



HAL
open science

Association between Polymorphisms in the Pfm^{dr6} Gene and Ex Vivo Susceptibility to Quinine in *Plasmodium falciparum* Parasites from Dakar, Senegal

Mathieu Gendrot, Silman Diawara, Marylin Madamet, Mame Bou Kounta, Sebastien Briolant, Khalifa Ababacar Wade, Mansour Fall, Nicolas Benoit, Aminata Nakoulima, Remy Amalvict, et al.

► To cite this version:

Mathieu Gendrot, Silman Diawara, Marylin Madamet, Mame Bou Kounta, Sebastien Briolant, et al.. Association between Polymorphisms in the Pfm^{dr6} Gene and Ex Vivo Susceptibility to Quinine in *Plasmodium falciparum* Parasites from Dakar, Senegal. *Antimicrobial Agents and Chemotherapy*, 2017, 61 (3), 10.1128/AAC.01183-16 . hal-01521240

HAL Id: hal-01521240

<https://hal.science/hal-01521240>

Submitted on 7 May 2018

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.



Association between Polymorphisms in the *Pfmdr6* Gene and *Ex Vivo* Susceptibility to Quinine in *Plasmodium falciparum* Parasites from Dakar, Senegal

Mathieu Gendrot,^{a,b} Silman Diawara,^c Marylin Madamet,^{b,d,e} Mame Bou Kounta,^f Sébastien Briolant,^{a,b,h,i} Khalifa Ababacar Wade,^f Mansour Fall,^g Nicolas Benoit,^{b,d,e} Aminata Nakoulima,^j Rémy Amalvict,^{b,d,e} Yaya Diémé,^c Bécaye Fall,^c Boubacar Wade,^k Bakary Diatta,^{g,k} Bruno Pradines^{a,b,c,e}

Unité de Parasitologie et d'Entomologie, Département des Maladies Infectieuses, Institut de Recherche Biomédicale des Armées, Marseille, France^a; Aix Marseille Université, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UM 63, CNRS 7278, IRD 198, INSERM 1095, Marseille, France^b; Laboratoire d'étude de la chimiosensibilité du paludisme, Fédération des laboratoires, Hôpital Principal de Dakar, Dakar, Senegal^c; Equipe résidente de recherche en infectiologie tropicale, Institut de Recherche Biomédicale des Armées, Hôpital d'instruction des armées, Marseille, France^d; Centre national de référence du Paludisme, Marseille, France^e; Service des Urgences, Hôpital Principal de Dakar, Dakar, Senegal^f; Service de Réanimation Médicale, Hôpital Principal de Dakar, Dakar, Senegal^g; Direction Interarmées du Service de Santé, Cayenne, Guyane, France^h; Laboratoire de Parasitologie, Institut Pasteur de la Guyane, Cayenne, Guyane, Franceⁱ; Service de Pédiatrie, Hôpital Principal de Dakar, Dakar, Senegal^j; Chefferie, Hôpital Principal de Dakar, Dakar, Senegal^k

ABSTRACT Polymorphisms and the overexpression of transporter genes, especially of the ATP-binding cassette superfamily, have been involved in antimalarial drug resistance. The objective of this study was to use 77 Senegalese *Plasmodium falciparum* isolates to evaluate the association between the number of Asn residues in the polymorphic microsatellite region of the *Plasmodium falciparum* multidrug resistance 6 gene (*Pfmdr6*) and the *ex vivo* susceptibility to antimalarials. A significant association was observed between the presence of 7 or 9 Asn repeats and reduced susceptibility to quinine.

KEYWORDS malaria, *Plasmodium falciparum*, antimalarial drug, *in vitro*, resistance, molecular marker

The resistance of malaria to most antimalarial drugs has developed in Southeast Asia and has spread to Africa. The World Health Organization (WHO) has recommended artemisinin-based combination therapy (ACT) as the first-line treatment for malaria since 2005. As recently described in Southeast Asia, the emergence of *Plasmodium falciparum* resistance to artemisinin and its derivatives manifests as delayed parasite clearance following treatment with either artesunate (AS) monotherapy or ACT (1, 2). In areas where artemisinin resistance is emerging, the partner drugs within the combination are under increasing pressure for the selection of resistance. In this context, the identification of molecular markers of resistance to these partner drugs is urgently needed to monitor the emergence and spread of resistance to antimalarial drugs.

Polymorphisms and the overexpression of transporter genes, especially of the ATP-binding cassette (ABC) superfamily, have been involved in antimalarial drug resistance (3). The N86Y mutation of the *P. falciparum* multidrug resistance 1 gene (*Pfmdr1*) is associated with *in vitro* susceptibility to dihydroartemisinin (DHA), lumefantrine (LMF), monodesethylamodiaquine (DQ), and mefloquine (MQ) (4). Field studies in East Africa have also shown selection of the N86 allele in recurrent infections after treatment with artemether plus lumefantrine (5, 6) or artesunate plus mefloquine (7). A single

Received 3 June 2016 Returned for modification 29 August 2016 Accepted 12 December 2016

Accepted manuscript posted online 4 January 2017

Citation Gendrot M, Diawara S, Madamet M, Kounta MB, Briolant S, Wade KA, Fall M, Benoit N, Nakoulima A, Amalvict R, Diémé Y, Fall B, Wade B, Diatta B, Pradines B. 2017. Association between polymorphisms in the *Pfmdr6* gene and *ex vivo* susceptibility to quinine in *Plasmodium falciparum* parasites from Dakar, Senegal. *Antimicrob Agents Chemother* 61:e01183-16. <https://doi.org/10.1128/AAC.01183-16>.

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Bruno Pradines, bruno.pradines@gmail.com.

mutation (F423Y) in the heavy metal transporter *Pfmdr2* was linked to *in vitro* resistance to pyrimethamine (8). Additionally, deletion of *Pfmdr2* in *P. falciparum* parasites resulted in a minor decrease in susceptibility to mefloquine, quinine (QN), and atovaquone (9). Repetitive amino acid motifs in *Pfmdr5* were associated with reduced *in vitro* susceptibility to lumefantrine (10). In another report, the presence of 9 Asn residues in the polymorphic microsatellite region of *Pfmdr6* (amino acid positions 103 to 109 in 3D7) appeared to modulate the *in vitro* dihydroartemisinin susceptibility of parasites from the China-Myanmar border area (11). Further, the presence of 7 Asn repeats was significantly associated with reduced susceptibility to lumefantrine. Another study on 16 reference strains from different sources worldwide and parasites collected in Kenya showed that parasites with 6 Asn repeats were significantly less susceptible to lumefantrine, whereas those with 8 Asn repeats were less susceptible to piperazine (PPQ) (10). There was no association of 9 Asn repeats with reduced susceptibility to dihydroartemisinin.

The objective of this study was to evaluate the association between the Asn number in *Pfmdr6* (accession number [PF3D7_1352100](https://www.ncbi.nlm.nih.gov/nuclot/PF3D7_1352100)) from Senegalese isolates of *P. falciparum* and *ex vivo* susceptibilities to chloroquine (CQ), quinine (QN), monodesethylamodiaquine (DQ), mefloquine (MQ), lumefantrine (LMF), piperazine (PPQ), pyronaridine (PND), dihydroartemisinin (DHA), artesunate (AS), and doxycycline (DOX).

Seventy-seven *P. falciparum* isolates from *falciparum* malaria patients attending the Hôpital Principal de Dakar from November 2013 to January 2014, August 2014 to December 2014, and September to November 2015 were successfully evaluated (13 from 2013, 32 from 2014, and 32 from 2015). Seventy-four percent of the patients were recruited from the emergency department. The other patients were recruited from the pediatric department (8%), the intensive care unit (5%), and other units (13%). There was no information available on antimalarial treatment prior to admission. The patients were treated with quinine or artesunate or artemether-lumefantrine, with or without doxycycline, at the Hôpital Principal de Dakar. Informed verbal consent was obtained from the patients or their parents/guardians before blood collection. The study was approved by the ethical committee of the Hôpital Principal de Dakar.

Venous blood samples were collected in Vacutainer ACD tubes prior to patient treatment. A malaria diagnosis was confirmed using a thin blood smear and a rapid diagnostic test. Thin blood smears were stained using a RAL kit (Réactifs RAL, Paris, France) based on eosin and methylene blue and were examined to determine *P. falciparum* density and to confirm species monoinfection. The level of parasitemia ranged from 0.06% to 14.1%.

The susceptibility of the isolates was assessed without culture adaptation. For the *in vitro* MicroTests, 100 μ l of parasitized red blood cells (final parasitemia, 0.5%; final hematocrit, 1.5%) was aliquoted into 96-well plates predosed with antimalarial drugs (CQ, QN, MQ, DQ, LMF, DHA, AS, PPQ, PND, and DOX). The plates were incubated in a sealed bag for 72 h at 37°C with atmospheric generators for capnophilic bacteria using Genbag CO₂ at 5% CO₂ and 15% O₂ (bioMérieux, Marcy l'Etoile, France). The *ex vivo* HRP2 enzyme-linked immunosorbent assay (ELISA) using the commercial Malaria Ag competitive enzyme-linked immunosorbent assay (CELISA) kit (reference number KM2159; Cellabs Pty Ltd., Brookvale, Australia) was previously described (12). The batches of plates were tested and validated on the chloroquine-resistant W2 strain (Indochina) (MR4, VA, USA) in three to six independent experiments using the same conditions. The mean 50% inhibitory concentrations (IC₅₀s) in the *in vitro* chemosusceptibility assay for the chloroquine-resistant W2 strain for the different batches used during the 3 years were 292 nM for CQ, 275 nM for QN, 72 nM for DQ, 13.7 nM for LMF, 15.4 nM for MQ, 32.5 nM for PPQ, 26.4 nM for PND, 1.27 nM for DHA, 1.43 nM for AS, and 10.7 μ M for DOX.

Pfmdr6 (accession number [PF3D7_1352100](https://www.ncbi.nlm.nih.gov/nuclot/PF3D7_1352100)) was amplified by PCR using the following primer pair: 5'-GAG-AAG-TAA-TAG-AAT-AAG-CG-3' and 5'-CCC-ATA-CAT-AAA-ATC-TTC-CT-3'. The reaction mixture consisted of 200 ng of genomic DNA, 0.32 μ M of each primer, 1 \times final concentration of the reaction buffer [750 mM Tris-HCl, 200 mM

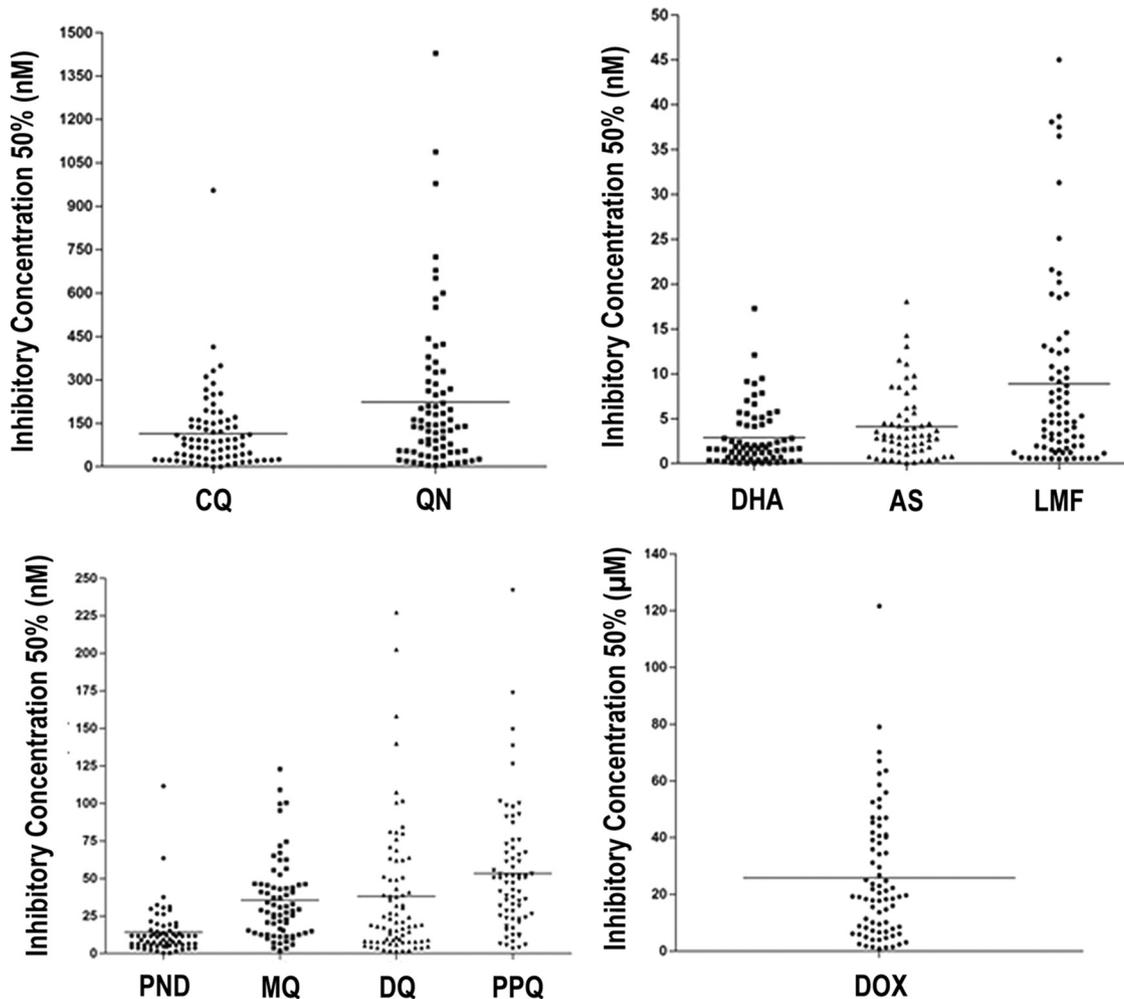


FIG 1 Distribution of *ex vivo* responses of 77 *P. falciparum* clinical field isolates from Dakar to dihydroartemisinin (DHA), artesunate (AS), lumefantrine (LMF), mefloquine (MQ), pyronaridine (PND), monodesethylamodiaquine (DQ), piperazine (PPQ), chloroquine (CQ), quinine (QN), and doxycycline (DOX).

(NH_4)₂SO₄, 0.1% (vol/vol) Tween 20, and stabilizer, pH 8.8], 2.5 mM MgCl₂, 200 μM deoxynucleoside triphosphate (dNTP) mixture, and 0.2 U of Red Diamond *Taq* polymerase (Eurogentec) in a final volume of 25 μl . The thermal cycler (T3 Biometra) was programmed as follows: 10 min at 94°C followed by 40 cycles of 30 s at 95°C, 45 s at 52°C, and 1 min at 72°C and a final extension for 10 min at 72°C. The purified amplicons were sequenced using the appropriate PCR primers on an ABI Prism 3100 analyzer (Applied Biosystems) according to the manufacturer's instructions. The sequences were analyzed using Vector NTI Advance software (version 11; Invitrogen, Cergy-Pontoise, France) to identify the number of asparagine repeats in *Pfmdr6* and their association with antimalarial drug resistance.

The IC₅₀s in the *ex vivo* chemosusceptibility assay ranged from 6.3 to 954.9 nM for CQ, 6.2 to 1,429.8 nM for QN, 1.9 to 227.3 nM for DQ, 0.6 to 45.0 nM for LMF, 3.4 to 123.0 nM for MQ, 3.9 to 241.9 nM for PPQ, 0.4 to 111.6 nM for PND, 0.1 to 17.3 nM for DHA, 0.1 to 18.1 nM for AS, and 0.9 to 121.6 μM for DOX. The distributions of the IC₅₀s for the 10 antimalarial drugs are shown in Fig. 1. Six different poly(Asn) repeat profiles, with 6 to 11 repeats, were detected in *Pfmdr6* among the 77 *P. falciparum* isolates from Dakar. The highest proportion of isolates in our study had 6 Asn repeats (41.6% of the samples), contrary to results for Asian isolates, in which 8 repeats predominated (76.5% of the samples) (11). The proportions of isolates with 8, 7, 9, 10, and 11 repeats were 31.1%, 14.3%, 10.4%, 1.3%, and 1.3%, respectively. There was no significant difference

TABLE 1 *Ex vivo* susceptibility of 77 *Plasmodium falciparum* isolates to quinine, chloroquine, monodesethylamodiaquine, lumefantrine, mefloquine, piperazine, pyronaridine, dihydroartemisinin, artesunate, and doxycycline according to the number of Asn repeats in *Pfmdr6*^a

Drug	Values according to no. of repeats						P value
	6 repeats	7 repeats	8 repeats	9 repeats	10 repeats	11 repeats	
Quinine							
IC ₅₀ geometric mean (nM) (no.)	99.5 (32)	216.4 (11)	82.9 (24)	219.7 (8)	248.0 (1)	218.0 (1)	0.0291
Median (nM)	123.8	294.0	115.6	171.7			
25 intercentile to 75 intercentile range	50.3–240.5	150.5–402.0	33.6–230.6	145.2–318.1			
% of resistant parasites	9.4	9.1	4.2	12.5			
Chloroquine							
IC ₅₀ geometric mean (nM) (no.)	67.7 (32)	38.4 (11)	80.9 (24)	30.5 (8)	311.0 (1)	266.0 (1)	0.1230
Median (nM)	93.2	65.6	94.0	31.2			
25 intercentile to 75 intercentile range	24.5–190.5	34.1–113.5	46.0–139.3	30.6–35.0			
% of resistant parasites	56.3	36.4	58.3	12.5			
Monodesethylamodiaquine							
IC ₅₀ geometric mean (nM) (no.)	19.7 (32)	24.9 (11)	22.1 (24)	11.8 (8)	71.0 (1)	43.2 (1)	0.5857
Median (nM)	23.8	19.1	23.0	11.8			
25 intercentile to 75 intercentile range	8.0–49.6	8.8–65.7	12.4–52.5	8.2–18.3			
% of resistant parasites	21.9	27.3	25.0	12.5			
Lumefantrine							
IC ₅₀ geometric mean (nM) (no.)	3.9 (32)	6.1 (11)	5.3 (24)	2.4 (8)	13.9 (1)	4.6 (1)	0.5017
Median (nM)	4.3	4.7	6.2	2.4			
25 intercentile to 75 intercentile range	1.4–12.4	2.3–23.0	2.4–9.5	0.6–7.8			
% of resistant parasites	0	0	0	0			
Mefloquine							
IC ₅₀ geometric mean (nM) (no.)	24.9 (31)	22.4 (10)	31.9 (22)	19.4 (8)	46.6 (1)	43.9 (1)	0.6229
Median (nM)	29.1	25.1	33.0	28.8			
25 intercentile to 75 intercentile range	13.8–41.1	15.6–40.8	10.4–36.4	10.4–36.4			
% of resistant parasites	41.9	40.0	50.0	50.0			
Piperazine							
IC ₅₀ geometric mean (nM) (no.)	42.7 (31)	24.2 (10)	38.7 (22)	44.1 (8)	51.5 (1)	51.7 (1)	0.4444
Median (nM)	50.4	33.6	37.5	47.1			
25 intercentile to 75 intercentile range	34.9–91.1	8.8–57.7	20.8–72.9	30.9–64.1			
% of resistant parasites	6.5	0	9.1	0			
Pyronaridine							
IC ₅₀ geometric mean (nM) (no.)	8.4 (30)	10.8 (10)	8.5 (22)	10.1 (8)	21.4 (1)	13.4 (1)	0.6135
Median (nM)	9.4	12.6	7.8	9.3			
25 intercentile to 75 intercentile range	6.4–14.0	8.8–26.8	5.4–14.0	6.4–16.2			
% of resistant parasites	0	10.0	4.5	0			
Dihydroartemisinin							
IC ₅₀ geometric mean (nM) (no.)	1.0 (30)	2.6 (10)	1.6 (22)	2.0 (8)	5.6 (1)	1.7 (1)	0.2913
Median (nM)	1.5	2.6	1.8	3.3			
25 intercentile to 75 intercentile range	0.1–2.3	1.6–5.1	0.7–3.2	1.3–4.4			
% of resistant parasites	3.3	0	4.5	0			
Artesunate							
IC ₅₀ geometric mean (nM) (no.)	1.8 (28)	2.1 (10)	2.8 (22)	3.8 (8)	4.5 (1)	4.2 (1)	0.4010
Median (nM)	2.2	2.8	3.1	4.4			
25 intercentile to 75 intercentile range	0.8–3.9	2.2–3.7	1.4–5.9	2.9–6.8			
% of resistant parasites	3.6	0	9.1	0			
Doxycycline							
IC ₅₀ geometric mean (μ M) (no.)	16.5 (32)	17.0 (11)	13.1 (23)	18.3 (8)	121.6 (1)	21.9 (1)	0.8828
Median (μ M)	20.5	17.9	14.7	22.9			
25 intercentile to 75 intercentile range	8.8–36.1	9.8–29.9	6.0–40.6	15.3–38.4			
% of resistant parasites	21.9	27.3	30.4	37.5			

^aThe threshold values for the reduced *in vitro* susceptibility or resistance were the following: 611 nM, 77 nM, 61 nM, 115 nM, 30 nM, 135 nM, 60 nM, 12 nM, 12 nM, and 37 μ M for quinine, chloroquine, monodesethylamodiaquine, lumefantrine, mefloquine, piperazine, pyronaridine, dihydroartemisinin, artesunate, and doxycycline, respectively (12, 13).

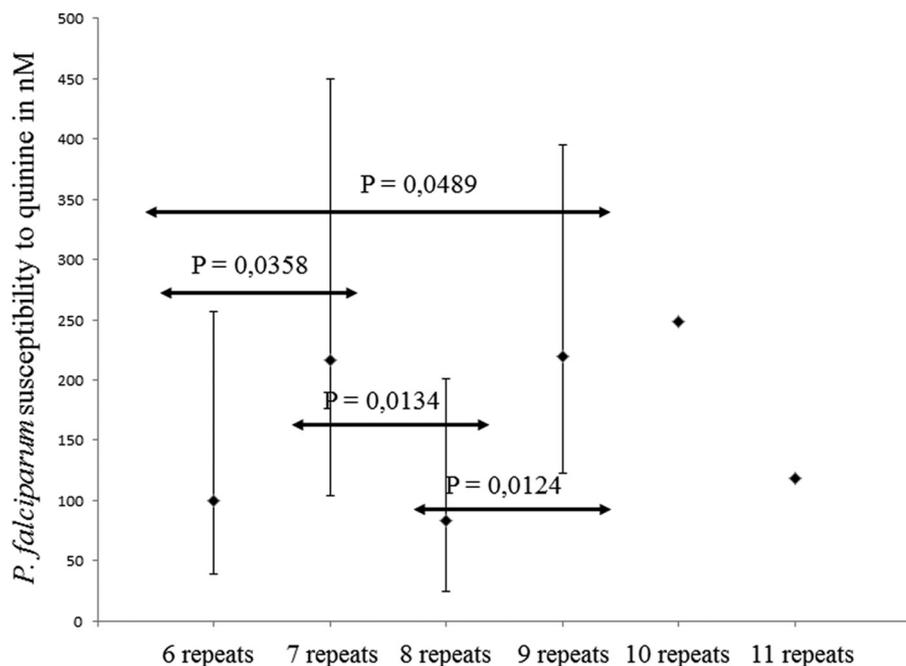


FIG 2 Distribution of quinine IC_{50} of 77 *P. falciparum* isolates according to the number of Asn repeats in *Pfmdr6*.

in *in vivo* susceptibility among the four different poly(Asn) repeat profiles for CQ, PPQ, PND, DQ, MQ, LMF, DHA, AS, and DOX (Table 1). We observed a significant association between 7 or 9 Asn repeats and reduced susceptibility to QN ($P = 0.0291$, Kruskal-Wallis) (Table 1 and Fig. 2). Six isolates were over the threshold of 611 nM for *in vitro* resistance to QN (12), but they were distributed in each group. There was no significant difference between the four groups (6, 7, 8, or 9 Asn repeats) in terms of percentage of isolates resistant to QN ($P = 0.849$). However, the isolate with the higher IC_{50} for quinine (1,429.8 nM) had 9 Asn repeats. This was the first observation of an association between *Pfmdr6* polymorphisms and reduced susceptibility to QN. The observations reported by Okombo et al. linking 6 and 8 Asn repeats with reduced susceptibility to LMF and PPQ, respectively (10), or by Wang et al. linking 7 and 9 Asn repeats with reduced susceptibility to LMF and DHA, respectively (11), were not replicated in our *P. falciparum* parasite population from Dakar. Additionally, the observations reported by Wang et al. and Okombo et al. were not replicated: reduced susceptibility to LMF was associated with 7 or 8 Asn repeats. A hypothesis explaining these differences is that Asn variations were mostly parasite population markers. The susceptibility to QN was associated with poly(Asn) repeat profiles of *Pfmdr6*. All of the patients treated with QN during the 3 years of collection were successfully treated. All of the isolates from these successfully treated patients contained 6 or 8 Asn repeats in the microsatellite region of *Pfmdr6*, which were associated with a low IC_{50} to QN. QN susceptibility was previously associated with microsatellite repeats in *P. falciparum* Na^+/H^+ exchanger 1 (*Pfnhe-1*) (14, 15). However, this association was location specific and differed between African and Asian *P. falciparum* isolates (15–17). The low number of *P. falciparum* isolates and the modest association limited the interpretation of the present results. It is difficult to extrapolate these findings to other parts of the world without new data sets. Another limitation was the use of standard *in vitro* tests for exploring resistance to artemisinin. The standard *in vitro* test is not adapted to follow resistance to artemisinin derivatives. The clinical resistance to artemisinin was manifested by an increase in the ring-stage survival rate after contact with artemisinin (ring survival test) (18). It is imperative to further assess more isolates from different geographical areas, to associate those results with functional biochemical studies, and to assess samples from clinical failure with QN to ascertain the role of this repeat polymorphism in *Pfmdr6*.

ACKNOWLEDGMENTS

We thank the patients and the staff of the Hôpital Principal de Dakar and Ndeye Fatou Diop and Maurice Gomis from the Hôpital Principal de Dakar for technical support.

This research was supported by the Délégation Générale pour l'Armement (grant PDH-2-NRBC-4-B1-402), the Schéma directeur Paludisme, Etat Major des Armées Françaises (grant LR 607a), and by the Ministère des Affaires Etrangères.

We declare that we have no competing interests.

REFERENCES

- Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NPJ, Lindegardh N, Socheat D, White NJ. 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 361:455–467. <https://doi.org/10.1056/NEJMoa0808859>.
- Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Seng S, Anderson JM, Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroeth S, Pukrittayakamee S, Jttamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runchaoren R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, et al. 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 371:411–423. <https://doi.org/10.1056/NEJMoa1314981>.
- Pradines B, Parquet V, Orlandi-Pradines E. 2009. ABC transporters in *Plasmodium* and their involvement in resistance to antimalarial drugs, p 113–128. In Ponte-Sucre A (ed), ABC transporters in microorganisms. Caister Academic Press, Wymondham, UK.
- Wurtz N, Fall B, Pascual A, Fall M, Baret E, Camara C, Nakoulima A, Diatta B, Ba Fall K, Mbaye PS, Dieme Y, Bercion R, Wade B, Pradines B. 2014. Role of *Pfmdr1* in *in vitro* susceptibility to chloroquine, quinine, monodesethylamodiaquine, mefloquine, lumefantrine and dihydroartemisinin. *Antimicrob Agents Chemother* 58:7032–7040. <https://doi.org/10.1128/AAC.03494-14>.
- Sisowath C, Stromberg J, Martensson A, Msellem M, Obondo C, Björkman A, Gil JP. 2005. *In vivo* selection of *Plasmodium falciparum* *Pfmdr1* 86N coding alleles by artemether-lumefantrine (coartem). *J Infect Dis* 191:1014–1017. <https://doi.org/10.1086/427997>.
- Dokomajilar C, Nsobya SL, Greenhouse B, Rosenthal PJ, Dorsey G. 2006. Selection of *Plasmodium falciparum* *Pfmdr1* alleles following therapy with artemether-lumefantrine in an area of Uganda where malaria is highly endemic. *Antimicrob Agents Chemother* 50:1893–1895. <https://doi.org/10.1128/AAC.50.5.1893-1895.2006>.
- Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, Patel R, Laing K, Looareesuwan S, White NJ, Nosten F, Krishna S. 2004. Mefloquine resistance in *Plasmodium falciparum* and increased *Pfmdr1* gene copy number. *Lancet* 364:438–447. [https://doi.org/10.1016/S0140-6736\(04\)16767-6](https://doi.org/10.1016/S0140-6736(04)16767-6).
- Briolant S, Bogreau H, Gil M, Bouchiba H, Baret E, Amalvict R, Rogier C, Pradines B. 2012. F423Y mutation in the *Pfmdr2* gene and the triple mutation (N51I, C59R, S108N) in *Pf dhfr* gene are independently associated with pyrimethamine resistance in *Plasmodium falciparum* isolates. *Antimicrob Agents Chemother* 56:2750–2752. <https://doi.org/10.1128/AAC.05618-11>.
- van der Velden M, Rijpma SR, Russel FGM, Sauerwein RW, Koenderink JB. 2015. *PfMDR2* and *PfMDR5* are dispensable for *Plasmodium falciparum* asexual parasite multiplication but change *in vitro* susceptibility to anti-malarial drugs. *Malar J* 14:76. <https://doi.org/10.1186/s12936-015-0581-y>.
- Okombo J, Abdi AI, Kiara SM, Mwai L, Pole L, Sutherland CJ, Nzila A, Ochola-Oyier LI. 2013. Repeat polymorphisms in the low-complexity regions of *Plasmodium falciparum* ABC transporters and associations with *in vitro* antimalarial responses. *Antimicrob Agents Chemother* 57:6196–6204. <https://doi.org/10.1128/AAC.01465-13>.
- Wang Z, Parker D, Meng H, Wu L, Li J, Zhao Z, Zhang R, Fan Q, Wang H, Cui L, Yang Z. 2012. *In vitro* sensitivity of *Plasmodium falciparum* from China-Myanmar border area to major ACT drugs and polymorphisms in potential target genes. *PLoS One* 7:e30927. <https://doi.org/10.1371/journal.pone.0030927>.
- Fall B, Diawara S, Sow K, Baret E, Diatta B, Fall KB, Mbaye PS, Fall F, Diémé Y, Rogier C, Wade B, Bercion R, Pradines B. 2011. *Ex vivo* susceptibility of *Plasmodium* isolates from Dakar, Senegal, to seven standard anti-malarial drugs. *Malar J* 10:310. <https://doi.org/10.1186/1475-2875-10-310>.
- Pascual A, Madamet M, Briolant S, Gaillard T, Amalvict R, Benoit N, Travers D, Pradines B, French National Reference Centre for Imported Malaria Study Group. 2015. Multinomial *in vitro* distribution of *Plasmodium falciparum* susceptibility to piperazine and pyronaridine. *Malar J* 14:49. <https://doi.org/10.1186/s12936-015-0586-6>.
- Henry M, Briolant S, Zettor A, Pelleau S, Baragatti M, Baret E, Mosnier J, Amalvict R, Fusai T, Rogier C, Pradines B. 2009. *Plasmodium falciparum* Na⁺/H⁺ exchanger 1 transporter is involved in reduced susceptibility to quinine. *Antimicrob Agents Chemother* 53:1926–1930. <https://doi.org/10.1128/AAC.01243-08>.
- Pelleau S, Bertaux L, Briolant S, Ferdig MT, Sinou V, Pradines B, Parzy D, Jambou R. 2011. Differential association of *Plasmodium falciparum* Na⁺/H⁺ exchanger polymorphism and quinine responses in field- and culture-adapted isolates of *Plasmodium falciparum*. *Antimicrob Agents Chemother* 55:5834–5841. <https://doi.org/10.1128/AAC.00477-11>.
- Briolant S, Pelleau S, Bogreau H, Hovette P, Zettor A, Castello J, Baret E, Amalvict R, Rogier C, Pradines B. 2011. *In vitro* susceptibility to quinine and microsatellite variations of the *Plasmodium falciparum* Na⁺/H⁺ exchanger (*Pfnhe-1*) gene: the absence of association in clinical isolates from the Republic of Congo. *Malar J* 10:37. <https://doi.org/10.1186/1475-2875-10-37>.
- Pascual A, Fall B, Wurtz N, Fall M, Camara C, Nakoulima A, Baret E, Diatta B, Fall KB, Mbaye PS, Diémé Y, Bercion R, Bogreau H, Briolant S, Rogier C, Wade B, Pradines B. 2013. *In vitro* susceptibility to quinine and microsatellite variations of the *Plasmodium falciparum* Na⁺/H⁺ exchanger transporter (*Pfnhe-1*) gene in 393 isolates from Dakar, Senegal. *Malar J* 12:189. <https://doi.org/10.1186/1475-2875-12-189>.
- Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, Lim P, Mao S, Sopha C, Sam B, Anderson JM, Duong S, Chuor CM, Taylor WR, Suon S, Mercereau-Puijalon O, Fairhurst RM, Menard D. 2013. 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* 13:1043–1049. [https://doi.org/10.1016/S1473-3099\(13\)70252-4](https://doi.org/10.1016/S1473-3099(13)70252-4).