

In Vivo Selection of *Plasmodium falciparum* *Pfcr*t and *Pfmdr*1 Variants by Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine in Burkina Faso

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***Plasmodium falciparum* *Pfcr*t-76 and *Pfmdr*1-86 gene polymorphisms were determined during a clinical trial in Burkina Faso comparing the efficacies of dihydroartemisinin-piperaquine (DHA-PPQ) and artemether-lumefantrine (AL). Significant selection of *Pfcr*t-K76 was observed after exposure to AL and DHA-PPQ, as well as selection of *Pfmdr*1-N86 after AL but not DHA-PPQ treatment, suggesting reverse selection on the *Pfcr*t gene by PPQ. These results support the rational use of DHA-PPQ in settings where chloroquine (CQ) resistance is high.**

Emergence of *Plasmodium falciparum* resistance to artemisinin-based combination therapy (ACT) in Asia (1, 2) represents a major threat to the recent gains in malaria control efforts. Partner drugs in ACTs increase the elimination of late-clearing parasites; however, if resistance develops against these partner drugs, treatment failures will probably increase (3).

Artemether-lumefantrine (AL) is currently the most widely used ACT in Africa (4), and dihydroartemisinin-piperaquine (DHA-PPQ) was recently recommended by the WHO for treatment of uncomplicated falciparum malaria (5). Piperaquine (PPQ) monotherapy was extensively used in China until 1978, when it was abandoned due to widespread resistance (6). PPQ has a long half-life of 3 to 4 weeks; therefore, its use may expose reinfecting and late-clearing parasites to a suboptimal blood concentration of PPQ, potentially leading to selection of resistant strains (6).

A *P. falciparum* chloroquine (CQ) resistance transporter gene mutation at codon 76 (*Pfcr*t-K76T) has been associated with CQ and amodiaquine (AQ) resistance (7, 8). Due to structural similarities between PPQ and CQ, there have been attempts to identify common markers of resistance (9, 10). However, the limited number of studies available did not observe selection of *Pfcr*t single nucleotide polymorphisms (SNPs) (11, 12).

Mutations in *P. falciparum* multidrug resistance gene 1 at codon 86 (*Pfmdr*1-N86Y) are associated with altered response to structurally unrelated antimalarials, including 4-aminoquinolines and aryl-aminoquinolines (13, 14). Significant selection of *Pfmdr*1-N86 has been consistently reported with AL in several settings (11, 12, 15). However, there is so far no *in vivo* evidence of *Pfmdr*1 selection after DHA-PPQ treatment, with the exception of the reported borderline selection of *Pfmdr*1-D1246 (12).

In Burkina Faso, artesunate-amodiaquine (AS-AQ) and AL were introduced in 2005 as first-line treatments of uncomplicated malaria (16) following high CQ resistance (CQR) (17). As part of a multicentric study on the safety and efficacy of DHA-PPQ, a clinical trial was conducted in Burkina Faso to test the noninferiority of DHA-PPQ compared to AL (18). We present here *Pfcr*t and *Pfmdr*1 SNP analysis of *P. falciparum* recurrent infections in

an attempt to assess whether DHA-PPQ selected for known polymorphisms in this area of known high CQR

A total of 301 children of ages 6 to 59 months were randomly allocated to either DHA-PPQ or AL treatment at a ratio of 2:1 and followed up for 42 days as published elsewhere (18). Blood spot filter paper specimens were collected on the day of enrollment and during follow-up. Genotyping to distinguish recrudescence from new infections was done as previously described (19). The detection of the *Pfcr*t-76 and *Pfmdr*1-86 SNPs was carried out by PCR followed by restriction fragment length polymorphism (RFLP) as previously described (7). A total of 272 (91 AL versus 181 DHA-PPQ samples) day 0 samples as well as 24 and 37 samples for recurrent *P. falciparum* infections in the AL and DHA-PPQ groups, respectively, were analyzed. The χ^2 test with Yates correction or the Fisher exact test was used for categorical variables as appropriate. The strength of association between markers and treatment outcomes was evaluated by odds ratios (OR). Mixed alleles were treated as mutant, while unsuccessful PCR results were excluded from the analysis. Statistical significance was set at ≤ 0.05 .

Totals of 267 (98.2%) and 272 (100%) day 0 blood samples were successfully genotyped for the *Pfcr*t-K76T and *Pfmdr*1-N86Y polymorphisms, respectively (Fig. 1). At baseline, the prevalences of the *Pfcr*t-76T mutants were similar between the two study arms: about 50% (48.3% for AL and 48.9% for DHA-PPQ) when con-

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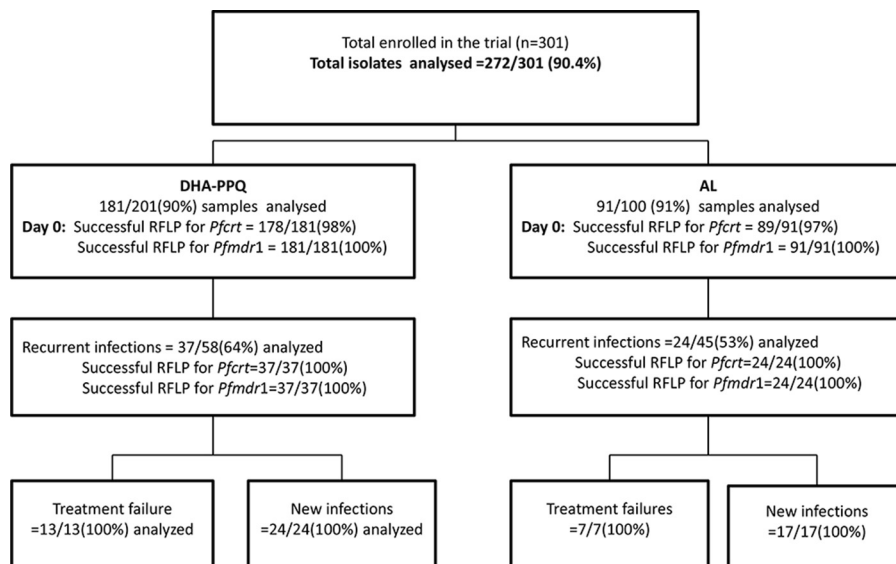


FIG 1 Trial profile indicating the number of samples analyzed at day 0 and among recurrences by treatment arm. The treatment outcomes are all PCR corrected.

sidering infections with only the mutant alleles and almost 70% when considering infections with mixed alleles (Table 1). The prevalences of the *Pfmdr*1-N86 allele at baseline were higher: 59.3% for AL and 64.1% for DHA-PPQ.

The prevalence of the *Pfcr*t-K76 was significantly higher in recurrent infections than at day 0, before treatment, both in the AL arm (79.2% versus 30.3%; $P < 0.001$) and in the DHA-PPQ arm (67.6% versus 29.2%; $P < 0.001$). Similarly, after treatment with AL, the prevalence of *Pfmdr*1-N86 in recurrent infections was significantly higher than that at day 0 (95.8% versus 59.3%; $P < 0.001$), while there was no difference in the DHA-PPQ arm

(Table 1). No association between *Pfcr*t-K76T or *Pfmdr*1-N86Y SNPs and treatment outcome was observed (Table 2).

Our results showed a high baseline prevalence of the *Pfcr*t-76T alleles, which corroborates previous reports from Burkina Faso (8). Interestingly, significant selection of the *Pfcr*t-K76 allele after DHA-PPQ treatment was unexpectedly observed as this is contrary to the hypothesis that PPQ and CQ share similar mechanisms of resistance given their structural similarities (20). In comparison, DHA-PPQ did not select for *Pfcr*t (K76) polymorphisms in previous studies carried out in Burkina Faso (11, 12). The reasons for the observed differences are un-

TABLE 1 Selection of *Pfcr*t and *Pfmdr*1 SNPs among *P. falciparum* recurrent infections by AL and DHA-PPQ

Treatment and allele ^a	SNP ^b	No. of infections/total:		χ^2 value	<i>P</i> value ^c	Comment
		Day 0	Recurrent			
AL						
<i>Pfcr</i> t	K76	27/89 (30.3)	19/24 (79.2)	16.7	<0.001	Selection for K76
	76T	43/89 (48.3)	1/24 (4.2)			
	Mixed (K76T)	19/89 (21.4)	4/24 (16.7)			
<i>Pfmdr</i> 1	N86	54/91 (59.3)	23/24 (95.8)	NA ^d	<0.001	Selection for N86
	86Y	17/91 (18.7)	1/24 (4.2)			
	Mixed (N86Y)	20/91 (22.0)	0/24 (0)			
DHA-PPQ						
<i>Pfcr</i> t	K76	52/178 (29.2)	25/37 (67.6)	18.0	<0.001	Selection for K76
	76T	87/178 (48.9)	9/37 (24.3)			
	Mixed (K76T)	39/178 (21.9)	3/37 (8.1)			
<i>Pfmdr</i> 1	N86	116/181 (64.1)	24/37 (64.9)	0.0	0.929	No evidence
	86Y	32/181 (17.7)	9/37 (24.3)			
	Mixed (N86Y)	33/181 (18.2)	4/37 (10.8)			

^a *Pfcr*t is the *P. falciparum* chloroquine resistance transporter gene, and *Pfmdr*1 is the *P. falciparum* multidrug resistance gene.

^b For *Pfcr*t, K76 is wild type and 76T is mutant, and for *Pfmdr*1, N86 is wild type and 86Y is mutant.

^c Significant values are in boldface.

^d NA, not applicable.

TABLE 2 Association between SNPs in *Pfcr*-76 and *Pfmdr1*-86 in *P. falciparum* isolates and treatment outcomes with AL and DHA-PPQ

Treatment and allele ^a	SNP	No. of samples with treatment outcome/total (%) ^b		OR (95% CI) ^c	P value
		Failed	Cured		
AL					
<i>Pfcr</i>	K76	2/7 (28.6)	25/82 (30.5)	0.91 (0.20–6.04)	0.92
	76T	5/7 (71.4)	57/82 (69.5)		
<i>Pfmdr1</i>	N86	7/7 (100.0)	49/84 (58.3)	NA ^d	NA
	86Y	0/7 (0.0)	35/84 (41.7)		
DHA-PPQ					
<i>Pfcr</i>	K76	3/13 (23.1)	49/165 (29.7)	0.71 (0.19–2.69)	0.62
	76T	10/13 (76.9)	116/165 (70.3)		
<i>Pfmdr1</i>	N86	8/13 (61.5)	109/167 (65.3)	0.85 (0.27–2.72)	0.79
	86Y	5/13 (38.5)	58/167 (34.7)		

^a *Pfcr* is the *P. falciparum* chloroquine resistance transporter gene, and *Pfmdr1* is the *P. falciparum* multidrug resistance gene.

^b The denominators represent number of samples for which the SNP was analyzable. Mixed alleles of the *Plasmodium falciparum* *Pfcr* gene or *Pfmdr1* gene were treated as mutants. PCR-adjusted treatment failure was defined according to the World Health Organization (27).

^c CI, confidence interval.

^d NA, not applicable.

known. However, an earlier *in vitro* study from Kenya (10) found that PPQ 50% inhibitory concentration (IC₅₀) values were not associated with *Pfcr* or *Pfmdr1* mutations. Conversely, *Pfcr* CQR haplotypes and a novel *Pfcr* mutation (C101F) were associated with resistance to PPQ in other studies (14, 20). Overall, the available evidence suggests high *in vitro* susceptibility of the CQR parasites to PPQ (9, 10, 21). This could be explained by the large bis-quinolone structure of PPQ postulated to inhibit the transporter-mediated drug efflux, thus maintaining high potency against CQR strains (22). Indeed, data from clinical trials confirm the outstanding efficacy of DHA-PPQ despite high CQR levels (18, 23). Thus, as DHA-PPQ becomes increasingly available for treatment and chemoprevention in settings where malaria is endemic, *Pfcr* polymorphism and its clinical implications should be further monitored.

Unlike recently reported results from Uganda (24), there was no significant selection of the *Pfmdr1*-86Y allele observed in our study after DHA-PPQ treatment, consistent with previous reports from Burkina Faso (11, 12). These observed differences may be explained by the different genetic backgrounds of the parasites in West and East Africa. Our results further confirmed that AL treatment selects for *Pfcr*-K76 and *Pfmdr1*-N86 as previously observed (15, 25). At present, AL remains efficacious, although the selected wild-type alleles have been repeatedly associated with diminished sensitivity to lumefantrine (12, 15, 26).

In conclusion, we observed significant selection of *Pfcr*-K76 following DHA-PPQ and AL treatment, suggesting that, despite structural similarities between PPQ and CQ, the drugs exert a different mechanism of selection on the *Pfcr* gene. These results support the rational use of DHA-PPQ in settings where CQ resistance is high.

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