View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Sapientia

Tropical Medicine and International Health doi:10.1111/j.1365-3156.2007.01843.x

VOLUME 12 NO 6 PP 736–742 JUNE 2007

The role of *pfmdr1* in *Plasmodium falciparum* tolerance to artemether-lumefantrine in Africa

Christin Sisowath[1], Pedro E. Ferreira[1,2], Leyla Y. Bustamante[3], Sabina Dahlström[1], Andreas Mårtensson[1,4], Anders Björkman[1], Sanjeev Krishna[3] and José P. Gil[1,2]

1 *Malaria Research Unit, Division of Infectious Diseases, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden*
2 *Centre of Structural and Molecular Biomedicine, University of Algarve, Gambelas, Portugal*
3 *Division of Cellular & Molecular Medicine, Centre for Infection, St. George's, University of London, London, UK*
4 *Emergency Medicine Unit, Department of Medicine, Kullbergska Hospital, Katrineholm, Sweden*

Summary

OBJECTIVE Artemether-lumefantrine (AL), presently the most favoured combination therapy against uncomplicated *Plasmodium falciparum* malaria in Africa, has recently shown to select for the *pfmdr1* 86N allele. The objective of this study was to search for the selection of other mutations potentially involved in artemether-lumefantrine tolerance and/or resistance, i.e. *pfmdr1* gene amplification, *pfmdr1* Y184F, S1034C, N1042D, D1246Y, *pfcrt* S163R and *PfATP6* S769N.

METHODS The above mentioned SNPs were analysed by PCR–restriction fragment length polymorphism and *pfmdr1* gene amplification by real-time PCR based protocols in parasites from 200 children treated with AL for uncomplicated *P. falciparum* malaria in Zanzibar.

RESULTS A statistically significant selection of *pfmdr1* 184F mostly in combination with 86N was seen in reinfections after treatment. No *pfmdr1* gene amplification was found.

CONCLUSION The results suggest that different *pfmdr1* alleles are involved in the development of tolerance/resistance to lumefantrine.

keywords malaria, *Plasmodium falciparum*, lumefantrine, resistance, *pfmdr1*

Introduction

Plasmodium falciparum malaria kills more than two million people annually, mostly in Africa. Artemisinin based combination therapy (ACT) is being promoted as the main policy in response to increasing resistance to common monotherapies. A fixed dose combination of the artemisinin derivative artemether and the arylaminoalcohol drug lumefantrine (Coartem; Novartis, AG) is presently the mostly favoured ACT and has, therefore, recently been introduced as first or second line treatment in several African countries.

Single nucleotide polymorphisms (SNPs) in the *P. falciparum* multidrug resistance gene 1 (*pfmdr1*) have been associated with altered *in vitro* and *in vivo* parasite response to arylaminoalcohols (Duraisingh & Cowman 2005), including lumefantrine (Duraisingh *et al.* 2000). We have previously reported that *in vivo* exposure to artemether-lumefantrine (AL) selects for *pfmdr1* 86N in new inoculations (reinfections) post-treatment (Sisowath *et al.* 2005). Due to this finding, it has been suggested that the selection of 86N may represent a marker of tolerance to lumefantrine (Hastings & Ward 2005), where tolerant parasites, being in the intermediate stage between sensitive and resistant, are killed by the high drug levels during treatment but can withstand residual lumefantrine concentrations and proliferate in the blood earlier than sensitive.

The genetic basis of *in vivo* resistance to AL remains unclear and, therefore, we have explored several additional factors of potential importance in development of AL tolerance/resistance such as: (1) Is amplification of the *pfmdr1* gene selected in recurrent infections after AL treatment in Zanzibar as it has been observed to influence mefloquine (Price *et al.* 2004) and lumefantrine (Price *et al.* 2006) susceptibility in Southeast Asia? (2) Is the selection of *pfmdr1* 86N allele just a marker of the selection of the gene amplification as this SNP is associated with amplification (Price *et al.* 2004; Uhlemann *et al.* 2005)? (3) Are other *pfmdr1* SNPs associated with arylaminoalcohol resistance (Woodrow & Krishna 2006) selected among the recurrent parasites? (4) Is the *P. falciparum* chloroquine resistance transporter (*pfcrt*) S163R SNP, previously associated with *in vitro* resistance to halofantrine

 © 2007 Blackwell Publishing Ltd

(Johnson *et al.* 2004) selected? (5) Is the product of the newly isolated sarco/endoplasmic reticulum Ca^{2+}-ATPase orthologue of *P. falciparum* (PfATP6) associated with the few recrudescences observed in our study, potentially due to a lower response to the artemether component of the drug combination? This has been suggested as a pharmacological target of artemisinin derivatives (Krishna *et al.* 2006), and a field study in South America has shown an association between the *PfATP6* S769N SNP and a significant decrease in sensitivity to artemether (Jambou *et al.* 2005).

The aim of this study was to address these questions in samples collected during a clinical trial of AL treatment in children with uncomplicated malaria in Zanzibar (Martensson *et al.* 2005; Sisowath *et al.* 2005), before AL was introduced as second line treatment on the islands.

Methods

Patients

In a previously published clinical trial conducted between October 2002 and February 2003 in Zanzibar, 200 children with microscopically confirmed uncomplicated *P. falciparum* malaria were treated under supervision with AL in standard doses according to body weight twice daily for 3 days (Martensson *et al.* 2005; Sisowath *et al.* 2005). Drug intake was not necessarily accompanied by a fatty meal, although this is considered to enhance drug absorption. All children were checked routinely for parasite presence on days 0, 1, 2, 3, 7, 14, 21, 28, 35, and 42, and at any time fever was recorded between the scheduled days. Finger prick blood samples were collected on filter paper (3MM; Whatman). Informed consent was obtained from parents or legal guardians of the enrolled children. The study was approved by the Zanzibar Health Research Council, the World Health Organization (WHO – SCRISM) in Geneva and the Karolinska Institutet Ethical Committees.

Molecular analysis

Blood samples from the day of enrolment (day 0) and day of recurrent infection were analysed at the Malaria Research Unit, Karolinska Institutet, Stockholm, and at the Division of Cellular and Molecular Medicine, St George's University of London.

Stepwise genotyping of *P. falciparum* merozoite surface protein 2 (*pfmsp2*) and 1 (*pfmsp1*) genes was performed to distinguish recrudescences from reinfections as described by Mugittu *et al.* (2006). Recurrent infections classified as recrudescences following *pfmsp2* genotyping (Sisowath *et al.* 2005) were subjected to additional *pfmsp1* genotyping, using established protocols with minor adjustments (Snounou *et al.* 1999). Recrudescence was defined as the presence of at least one matching allelic band from day 0 and day of recurrent infection for both the *pfmsp2* and *pfmsp1* marker. Reinfections were defined as the absence of matching bands either after *pfmsp2* or *pfmsp1* genotyping. A negative PCR result on either *pfmsp2* or *pfmsp1* analysis was considered as uncertain.

The *pfmdr1* N86Y (Sisowath *et al.* 2005), Y184F, S1034C, N1042D, D1246Y and the *pfcrt* S163R SNPs were analysed by conventional PCR-restriction fragment length polymorphism (RFLP) based protocols for all baseline and recurrent infections. The endonuclease restriction enzyme *Apo*I (New England Biolabs) was used for the recognition of *pfmdr1* 86N, *Tsp*509I (New England Biolabs), or the Fermentas isoschizomer *Tas*I was used for the recognition of 184F; *Dde*I (New England Biolabs) for 1034S; *Ase*I (New England Biolabs) or the isoschizomer *Vsp*I (Fermentas) for 1042N; *Eco*RV (New England Biolabs) for 1246Y; and *Hinf*I (New England Biolabs) for the recognition of *pfcrt* 163R. The *PfATP6* S769N SNP was analysed through a semi-nest PCR-RFLP for recrudescent infections defined by *pfmsp2* only, according to the definition of recrudescence used in our previous report (Sisowath *et al.* 2005). Oligonucleotide primers were: 1st fw 5′-AAT TCA TAA TAA GAT TCA AAA TAT GGG AAA-3′; 1st rev 5′-GAT CAA TAA TAC CTA ATC CAC CTA AAT AAA-3′; semi nest rev 5′-ATC TGT ATT CTT AAT ATT TAA ATC TTT AGT A-3′. The 769S allele was identified by the restriction enzyme *Rsa*I (New England Biolabs). Restriction fragments were analysed by electrophoresis in 2% agarose gels. PCR–RFLP results were confirmed by automated sequencing (Megabace 1000, Amersham Biosciences). *Taq* polymerase was from Promega. All PCR reactions were performed in GeneAmp® PCR System 2700 thermocyclers (Applied Biosystems).

Assessment of *pfmdr1* copy number was performed as previously described by TaqMan® probe based real-time PCR protocols at St George's University of London (ABI PRISM® 7700 Sequence Detection System; Applied Biosystems) and at the Karolinska Institutet in Stockholm (ABI PRISM® 7000 Sequence Detection System) (Price *et al.* 2004). The multiplex PCR reactions contained 1×TaqMan buffer (Applied Biosystems), 300 nM of each primer, 100 nM of each probe and 5–10 μl of DNA in a total reaction volume of 25 μl. All samples were run in triplicate. The *P. falciparum* clone 3D7 was used as a one copy calibrator and Dd2 was used as a multi-copy control. The results were analysed by the comparative $\Delta\Delta$Ct (cycle threshold) method. Results of triple replicate samples were

© 2007 Blackwell Publishing Ltd

excluded if: (1) more than one replicate exhibited a Ct > 35; (2) the triple replicate samples had Ct SD > 0.5 and the Ct difference between the two remaining replicates was > 0.7 after the removal of any outlier. The parasites were considered to have an amplified *pfmdr1* gene if copy number was > 1.5.

Statistical analysis

When determining the prevalence of individual SNP alleles, mixed infections contribute equally to each of the studied allele, as described in our previous report (Sisowath *et al.* 2005). As for the haplotype analysis of *pfmdr1* position 86 in combination with 184 or 1246, if any of the two analysed SNP positions in the haplotype analysis were both wild type and mutant allele, the infection was considered to consist of two different haplotypes. If both the SNPs had wild type and mutant alleles, the sample was excluded from that haplotype analysis. 95% confidence intervals were calculated with CIA (Confidence Interval Analysis) program. Yates' corrected chi-square testing was performed with the Microstat® software. Statistical significance was defined as a *P*-value ≤ 0.05.

Results

Out of 200 children included in the study, three children were lost to follow-up. Forty-five episodes with recurrent parasitaemia were recorded, the earliest on day 21. One recurrent episode was excluded because of a previous retreatment with another drug. As reported previously, 189 baseline and 39 recurrent infections were PCR positive for *pfmsp2*, *pfcrt* position 76 and *pfmdr1* 86 (Sisowath *et al.* 2005). By initial *pfmsp*2 analysis, 11 of the recurrent episodes were defined as recrudescences and 28 as reinfections. After additional *pfmsp1* analysis, only 2 out of 11 remained recrudescences, while 7 were reclassified as reinfections and 2 were considered uncertain due to negative PCR outcome with the *pfmsp1* marker.

None of the 186 successfully analysed baseline isolates had an amplified *pfmdr1* gene, neither had any recurrent infections. Of all the herein SNPs analysed, allelic variation was only observed in *pfmdr1* positions 184 and 1246. As for the remaining *pfmdr1* S1034C, N1042D, *pfcrt* S163R and *PfATP6* S769N SNPs, all parasite isolates had the wild type allele.

The *pfmdr1* 184F prevalence showed a 2.1 fold increase in reinfections from 16.6% to 35.5% ($P = 0.027$) (Table 1). When combining this result with our previously published data on N86Y, an association between the 86N/184F haplotype and reinfections was seen (Table 2). The statistically significant selection of this haplotype ($P = 0.001$) was followed by a similar decrease in the prevalence of 86Y/184Y ($P = 0.009$), while the remaining two haplotypes (86Y/184F and 86N/184Y) were not affected.

The *pfmdr1* 1246D allele prevalence was 66.8% prior to treatment and 83.9% among reinfecting parasites ($P = 0.086$) (Table 1). The selection of 1246D in combination with 86N was specifically significant in reinfecting parasites ($P = 0.001$). This selection was not at the cost of any specific allele (Table 2). Both *pfmdr1* 86N and 184F SNPs were selected mainly in the early reinfections (Figure 1).

Two recrudescences were found after stepwise genotyping. Both had *pfmdr1* 184Y alleles, while in positions 86/1246 the respective recrudescence had N/D and Y/Y alleles. The small number of recrudescences did not allow any meaningful analysis of selection potential.

Discussion

Artemether-lumefantrine presently represents the most important drug for the treatment of uncomplicated malaria in the post-chloroquine and sulfadoxine-pyrimethamine era in Africa. Understanding the molecular basis of progression from susceptibility via tolerance to resistance of *P. falciparum* against this combination is fundamental

Table 1 *Pfmdr1* SNPs N86Y, Y184F and D1246Y before treatment and in reinfections.

	Day 0		Reinfections		
pfmdr1 SNPs	*n* A + B/C + B	95% CI	*n* A + B/C + B	95% CI	*P*-value
86N	29 + 20/189 + 20	23.4 (17.7–29.2)	15 + 3/35 + 3	47.4 (31.0–64.2)	0.004
184F	22 + 8/173 + 8	16.6 (11.2–22.0)	10 + 1/30 + 1	35.5 (19.2–54.6)	0.027
1246D	108 + 47/185 + 47	66.8 (60.8–72.9)	25 + 1/30 + 1	83.9 (66.3–94.5)	0.086

For the SNP analysis a mixed infection was considered to contribute equally for both SNP alleles. To calculate the frequency of the studied allele, the number of mixed infections harbouring both wild type and mutant alleles (B) was added to the number of infections carrying only the allele of interest (A). Mixed infections (B) was also added to the total numbers of successfully analysed infections (C).

© 2007 Blackwell Publishing Ltd

Table 2 *Pfmdr1* 86/184 and 86/1246 haplotypes before treatment and in reinfections

	Day 0		Reinfections		
pfmdr1 haplotypes	*n*	95% CI	*n*	95% CI	*P*-value
86N + 184Y	27/190	14.2 (9.3–19.2)	6/33	18.2 (7.0–35.5)	0.743
86Y + 184Y	134/190	70.5 (64.0–77.0)	15/33	45.5 (28.1–63.7)	0.009
86N + 184F	16/190	8.4 (4.9–13.3)	10/33	30.3 (15.6–48.7)	0.001
86Y + 184F	13/190	6.8 (3.7–11.4)	2/33	6.1 (0.7–20.2)	0.833
86N + 1246Y	3/230	1.3 (0.3–3.8)	0/33	0 (0–10.6)	0.829
86Y + 1246Y	67/230	29.1 (23.3–35.0)	5/33	15.2 (5.1–31.9)	0.140
86N + 1246D	39/230	17.0 (12.1–21.8)	14/33	42.4 (25.5–60.8)	0.001
86Y + 1246D	121/230	52.6 (46.2–59.1)	14/33	42.4 (25.5–60.8)	0.364

For the haplotype analysis only infections being successfully analysed for both the amino acid positions of the haplotype (86 and 184 or 86 and 1246) were included. A mixed infection in one of the SNP was considered as two different haplotypes. If both the SNPs were mixed infections the sample was excluded from that haplotype analysis.

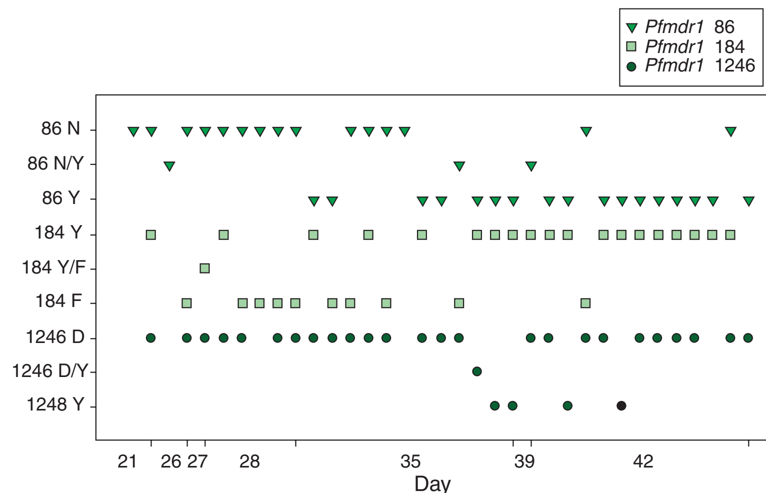

Figure 1 Distribution of *pfmdr1* N86Y, Y184F and D1246Y SNPs of the 28 *pfmsp2* adjusted *Plasmodium falciparum* reinfections during the 42-day follow-up period. Infections with *pfmdr1* 86N alleles are being selected particularly in the early reinfections, while in the later reinfections on day 42 the 86Y and 86N frequencies have returned towards the baseline frequency. A similar finding was seen for *pfmdr1* Y184F.

for the establishment of measures to protect it from premature dismissal. Understanding the molecular basis for tolerance and resistance and having molecular markers for its surveillance will provide more solid ground for appropriate drug polices. Markers of tolerance, indicating emerging resistance, will enable decision makers to change to more effective drugs before resistance has reached deleterious levels.

We have previously identified the *pfmdr1* 86N allele as a potential marker of tolerance to lumefantrine (Sisowath *et al.* 2005). In this follow-up work, we have re-examined samples from the same trial to assess the possible contribution of other tentative molecular factors in the *P. falciparum* response to exposure of both lumefantrine and artemether.

In particular, *pfmdr1* gene amplification has been established as a contributor to altered *in vivo* parasite response to arylaminoalcohols (Cowman *et al.* 1994; Duraisingh *et al.* 2000; Price *et al.* 2004; Price *et al.* 2006). Our results support that *pfmdr1* amplification is presently rare in this region of Africa, because these are in agreement with other recent observations from Uganda (Dokomajilar *et al.* 2006) and Kenya (Holmgren *et al.* 2006), whereas in Southeast Asia, the prevalence can be up to 50% (Price *et al.* 2006). The reasons for this current rarity of *pfmdr1* amplification in East Africa are unclear, but may be related to a parasite fitness cost associated with counter selection by the vast use of chloroquine on the continent. Parasites selected for high levels of chloroquine resistance have been shown to deamplify *pfmdr1* (Barnes *et al.* 1992). It is also possible that the lack of selection of *pfmdr1* amplification by AL may be due to low selection pressure or a result of the combination *per se*, including the documented synergistic effect of the two compounds

© 2007 Blackwell Publishing Ltd

(Hassan Alin *et al.* 1999). In Gabon, low frequencies of *pfmdr1* amplification were found after treatment with mefloquine as monotherapy (Uhlemann *et al.* 2005), suggesting that selection of gene amplification may occur if lumefantrine would be used alone in Africa.

The two recrudescent infections found in this study could not be explained by enhanced *pfmdr1* copy number. Recent data from *in vivo* trials in Southeast Asia (Price *et al.* 2006) showed that *pfmdr1* amplification was only associated with treatment failure following a four-dose regimen of AL and low lumefantrine plasma levels (Price *et al.* 2006). Our study used the standard six dose regimen, but we do not know if the two treatment failures were caused by insufficient drug bioavailability since lumefantrine plasma concentrations are not available.

A wide range of susceptibility levels to arylaminoalcohols has been seen without the presence of *pfmdr1* amplification (Basco *et al.* 1995; Ritchie *et al.* 1996; Chaiyaroj *et al.* 1999; Johnson *et al.* 2004; Price *et al.* 2004), suggesting that other factors such as SNPs could be involved in the development of tolerance and resistance.

In our study, there was a selection of *pfmdr1* genes carrying the 1246D allele among reinfecting parasites, although not statistically significant ($P = 0.086$) possibly due to the established high frequency already at baseline. A significant selection of the combined 86N/1246D haplotype was, however, observed ($P = 0.001$). The 1246D SNP may thus represent a secondary marker of the response to lumefantrine. *Pfmdr1* 184F harbouring parasites were also found to be selected, specifically in early reinfections (Figure 1). Again, we observed a co-selection with 86N; the reason for the association is presently unclear, but may involve a compensating mechanism or a combined contribution to decreased drug susceptibility, e.g. enhanced substrate (drug) binding due to combined changes in the amino acid side chains. The selection of the 1246D and 184F alleles after AL treatment is in agreement with recent findings from Uganda where ACT has not yet been implemented (Dokomajilar *et al.* 2006). Whether the *pfmdr1* 1246D, 184F and 86N SNPs all take part in a development of resistance to lumefantrine or rather represent compensating mechanisms to maintain or increase parasite fitness remains unclear.

The *pfcrt* S163R SNP, a recently proposed marker of *in vitro* resistance to arylaminoalcohols, was not found, confirming the previous reports of its rarity *in vivo* (Johnson *et al.* 2004). It is notable that this SNP was selected *in vitro* from a background of *pfmdr1* 86Y (strain K1) (Ritchie *et al.* 1996), which is not usually associated with decreased arylaminoalcohol sensitivity *in vivo* (Price *et al.* 2004; Sisowath *et al.* 2005).

Finally, for the first time, the *PfATP6* 769N allele, recently associated to decrease artemisinin susceptibility (Jambou *et al.* 2005), was investigated in an ACT efficacy trial. However, this SNP was detected neither at baseline nor among the previously *pfmsp2* defined 11 recrudescences. This mutation may represent a geographically specific variant as it has so far only been detected in South America (Jambou *et al.* 2005).

In conclusion, our results confirm the involvement of *pfmdr1* in lumefantrine tolerance *in vivo*. The path to resistance to AL may involve a marked selection of 86N/184F and 86N/1246D haplotypes. *Pfmdr1* amplification was not present in our study, but may potentially represent a further development towards resistance with increased selection pressure of lumefantrine in Africa.

Acknowledgements

We thank Prof Nicolas White (Mahidol University, Bangkok, Thailand/Oxford University, UK) for his valuable research suggestions. This work was partially supported by the European Union 5th Framework Project (contract QLK2-CT20021-1503-RESMALCHIP).

References

Barnes DA, Foote SJ, Galatis D, Kemp DJ & Cowman AF (1992) Selection for high-level chloroquine resistance results in deamplification of the pfmdr1 gene and increased sensitivity to mefloquine in *Plasmodium falciparum. The EMBO Journal* **11**, 3067–3075.

Basco LK, Le Bras J, Rhoades Z & Wilson CM (1995) Analysis of pfmdr1 and drug susceptibility in fresh isolates of *Plasmodium falciparum* from subsaharan Africa. *Molecular and Biochemical Parasitology* **74**, 157–166.

Chaiyaroj SC, Buranakiti A, Angkasekwinai P, Looressuwan S & Cowman AF (1999) Analysis of mefloquine resistance and amplification of pfmdr1 in multidrug-resistant *Plasmodium falciparum* isolates from Thailand. *The American Journal of Tropical Medicine and Hygiene* **61**, 780–783.

Cowman AF, Galatis D & Thompson JK (1994) Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the pfmdr1 gene and cross-resistance to halofantrine and quinine. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 1143–1147.

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. *Antimicrobial Agents and Chemotherapy* **50**, 1893–1895.

Duraisingh MT & Cowman AF (2005) Contribution of the pfmdr1 gene to antimalarial drug-resistance. *Acta Tropica* **94**, 181–190.

© 2007 Blackwell Publishing Ltd

Duraisingh MT, Roper C, Walliker D & Warhurst DC (2000) Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the pfmdr1 gene of *Plasmodium falciparum*. *Molecular Microbiology* **36**, 955–961.

Hassan Alin M, Bjorkman A & Wernsdorfer WH (1999) Synergism of benflumetol and artemether in *Plasmodium falciparum*. *The American Journal of Tropical Medicine and Hygiene* **61**, 439–445.

Hastings IM & Ward SA (2005) Coartem (artemether-lumefantrine) in Africa: the beginning of the end? *The Journal of Infectious Diseases* **192**, 1303–1304; author reply 1304–1305.

Holmgren G, Bjorkman A & Gil JP (2006) Amodiaquine resistance is not related to rare findings of pfmdr1 gene amplifications in Kenya. *Tropical Medicine and International Health* **11**, 1808–1812.

Jambou R, Legrand E, Niang M *et al.* (2005) Resistance of *Plasmodium falciparum* field isolates to in-vitro artemether and point mutations of the SERCA-type PfATPase6. *Lancet* **366**, 1960–1963.

Johnson DJ, Fidock DA, Mungthin M *et al.* (2004) Evidence for a central role for PfCRT in conferring *Plasmodium falciparum* resistance to diverse antimalarial agents. *Molecular Cell* **15**, 867–877.

Krishna S, Woodrow CJ, Staines HM, Haynes RK & Mercereau-Puijalon O (2006) Re-evaluation of how artemisinins work in light of emerging evidence of in vitro resistance. *Trends in Molecular Medicine* **6**, 6.

Martensson A, Stromberg J, Sisowath C *et al.* (2005) Efficacy of artesunate plus amodiaquine versus that of artemether-lumefantrine for the treatment of uncomplicated childhood *Plasmodium falciparum* malaria in Zanzibar, Tanzania. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **41**, 1079–1086. Epub 2005 September 1013.

Mugittu K, Adjuik M, Snounou G *et al.* (2006) Molecular genotyping to distinguish between recrudescents and new infections in treatment trials of *Plasmodium falciparum* malaria conducted in Sub-Saharan Africa: adjustment of parasitological outcomes and assessment of genotyping effectiveness. *Tropical Medicine and International Health* **11**, 1350–1359.

Price RN, Uhlemann AC, Brockman A *et al.* (2004) Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. *Lancet* **364**, 438–447.

Price RN, Uhlemann AC, Vugt MV *et al.* (2006) Molecular and Pharmacological Determinants of the Therapeutic Response to Artemether-Lumefantrine in Multidrug-Resistant *Plasmodium falciparum* Malaria. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **42**, 1570–1577. Epub 2006 April 1526.

Ritchie GY, Mungthin M, Green JE, Bray PG, Hawley SR & Ward SA (1996) In vitro selection of halofantrine resistance in *Plasmodium falciparum* is not associated with increased expression of Pgh1. *Molecular and Biochemical Parasitology* **83**, 35–46.

Sisowath C, Stromberg J, Martensson A *et al.* (2005) In vivo selection of *Plasmodium falciparum* pfmdr1 86N coding alleles by artemether-lumefantrine (Coartem). *The Journal of Infectious Diseases* **191**, 1014–1017 Epub 2005 February 1018.

Snounou G, Zhu X, Siripoon N *et al.* (1999) Biased distribution of msp1 and msp2 allelic variants in *Plasmodium falciparum* populations in Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 369–374.

Uhlemann AC, Ramharter M, Lell B, Kremsner PG & Krishna S (2005) Amplification of *Plasmodium falciparum* multidrug resistance gene 1 in isolates from Gabon. *The Journal of Infectious Diseases* **192**, 1830–1835 Epub 2005 October 1837.

Woodrow CJ & Krishna S (2006) Antimalarial drugs: recent advances in molecular determinants of resistance and their clinical significance. *Cellular and Molecular Life Sciences* **15**, 15.

Corresponding Author Christin Sisowath, Malaria Research Unit, Division of Infectious Diseases, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden. Tel.: +46 8 51776739; Fax: +46 8 51776740; E-mail: christin.sisowath@ki.se

Rôle de pfmdr1 dans la tolérance de *Plasmodium falciparum* à l'artemether-lumefantrine en Afrique

OBJECTIF L'artemether-lumefantrine (AL), la thérapie de combinaison actuellement préférée pour la malaria non compliquée à *Plasmodium falciparum* en Afrique a été récemment rapportée comme sélectionnant pour l'allèle 86N de *pfmdr1*. L'objectif de cette étude est de rechercher la sélection d'autres mutations potentiellement impliquées dans la tolérance et/ou la résistance à AL, c'est-à-dire l'amplification du gène *pfmdr1*, *pfmdr1* Y184F, S1034C, N1042D, D1246Y, *pfcrt* S163R et *PfATP6* S769N.

MÉTHODES Les SNP ci-dessus mentionnés ont été analysés par PCR-RFLP et l'amplification du gène *pfmdr1* par des protocoles de PCR en temps réel chez des parasites provenant de 200 enfants traités avec AL pour la malaria non compliquée à *P. falciparum* à Zanzibar.

RÉSULTATS Une sélection statistiquement significative de *pfmdr1* 184F, en combinaison souvent avec 86N a été observée dans les réinfections après traitement. Aucune amplification du gène *pfmdr1* n'a été observée.

CONCLUSION Les résultats suggèrent que les différents allèles *pfmdr1* sont impliqués dans le développement de la tolérance/ résistance au lumefantrine.

mots clés malaria, *Plasmodium falciparum*, lumefantrine, résistance, pfmdr1

© 2007 Blackwell Publishing Ltd

El papel de pfmdr1 en la tolerancia de *Plasmodium falciparum* a artemeter-lumefantrina en África

objetivo Artemeter-lumefantrina (AL), actualmente la terapia de combinación más favorecida contra la malaria no complicada por *Plasmodium falciparum* en África, ha demostrado recientemente seleccionar el alelo 86N de *pfmdr1*. El objetivo de este estudio era buscar la selección de otras mutaciones potencialmente involucradas en la tolerancia y/o resistencia a AL, por ejemplo amplificación del gen *pfmdr1*, *pfmdr1* Y184F, S1034C, N1042D, D1246Y, *pfcrt* S163R y *PfATP6* S769N.

métodos Se analizaron las variantes genéticas que afectan un único nucleótido (*singular nuclear polymorphisms* - SNP) mediante PCR-RFLP y la amplificación del gen *pfmdr1* se realizó mediante protocolos basados en PCR a tiempo real en parásitos provenientes de 200 niños tratados con AL para malaria no complicada por *P. falciparum* en Zanzibar.

resultados Se observó una selección estadísticamente significativa de *pfmdr1* 184F principalmente en combinación con 86N en reinfecciones después del tratamiento. No se encontró ninguna amplificación del gen *pfmdr1*.

conclusión Los resultados sugieren que diferentes alelos de *pfmdr1* están involucrados en el desarrollo de tolerancia / resistencia a la lumefantrina.

palabras clave malaria, *Plasmodium falciparum*, lumefantrina, resistencia, *pfmdr1*

© 2007 Blackwell Publishing Ltd