2. Adjusted odds ratios for recurrent infection vs. ACPR by treatment and SNP

Marker	Genotype classified as exposure	Cases exposed	Cases unexposed	Controls exposed	Controls unexposed	Treatment	Adjusted model		
							OR	95% CI	P-value
pfmdr1[†]									
N86Y									
	N	586	37	743	88	AL	0.22	(0.05, 0.94)	**<0.001**
	Y	40	55	68	347	ASAQ	1.98	(0.64, 6.16)	0.24
	Y	56	120	21	267	DP	0.92	(0.23, 3.70)	0.91
Y184F									
	F	327	299	465	368	AL	1.13	(0.79, 1.62)	0.48
	Y	69	27	218	197	ASAQ	0.85	(0.33, 2.19)	0.74
	Y	135	41	182	106	DP	1.15	(0.60, 2.21)	0.68
D1246Y									
	D	602	17	800	19	AL	1.17	(0.39, 3.56)	0.67
	Y	21	78	7	395	ASAQ	3.09	(0.29, 32.43)	0.35
	Y	22	154	9	279	DP	1.29	(0.33, 5.00)	0.71

[†]*pfmdr1*=Plasmodium falciparum multi drug resistance 1 (pfmdr1) gene
ACPR, adequate and clinical parsitological response; SNP, single nucleotide polymorphism; AL, artemether-lumefantrine; ASAQ, artesunate-amodiaquine; DP, dihydroartemisinin-piperaquine

Table 3. Adjusted odds ratios for recrudescence vs. ACPR by treatment and SNP

Marker	Genotype classified as exposure	Cases exposed	Cases unexposed	Controls exposed	Controls unexposed	Treatment	Adjusted model		
							OR	95% CI	P-value
pfmdr1[†]									
N86Y									
	N	129	5	743	88	AL	0.09	(0.01, 0.72)	**0.02**
	Y	9	12	68	347	ASAQ	1.54	(0.13, 18.20)	0.73
	Y	9	22	21	267	DP	-	-	-
Y184F									
	F	75	59	465	368	AL	0.84	(0.47, 1.49)	0.55
	Y	14	7	218	197	ASAQ	0.13	(0.14, 1.26)	0.08
	Y	25	6	182	106	DP	0.94	(0.26, 3.45)	0.92
D1246Y									
	D	129	3	800	19	AL	-	-	-
	Y	4	17	7	395	ASAQ	-	-	-
	Y	2	29	9	279	DP	-	-	-

[†]*pfmdr1*=Plasmodium falciparum multi drug resistance 1 (pfmdr1) gene.
ACPR, adequate and clinical parsitological response; SNP, single nucleotide polymorphism; AL, artemether-lumefantrine; ASAQ, artesunate-amodiaquine; DP, dihydroartemisinin-piperaquine; -, results unstable, not presented due to small number of people at some combination of exposure and case-control status