HAL open science

Are k13 and plasmepsin II genes, involved in Plasmodium falciparum resistance to artemisinin derivatives and piperaquine in Southeast Asia, reliable to monitor resistance surveillance in Africa?

Francis Foguim Tsombeng, Mathieu Gendrot, Marie Gladys Robert, Marylin Madamet, Bruno Pradines

To cite this version:

Francis Foguim Tsombeng, Mathieu Gendrot, Marie Gladys Robert, Marylin Madamet, Bruno Pradines. Are k13 and plasmepsin II genes, involved in Plasmodium falciparum resistance to artemisinin derivatives and piperaquine in Southeast Asia, reliable to monitor resistance surveillance in Africa?. Malaria Journal, 2019, 18 (1), pp.285. 10.1186/s12936-019-2916-6 . hal-02272228

HAL Id: hal-02272228

https://hal.science/hal-02272228v1

Submitted on 23 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License

Foguim Tsombeng *et al. Malar J* (2019) 18:285
https://doi.org/10.1186/s12936-019-2916-6

Malaria Journal

OPINION

Open Access

Are *k13* and *plasmepsin II* genes, involved in *Plasmodium falciparum* resistance to artemisinin derivatives and piperaquine in Southeast Asia, reliable to monitor resistance surveillance in Africa?

Francis Foguim Tsombeng[1,2,3], Mathieu Gendrot[1,2,3], Marie Gladys Robert[1,2,3], Marylin Madamet[1,2,3,4] and Bruno Pradines[1,2,3,4*]

Abstract

Mutations in the propeller domain of *Plasmodium falciparum kelch 13* (*Pfk13*) gene are associated with artemisinin resistance in Southeast Asia. Artemisinin resistance is defined by increased ring survival rate and delayed parasite clearance half-life in patients. Additionally, an amplification of the *Plasmodium falciparum plasmepsin II* gene (*pfpm2*), encoding a protease involved in hemoglobin degradation, has been found to be associated with reduced in vitro susceptibility to piperaquine in Cambodian *P. falciparum* parasites and with dihydroartemisinin–piperaquine failures in Cambodia. The World Health Organization (WHO) has recommended the use of these two genes to track the emergence and the spread of the resistance to dihydroartemisinin–piperaquine in malaria endemic areas. Although the resistance to dihydroartemisinin–piperaquine has not yet emerged in Africa, few reports on clinical failures suggest that *k13* and *pfpm2* would not be the only genes involved in artemisinin and piperaquine resistance. It is imperative to identify molecular markers or drug resistance genes that associate with artemisinin and piperaquine in Africa. *K13* polymorphisms and *Pfpm2* copy number variation analysis may not be sufficient for monitoring the emergence of dihydroartemisinin–piperaquine resistance in Africa. But, these markers should not be ruled out for tracking the emergence of resistance.

Keywords: Malaria, *Plasmodium falciparum*, Anti-malarial drug, Resistance, In vitro, Dihydroartemisinin, Piperaquine, Plasmepsin II, K13

Background

According to the World Health Organization (WHO), artemisinin-based combination therapy (ACT) has been recommended as treatment of uncomplicated falciparum malaria since 2001. However, *Plasmodium falciparum* parasites resistant to artemisinin derivatives emerged in Southeast Asia, and more particularly in western Cambodia, Myanmar, Thailand and Laos [1–6]. More recently, the emergence of *P. falciparum* resistance to dihydroartemisinin–piperaquine was observed in Cambodia, where recrudescent infections had rapidly increased [7–9], and then in Vietnam [10, 11]. However, dihydroartemisinin–piperaquine is little-used in African countries for the treatment of uncomplicated malaria, where artemether–lumefantrine and/or artesunate–amodiaquine are currently used. Only Senegal has adopted dihydroartemisinin–piperaquine as a third alternative first-line regimen. Dihydroartemisinin–piperaquine has

*Correspondence: bruno.pradines@gmail.com
[1] Unité Parasitologie et Entomologie, Département Microbiologie et maladies infectieuses, Institut de Recherche Biomédicale des Armées, 19-21 Boulevard Jean Moulin, 13005 Marseille, France
Full list of author information is available at the end of the article

© The Author(s) 2019. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

emerged as a potential combination for chemoprevention in pregnant women and children in Africa [12–14].

According to the WHO, the resistance to dihydroartemisinin–piperaquine has not yet emerged in Africa. There is currently no evidence of failing efficacy of dihydroartemisinin–piperaquine in Africa. The latest published studies showed that PCR-corrected adequate clinical and parasitological response (APCR) at day 42 ranged between 94.6 and 100% for the treatment of uncomplicated *P. falciparum* malaria in children treated between 2011 and 2017 in Africa (Tanzania, Rwanda, Mali, Guinea, Burkina Faso, Angola, Niger) [15–20]. However, there are some rare cases of clinical failures with dihydroartemisinin–piperaquine in Africa [15]. Two cases of late treatment failure after 30 and 32 days were reported in Italian travelers returning from Ethiopia and treated with dihydroartemisinin–piperaquine [21, 22]. Additionally, some clinical failures in travelers returning from Africa, and confirmed by an expected plasmatic level of dihydroartemisinin–piperaquine, were obtained in the French national reference centre for malaria (unpublished personal data). The genes involved in resistance to artemisinin derivatives and piperaquine in Southeast Asia do not properly explain these few clinical failures observed in Africa [22–27]. It is imperative to monitor the emergence of dihydroartemisinin–piperaquine resistance in Africa. But, are *k13* and *plasmepsin II* genes reliable to survey resistance in Africa?

Artemisinin derivative resistance

The emergence and spread of resistance to artemisinin derivatives were observed in Southeast Asia [1–6]. This resistance was associated with delayed parasite clearance half-lives (> 5 h) after artemisinin-based monotherapy treatment or ACT [1, 4, 28, 29]. Additionally, slow in vivo parasite clearance half-live was correlated with in vitro resistance manifested an increase in the ring-stage survival rate after contact with 700 nM of artemisinin for 6 h, evaluated with a new phenotypic assay, the in vitro ring-stage survival assay or RSA [30–33].

Different molecular markers, associated with in vitro resistance to artemisinin derivatives measured by standard phenotypic assays, were previously proposed. Polymorphisms in the *pfATPase6* gene, encoding the *P. falciparum* sarco-endoplasmic reticulum calcium-ATPase PfATPase 6 protein, were first associated with in vitro resistance [34], but not with in vivo delayed parasite clearance in *P. falciparum* parasites from the Thai-Cambodia border [35]. Amplification of the *P. falciparum* multidrug resistance 1 gene (*pfmdr1*) was also associated with in vitro reduced susceptibility to artemisinin derivatives [36–38], but never with delayed parasite clearance [39]. Additionally, mutations on *pfmdr1* genes were shown to be correlated with in vitro reduced susceptibility to artemisinin derivatives [40–42]. The involvement of polymorphisms in potential genes was evaluated, such *as pfubp-1* encoding the *P. falciparum* ubiquitin specific protease 1 [43–45], the gene encoding the RING E3 protein ubiquitin ligase [46, 47], *pfap2mu* encoding the *P. falciparum* adaptor protein complex 2 mu subunit [44, 48], *pfmdr5* encoding the *P. falciparum* multidrug resistance 5 protein [49] or *pfmdr6* encoding the *P. falciparum* multidrug resistance 6 protein [49–51]. Only mutations *pfap2mu* S160N and *pfubp1* E1525D/Q were found in cases of African imported *P. falciparum* malaria with clinical failure with ACT [25]. Whole-genome sequencing of the artemisinin-susceptible F32-Tanzania strain and the artemisinin-resistant F32-ART line, obtained after 5 years of artemisinin pressure, led to identification of several mutations (M476I, C580Y, R539T, Y493H, I543T and P574L) in the propeller domain of the *kelch 13* (*k13*) gene (PF3D7_1343700) that are associated with in vitro resistance to artemisinin [31, 52, 53]. These mutations were associated with artemisinin-resistant (high survival rate) Cambodian isolates evaluated with RSA [1, 31, 32]. Additionally, these mutations were also associated with in vivo delayed parasite clearance half-lives (> 5 h) in Southeast Asia, including Cambodia, Vietnam, Thailand, Myanmar and China [1, 31, 54] or parasitaemia still positive on day 3 after 7 days of artesunate monotherapy or 3 days of ACT [23]. Another mutation, F446I, was predominant in Myanmar and associated with high survival rate and *P. falciparum* in vivo delayed clearance [55–58].

According to the WHO, the proportion of patients still parasitaemic on day 3 (10%) or with a parasite slow clearance half-life above 5 h (10%) after artesunate monotherapy or treatment with ACT, or carrying *k13* mutations associated with artemisinin resistance in Asia are indicators to identify emergence of suspected artemisinin resistance [59]. Resistance to artemisinin is confirmed when at least 5% of the patients carry parasites with *k13* resistance-associated mutations are still parasitaemic on day 3 or show slow parasite clearance [59]. The WHO has recommended evaluate *k13* resistance-associated mutations to track emergence and spread of artemisinin resistance in Africa.

The main *k13* mutations involved in artemisinin resistance in Southeast Asia are not yet reported in Africa certainly due to an absence of artemisinin resistance in Africa [23, 60–68]. Artemisinin resistance due to *k13* mutations has not disseminated to African countries yet. However, clinical failures with ACT, although rare, were reported in Africa (Angola, Senegal, Zaire) or in imported falciparum cases from Africa (Angola, Ethiopia, Liberia, Uganda) and were not associated with *k13* resistance-associated mutations [21–25, 69–71]. In some cases, pharmacokinetic data were associated and allowed

to exclude sub-therapeutic drug exposure to dihydroartemisinin [21, 22]. On Senegalese patients, parasites were still detected on day 3 after ACT treatment and were wild-type for K13 [24]. An isolate from Equatorial Guinea collected from patient with early treatment failure after artemisinin–piperaquine showed in vitro survival rate higher than the rate observed in the control strains but lower than rate in Asian artemisinin-resistant strain with a C580Y mutation [72]. However, none of the mutations described in artemisinin resistance in Asia was detected. A new mutation (M579I) was identified.

Additionally, 98.5% of Cambodian patients with isolates carrying C580Y or Y493H mutations on day 1 were negative on day 3 after dihydroartemisinin–piperaquine treatment [73]. Cambodian parasites with in vitro survival rates above the cut-off of 1% can lack the *k13* mutations involved in artemisinin resistance in Cambodia [74]. Chinese patients with R539T mutant parasites imported from Angola and P574L mutant parasites from Equatorial Guinea all recovered after treatment with dihydroartemisinin–piperaquine [75].

These data suggest that other mechanisms than *k13* mutations may explain artemisinin resistance, and more particularly in Africa. Mutations on *falcipain 2a* gene, encoding a cysteine protease and haemoglobinase and *atg18* gene, encoding the autophagy-related protein 18, might be associated with artemisinin resistance in parasites from the China Myanmar-border [76–78]. Additionally, mutations in the actin-binding protein coronin (R100K, E107V or G50E) conferred high in vitro survival rate in Senegalese *P. falciparum* strains, and this in the absence of mutation on the *k13* propeller gene [79].

Piperaquine resistance

Emergence of *P. falciparum* resistance to dihydroartemisinin–piperaquine was observed in Cambodia, where the prevalence of recrudescent infections rapidly increased [7–9], and then in Vietnam [10, 11]. Additionally, in vitro resistance to piperaquine was detected in Cambodia and increased rapidly between 2013 and 2015 [80]. Duplication of the *Plasmodium falciparum plasmepsin II* gene (*pfpm2*) (PF3D7_1408000), encoding a protease involved in haemoglobin degradation, has been found to be associated with reduced in vitro susceptibility to piperaquine in Cambodian *P. falciparum* parasites and with dihydroartemisinin–piperaquine failures in Cambodia [81, 82]. A new in vitro test, the piperaquine survival assay (PSA), was developed to follow piperaquine resistance [83]. *Plasmodium falciparum* dihydroartemisinin–piperaquine failures in Cambodia were associated with piperaquine survival rate above 10% or high piperaquine IC_{50} above 90 nM estimated by in vitro standard assay [81–83].

However, the involvement of *pfpm2* in piperaquine resistance seems controversial in Africa. In Mali, the presence of *P. falciparum* isolates with *pfpm2* duplications was confirmed in only 7 out of 65 clinical failures with dihydroartemisinin–piperaquine [26]. Three patients harbouring parasites with two copies of *pfpm2* in Tanzania were successfully treated with dihydroartemisinin–piperaquine [84]. Additionally, only a single copy of *pfpm2* was detected in two isolates collected in imported malaria cases from Ethiopia and Cameroon after dihydroartemisinin–piperaquine failures [22, 27]. The use of dihydroartemisinin–piperaquine as intermittent preventive treatment during pregnancy did not select for *pfpm2* duplication in Uganda [85]. Ex vivo susceptibility to piperaquine in imported *P. falciparum* parasites from Africa, in Ugandan and Senegalese isolates was not associated with variation in *pfpm2* copy number ([86–88], unpublished personal data). Additionally, a recent publication showed that overexpression of *pfpm2* did not change the susceptibility of the 3D7 *P. falciparum* strain to piperaquine [89].

All these data suggest that *pfpm2* would not be the only gene that explains the resistance to piperaquine in Africa. The *P. falciparum* chloroquine resistance transporter gene (*pfcrt*) may be a causal gene because piperaquine is a dimer of chloroquine. Mutations in *pfcrt* could be involved in piperaquine resistance. However, the K76T mutation involved in chloroquine resistance was not associated with in vitro and ex vivo resistance to piperaquine [90, 91]. Novel mutations in *pfcrt*, like H97Y, F145I, M343L, C350R or G353V, seem to confer in vitro resistance to piperaquine in *P. falciparum* parasites [92–94]. However, there is no direct evidence of piperaquine inhibiting PfCRT.

Conclusion

Mutations in K13 (C580Y, R539T, Y493H, I543T and P574L) and *pfpm2* duplications in *P. falciparum* are associated with in vitro resistance and clinical failures with dihydroartemisinin–piperaquine in Southeast Asia. Although the resistance to dihydroartemisinin–piperaquine has not yet emerged in Africa, the first data on clinical failures and in vitro reduced susceptibility suggest that *k13* and *pfpm2* would be not the only genes involved in artemisinin and piperaquine resistance. It is imperative to identify new genes to explain resistance to artemisinin and piperaquine in Africa. It is necessary to maintain tracking of the emergence and spread of *k13* and *pfpm2* mutant parasites in Africa, which could be imported from Asia. This surveillance must be associated with the tracking of dihydroartemisinin–piperaquine clinical failures in Africa due to resistant parasites. Too few studies associate drug plasmatic measures to verify good compliance and pharmacokinetic to confirm resistance. African parasites may have their own genetic background preference to select

dihydroartemisinin–piperaquine resistance which surely differs from Southeast Asian parasites. These isolates should be characterized by assessing *k13* polymorphisms, *pfpm2* copy number variation, but also other potential marker of resistance. The identification of new genes involved in dihydroartemisinin–piperaquine resistance in Africa could be performed by systematic analysis of African resistant parasites by genome wide association study (GWAS).

Abbreviations

ACT: artemisinin-based combination therapy; ACPR: adequate clinical and parasitological response; GWAS: genome wide association study; PCR: polymerase chain reaction; *pfap2mu*: *P. falciparum* adaptor protein complex 2 mu gene; *pfatg18*: *P. falciparum* autophagy-related gene 18; *pfATPase6*: *P. falciparum* sarco-endoplasmic reticulum calcium-ATPase 6 gene; *pfcrt*: *P. falciparum* chloroquine resistance transporter gene; *pfmdr1*: *P. falciparum* multidrug resistance 1 gene; *pfmdr5*: *P. falciparum* multidrug resistance 5 gene; *pfmdr6*: *P. falciparum* multidrug resistance 6 gene; *pfubp-1*: *P. falciparum* ubiquitin specific protease 1 gene; PSA: in vitro piperaquine survival assay; RSA: in vitro ring-stage survival assay; WHO: World Health Organization.

Acknowledgements

Not applicable.

Authors' contributions

FFT, MG, MGR, MM, and BP drafted the manuscript. All the authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Unité Parasitologie et Entomologie, Département Microbiologie et maladies infectieuses, Institut de Recherche Biomédicale des Armées, 19-21 Boulevard Jean Moulin, 13005 Marseille, France. [2] Aix Marseille Univ, IRD, SSA, AP-HM, VITROME, Marseille, France. [3] IHU Méditerranée Infection, Marseille, France. [4] Centre National de Référence du Paludisme, Institut de Recherche Biomédicale des Armées, Marseille, France.

Received: 4 March 2019 Accepted: 17 August 2019
Published online: 23 August 2019

References

1. 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.
2. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in western Cambodia. New Engl J Med. 2008;359:2619–20.
3. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2009;361:455–67.
4. Phyo AP, Nkhoma S, Stepniewska K, Ashley EA, Nair S, McGready R, et al. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. Lancet. 2012;379:1960–6.
5. Bouillé M, Witkowski B, Duru V, Sriprawat K, Nair SK, McDew-White M, et al. Artemisinin-resistant *Plasmodium falciparum* K13 mutant alleles, Thailand-Myanmar border. Emerg Infect Dis. 2016;22:1503–5.
6. Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, Rekol H, et al. The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. Lancet Infect Dis. 2017;17:491–7.
7. Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, et al. Dihydroartemisinin–piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: a multisite prospective cohort study. Lancet Infect Dis. 2016;16:357–65.
8. Leang R, Taylor WRJ, Bouth DM, Song L, Tarning J, Char MC, et al. Evidence of *Plasmodium falciparum* malaria multidrug resistance to artemisinin and piperaquine in Western Cambodia: dihydroartemisinin–piperaquine open-label multicenter clinical assessment. Antimicrob Agents Chemother. 2015;59:4719–26.
9. Spring MD, Lin JT, Manning JE, Vanachayangkul P, Somethy S, Bun R, et al. Dihydroartemisinin–piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: an observational cohort study. Lancet Infect Dis. 2015;15:683–91.
10. Phuc BQ, Rasmussen C, Duong TT, Dong LT, Loi MA, Ménard D, et al. Treatment failure of dihydroartemisinin/piperaquine for *Plasmodium falciparum* malaria, Vietnam. Emerg Infect Dis. 2017;23:715–7.
11. Thanh NV, Thuy-Nhien N, Tuyen NT, Tong NT, Nha-Ca NT, Dong LT, et al. Rapid decline in the susceptibility of *Plasmodium falciparum* to dihydroartemisinin–piperaquine in the south of Vietnam. Malar J. 2017;16:27.
12. Kwambai TK, Dhabangi A, Idro R, Opoka R, Kariuki S, Samuels AM, et al. Malaria chemoprevention with monthly dihydroartemisinin–piperaquine for the post-discharge management of severe anaemia in children aged less than 5 years in Uganda and Kenya: study protocol for a multicentre, two-arm, randomised, placebo-controlled, superiority trial. Trials. 2018;19:610.
13. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, et al. Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin–piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomized, controlled, superiority trial. Lancet. 2019;393:1428–39.
14. Jagannathan P, Kakuru A, Okiring J, Muhindo MK, Natureeba P, Nakalembe M, et al. Dihydroartemisinin–piperaquine for intermittent preventive treatment of malaria during pregnancy and risk of malaria in early childhood: a randomized controlled trial. PLoS Med. 2018;15:e1002606.
15. The West African Network for Clinical Trials of Antimalarial Drugs (WANECAM). Pyronaridine–artesunate or dihydroartemisinin–piperaquine versus current first-line therapies for repeated treatment of uncomplicated malaria: a randomized, multicentre, open-label, longitudinal, controlled, phase 3b/4 trial. Lancet. 2018;391:1378–90.
16. Mandara CI, Francis F, Chiduo MG, Ngasala B, Mandike R, Mkude S, et al. High cure rates and tolerability of artesunate-amodiaquine and dihydroartemisinin–piperaquine for the treatment of uncomplicated falciparum malaria in Kibaha and Kigoma, Tanzania. Malar J. 2019;18:99.
17. Madara CI, Kavishe RA, Gesase S, Mghamba J, Ngadaya E, Mmbuji P, et al. High efficacy of artemether-lumefantrine and dihydroartemisinin–piperaquine for the treatment of uncomplicated falciparum malaria in Muheza and Kigoma Districts, Tanzania. Malar J. 2018;17:261.
18. Uwimana A, Penkunas MJ, Nisingizwe MP, Warsame M, Umalissa N, Uyizeye D, et al. Efficacy of artemether-lumefantrine versus dihydroartemisinin–piperaquine for the treatment of uncomplicated malaria among children in Rwanda: an open-label, randomized controlled trial. Trans R Soc Trop Med Hyg. 2019;113:312–9.
19. Davlantes E, Dimbu PR, Ferreira CM, Joao MF, 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.
20. Grandesso F, Guindo O, Messe LW, Makarimi R, Traore A, Dama S, et al. Efficacy of artesunate–amodiaquine, dihydroartemisinin–piperaquine

and artemether–lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Maradi, Niger. Malar J. 2018;17:52.
21. Gobbi F, Buonfrate D, Menegon M, Lunardi G, Angheben A, Severini C, et al. Failure of dihydroartemisinin–piperaquine treatment of uncomplicated *Plasmodium falciparum* malaria in a traveller coming from Ethiopia. Malar J. 2016;15:525.
22. Russo G, L'Episcopia M, Menegon M, Souza SS, Dongho BGD, Vullo V, et al. Dihydroartemisinin–piperaquine treatment failure in uncomplicated *Plasmodium falciparum* malaria case imported from Ethiopia. Infection. 2018;46:867–70.
23. Ménard D, Khim N, Beghain J, Adegnika AA, Alam MS, Amodu O, et al. A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. N Engl J Med. 2016;374:2453–64.
24. Madamet M, Kouta MB, Wade KA, Lo G, Diawara S, Fall M, et al. Absence of association between polymorphisms in the K13 gene and the presence of *Plasmodium falciparum* parasites at day 3 after treatment with artemisinin derivatives in Senegal. Int J Antimicrob Agents. 2017;49:754–6.
25. Sutherland CJ, Lansdell P, Sanders M, Muwanguzi J, van Schalkwyk DA, Kaur H, et al. *pfk13*-independent treatment failure in four imported cases of *Plasmodium falciparum* malaria treated with artemether–lumefantrine in the United Kingdom. Antimicrob Agents Chemother. 2017;61:e02382-16.
26. Inoue J, Silva M, Fofana B, Sanogo K, Martensson A, Sagara I, et al. *Plasmodium falciparum plasmepsin 2* duplications, West Africa. Emerg Infect Dis. 2018;24:1591–3.
27. Malvy D, Torrentino-Madamet M, L'Ollivier C, Receveur MC, Jeddi F, Delhaes L, et al. *Plasmodium falciparum* recrudescence two years after treatment of an uncomplicated infection without return to an area where malaria is endemic. Antimicrob Agents Chemother. 2018;62:e01892-17.
28. Flegg JA, Guerin PJ, White NJ, Stepniewska K. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. Malar J. 2011;10:339.
29. WWARN Parasite Clearance Study Group, Abdulla S, Ashley EA, Bassat Q, Bethell D, Björkman A, et al. Baseline data of parasite clearance in patients with falciparum malaria treated with artemisinin derivative: an individual patient data meta-analysis. Malar J. 2015;14:359.
30. Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, et al. Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: in vitro and ex vivo drug-response studies. Lancet Infect Dis. 2013;13:1043–9.
31. 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.
32. Amaratunga C, Witkowski B, Dek D, Try V, Khim N, Miotto O, et al. *Plasmodium falciparum* founder populations in western Cambodia have reduced artemisinin sensitivity in vitro. Antimicrob Agents Chemother. 2014;58:4935–7.
33. Amaratunga C, Witkowski B, Khim N, Ménard D, Fairhurst RM. Artemisinin resistance in *Plasmodium falciparum*. Lancet Infect Dis. 2014;14:449–50.
34. Jambou R, Legrand E, Niang M, Khim N, Lim P, Volney B, et al. Resistance of *Plasmodium falciparum* field isolates to in vitro artemether and point mutations of the SERCA-type PfATPase6. Lancet. 2005;366:1960–3.
35. 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;51:82–9.
36. Sidhu AB, Uhleman AC, Valderramos SG, Valderramos JC, Krishna S, Fidock DA. Decreasing *pfmdr1* copy number in *Plasmodium falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin. J Infect Dis. 2006;194:528–35.
37. Pascual A, Fall B, Wurtz N, Fall M, Camara C, Nakoulima A, et al. *Plasmodium falciparum* with multidrug resistance 1 gene duplications, Senegal. Emerg Infect Dis. 2013;19:814–5.
38. Chavchich M, Gerena L, Peters J, Chen N, Cheng Q, Kyle DE. Role of *pfmdr1* amplification and expression in induction of resistance to artemisinin derivatives in *Plasmodium falciparum*. Antimicrob Agents Chemother. 2010;54:2455–64.
39. Ngalah BS, Ingasia LA, Cheruiyot AC, Chebon LJ, Juma DW, Muiruri P, et al. Analysis of major genome loci underlying artemisinin resistance and pfmdr1 copy number in pre- and post-ACTs in western Kenya. Sci Rep. 2015;5:8308.
40. Duraisingh MT, Roper C, Walliker D, Warhurst DC. Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the *pfmdr1* gene of *Plasmodium falciparum*. Mol Microbiol. 2000;36:955–61.
41. Wurtz N, Fall B, Pascual A, Fall M, Baret E, Camara C, et al. Role of *Pfmdr1* in in vitro *Plasmodium falciparum* susceptibility to chloroquine, quinine, monodesethylamodiaquine, mefloquine, lumefantrine, and dihydroartemisinin. Antimicrob Agents Chemother. 2014;58:7032–40.
42. Njokah MJ, Kangethe JN, Kinyua J, Kariuki D, Kimani FT. In vitro selection of *Plasmodium falciparum Pfcrt* and *Pfmdr1* variants by artemisinin. Malar J. 2016;15:381.
43. Imwong M, Dondorp AM, Nosten F, Yi P, Mungthin M, Hanchana S, et al. Exploring the contribution of candidate genes to artemisinin resistance in *Plasmodium falciparum*. Antimicrob Agents Chemother. 2010;54:2886–92.
44. Henriques G, Hallett RL, Beshir KB, Gadalla NB, Johnson RE, Burrow R, et al. Directional selection at the pfmdr1, pfcrt, pfubp1, and pfap2mu loci of *Plasmodium falciparum* in Kenya children treated with ACT. J Infect Dis. 2014;201:2001–8.
45. Borrman S, Straimer J, Mwai L, Abdi A, Rippert A, Okombo J, et al. Genome-wide screen identifies new candidate genes associated with artemisinin susceptibility in *Plasmodium falciparum* in Kenya. Sci Rep. 2013;3:3318.
46. Gendrot M, Fall B, Madamet M, Fall M, Wade KA, Amalvict R, et al. Absence of association between polymorphisms in the RING E3 ubiquitin protein ligase gene and ex vivo susceptibility to conventional antimalarial drugs in *Plasmodium falciparum* isolates from Dakar, Senegal. Antimicrob Agents Chemother. 2016;60:5010–3.
47. Gendrot M, Foguim FT, Robert MG, Amalvict R, Mosnier J, Benoit M, et al. The D113N mutation in the RING E3 ubiquitin protein ligase gene is not associated with ex vivo susceptibility to common anti-malarial drugs in African *Plasmodium falciparum* isolates. Malar J. 2018;17:108.
48. Adams T, Ennuson NAA, Quashie NB, Futagbi G, Matrevi S, Hagan OCK, et al. Prevalence of *Plasmodium falciparum* delayed clearance associated polymorphisms in adaptor protein complex 2 mu subunit (*pfap2mu*) and ubiquitin specific protease 1 (*pfubp1*) genes in Ghanaian isolates. Parasit Vectors. 2018;11:175.
49. Okombo J, Abdi AI, Kiara SM, Mwai L, Pole L, Sutherland CJ, et al. Repeat polymorphisms in the low-complexity regions of *Plasmodium falciparum* ABC transporters and associations with in vitro antimalarial responses. Antimicrob Agents Chemother. 2013;57:6196–204.
50. Wang Z, Parker D, Meng H, Wu L, Li J, Zhao Z, et al. In vitro sensitivity of *Plasmodium falciparum* from China-Myanmar border area to major ACT drugs and polymorphisms in potential genes. PLoS ONE. 2012;7:e30927.
51. Gendrot M, Diawara S, Madamet M, Kounta MB, Briolant S, Wade KA, et al. Association between polymorphisms in the *Pfmdr6* gene and ex vivo susceptibility to quinine in *Plasmodium falciparum* parasites from Dakar, Senegal. Antimicrob Agents Chemother. 2017;61:e01183-16.
52. Witkowski B, Lelièvre J, Barragan MJ, Laurent V, Su XZ, Berry A, et al. Increased tolerance to artemisinin in *Plasmodium falciparum* is mediated by a quiescence mechanism. Antimicrob Agents Chemother. 2010;54:1872–7.
53. Straimer J, Gnädig NF, Witkowski B, Amaratunga C, Duru V, Pramundita Ramadani A, et al. K-13propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. Science. 2015;347:428–31.
54. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, et al. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. J Infect Dis. 2015;211:670–9.
55. Huang F, Takala-Harrison S, Jacob CG, Liu H, Sun X, Yang H, et al. A single mutation in K13 predominates in Southern China and is associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment. J Infect Dis. 2015;212:1629–35.
56. Wang Z, Wang Y, Cabrera M, Zhang Y, Gupta B, Wu Y, et al. Artemisinin resistance at the China-Myanmar border and association with mutations in the K13 gene. Antimicrob Agents Chemother. 2015;59:6952–9.
57. Bonnington CA, Phyo AP, Ashley EA, Imwong M, Sriprawat K, Parker DM, et al. *Plasmodium falciparum* Kelch 13 mutations and treatment response in patients in Hpa-Pun District, Northern Kayin State, Myanmar. Malar J. 2017;16:480.
58. Wang J, Huang Y, Zhao Y, Zhang D, Pan W. Introduction of F446I mutation in the K13 propeller gene leads to increased ring survival rates in *Plasmodium falciparum* isolates. Malar J. 2018;17:248.

59. WHO. Status report on artemisinin resistance (September 2014). Geneva: World Health Organization; 2014.
60. Torrentino-Madamet M, Fall B, Benoit N, Camara C, Amalvict R, Fall M, et al. Limited polymorphisms in *k13* gene in *Plasmodium falciparum* isolates from Dakar, Senegal in 2012–2013. Malar J. 2014;13:472.
61. Boussaroque A, Fall A, Madamet M, Camara C, Benoit N, Fall M, et al. Emergence of mutations in the K13 Propeller gene of *Plasmodium falciparum* isolates from Dakar, Senegal in 2013–2014. Antimicrob Agents Chemother. 2015;60:624–7.
62. Hawkes M, Conroy AL, Opoka RO, Namasopo S, Zhong K, Liles WC, et al. Slow clearance of *Plasmodium falciparum* in severe pediatric malaria, Uganda, 2011–2013. Emerg Infect Dis. 2015;21:1237–9.
63. Kamau E, Campino S, Amenga-Etego L, Drury E, Ishengoma D, Johnson K, et al. K13-propeller polymorphisms in *Plasmodium falciparum* parasites from sub-saharan Africa. J Infect Dis. 2015;211:1352–5.
64. Ouattara A, Kone A, Adams M, Fofana B, Maiga AW, Hampton S, et al. Polymorphisms in the K13-propeller gene in artemisinin-susceptible *Plasmodium falciparum* parasites from Bougoula-Hameau and Bandiagara, Mali. Am J Trop Med Hyg. 2015;92:1202–6.
65. Taylor SM, Parobek CM, DeConti DK, Kayentao K, Coulibaly SO, Greenwood BM, et al. Absence of putative artemisinin resistance mutations among *Plasmodium falciparum* in sub-Saharan Africa: a molecular epidemiologic study. J Infect Dis. 2015;211:680–8.
66. Torrentino-Madamet M, Collet L, Lepère JF, Benoit N, Amalvict R, Ménard D, et al. K13-propeller polymorphisms in *Plasmodium falciparum*isolates from patients in Mayotte in 2013 and 2014. Antimicrob Agents Chemother. 2015;59:7878–81.
67. Muwanguzi J, Henriques G, Sawa P, Bousema T, Sutherland CJ, Beshir KB. Lack of K13 mutations in *Plasmodium falciparum* persisting after artemisinin combination therapy treatment of Kenyan children. Malar J. 2016;15:36.
68. Voumbo-Matoumona DF, Kouna LC, Madamet M, Maghendji-Nzondo S, Pradines B, Lekana-Douki JB. Prevalence of *Plasmodium falciparum* antimalarial drug resistance genes in Southeastern Gabon from 2011 to 2014. Infect Drug Resist. 2018;11:1329–38.
69. Dieye B, Affara M, Sangare L, Joof F, Ndiaye YD, Gomis JF, et al. West Africa international centers of excellence for malaria research: drug resistance patterns to artemether-lumefantrine in Senegal, Mali, and The Gambia. Am J Trop Med Hyg. 2016;95:1054–60.
70. 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. 2015;59:437–43.
71. 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.
72. Lu F, Culleton R, Zhang M, Ramaprasad A, von Seidlein L, Zhou H, et al. Emergence of indigenous artemisinin-resistant *Plasmodium falciparum* in Africa. New Engl J Med. 2017;376:991–3.
73. Kheang ST, Sovannaroth S, Ek S, Chy S, Chhun P, Mao S, et al. Prevalence of k13 mutation and day-3 positive parasitaemia in artemisinin-resistant malaria endemic area of Cambodia: a cross-sectional study. Malar J. 2017;16:372.
74. Mukherjee A, Bopp S, Magistrado P, Wong W, Daniels R, Demas A, et al. Artemisinin resistance without *pfkelch13* mutations in *Plasmodium falciparum* isolates from Cambodia. Malar J. 2017;16:195.
75. Yang C, Zhang H, Zhou R, Qian D, Liu Y, Zhao Y, et al. Polymorphisms of *Plasmodium falciparum* k13-propeller gene among migrant workers returning to Henan Province, China from Africa. BMC Infect Dis. 2017;17:560.
76. Siddiqui FA, Cabrera M, Wang M, Brashear A, Kemirembe K, Wang Z, et al. *Plasmodium falciparum* Falcipain-2-a polymorphisms in Southeast Asia and their association with artemisinin resistance. J Infect Dis. 2018;218:434–42.
77. Wang Z, Cabrera M, Yang L, Gupta B, Liang X, Kemirembe K, et al. Genome-wide association analysis identifies genetic loci associated with resistance to multiple antimalarials in *Plasmodium falciparum* from China-Myanmar border. Sci Rep. 2016;3:33891.
78. Breglio KF, Amato R, Eastman R, Lim P, Sa JM, Guha R, et al. A single nucleotide polymorphism in the *Plasmodium falciparum* atg18 gene associates with artemisinin resistance and confers enhanced parasite survival under nutrient deprivation. Malar J. 2018;17:391.
79. Demas AR, Sharma AI, Wong W, Early AM, Redmond S, Bopp S, et al. Mutations in *Plasmodium falciparum* actin-binding protein coronin confer reduced artemisinin susceptibility. Proc Natl Acad Sci USA. 2018;115:12799–804.
80. Chaorattanakawee S, Lon C, Jongsakul K, Gawee J, Sok S, Sundrakes S, et al. Ex vivo piperaquine resistance developed rapidly in *Plasmodium falciparum* isolates in northern Cambodia compared to Thailand. Malar J. 2016;15:519.
81. Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, et al. A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: a phenotype-genotype association study. Lancet Infect Dis. 2016;17:174–83.
82. Amato R, Lim P, Miotto O, Amaratunga C, Dek D, Pearson RD, et al. Genetic markers associated with dihydroartemisinin-piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: a genotype-phenotype association study. Lancet Infect Dis. 2016;17:164–73.
83. Duru V, Khim N, Leang R, Kim S, Domergue A, Kloeung N, et al. *Plasmodium falciparum* dihydroartemisinin-piperaquine failures in Cambodia are associated with mutant K13 parasites presenting high survival rates in novel piperaquine in vitro assays: retrospective and prospective investigations. BMC Med. 2015;13:305.
84. Kakolwa MA, Mahende MK, Ishengoma DS, Mandara CI, Ngasala B, Kamugisha E, et al. Efficacy and safety of artemisinin-based combination therapy and molecular markers for artemisinin and piperaquine resistance in Mailand Tanzania. Malar J. 2018;17:369.
85. Conrad MD, Mota D, Foster M, Tukwasibwe S, Legac J, Tumwebase P, et al. Impact of intermittent preventive treatment during pregnancy on *Plasmodium falciparum* drug resistance-mediating polymorphisms in Uganda. J Infect Dis. 2017;2016:1008–17.
86. Rasmussen SA, Ceja FG, Conrad MD, Tumwebase PK, Byaruhanga O, Katairo T, et al. Changing antimalaria drug sensitivities in Uganda. Antimicrob Agents Chemother. 2017;61:e01516-17.
87. Robert MG, Foguim Tsombeng F, Gendrot M, Mosnier J, Amalvict R, Benoit N, et al. Absence of a high level of duplication of the Plasmepsin II gene in Africa. Antimicrob Agents Chemother. 2018;62:e00374-18.
88. Robert MG, Foguim Tsombeng F, Gendrot M, Diawara S, Madamet M, Kounta MB, et al. Baseline ex vivo and molecular responses of *Plasmodium falciparum* isolates to piperaquine before implementation of dihydroartemisinin-piperaquine in Senegal. Antimicrob Agents Chemother. 2019;63:e02445-18.
89. Loesbanluechai D, Kotanan N, de Cozar C, Kochakarn T, Ansbro MR, Chotivanich K, et al. Overexpression of plasmepsin II and plasmepsin III does not directly cause reduction in *Plasmodium falciparum* sensitivity to artesunate, chloroquine and piperaquine. Int J Parasitol Drugs Drug Resist. 2019;9:16–22.
90. Briolant S, Henry M, Oeuvray C, Amalvict R, Baret E, Didillon E, et al. Absence of association between in vitro responses and polymorphisms in the *pfcrt*, *pfmdr1*, *pfmrp*, and *pfnhe* genes in *Plasmodium falciparum*. Antimicrob Agents Chemother. 2010;54:3537–44.
91. Pascual A, Madamet M, Bertaux L, Amalvict R, Benoit N, Travers D, et al. In vitro piperaquine susceptibility is not associated with the *Plasmodium falciparum* chloroquine resistance transporter gene. Malar J. 2013;12:431.
92. Pelleau S, Moss EL, Dhingra SK, Volney B, Casteras J, Gabryszewski SJ, et al. Adaptative evolution of malaria parasites in French Guiana: reversal of chloroquine resistance by acquisition of a mutation in *pfcrt*. Proc Natl Aca Sci USA. 2015;112:11672–7.
93. Agrawal S, Moser KA, Morton L, Cummings MP, Parihar A, Dwivedi A, et al. A novel mutation in the *Plasmodium falciparum* chloroquine resistance transporter is associated with decreased piperaquine sensitivity. J Infect Dis. 2017;216:468–76.
94. Ross LS, Dhingra SK, Mok S, Yeo T, Wicht KJ, Kümpornsin K, et al. Emerging Southeast Asian PFCRT mutations confer *Plasmodium falciparum* resistance to the first-line antimalarial piperaquine. Nat Commun. 2018;9:3314.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.