Tropical Medicine and International Health doi:10.1111/j.1365-3156.2007.01995.x

VOLUME 13 NO 2 PP 238–240 FEBRUARY 2008

Short Communication

Chloroquine-resistance molecular markers (*Pfcrt* T76 and *Pfmdr-1* Y86) and amodiaquine resistance in Burkina Faso

Halidou Tinto[1,2], Lougué Guekoun[2], Issaka Zongo[1], Robert Tinga Guiguemdé[2], Umberto D'Alessandro[3] and Jean Bosco Ouédraogo[1,2]

1 *Institut de Recherche en Sciences de la Santé (IRSS), Bobo Dioulasso, Burkina Faso*
2 *Centre Muraz, Bobo Dioulasso, Burkina Faso*
3 *Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium*

Summary We investigated the relationship between the two main molecular markers for chloroquine resistance (*Pfcrt* T76 and *Pfmdr-1* Y86) and the clinical efficacy of amodiaquine in Burkina Faso. Before treatment, the prevalence of *Pfcrt* T76, *Pfmdr-1* Y86 or both mutations in the same infection was significantly higher in patients who experienced a recrudescence than in those who successfully responded to the treatment. Therefore, these two molecular markers could be useful in monitoring amodiaquine resistance, particularly in countries where this drug is used in combination with artesunate as first- or second-line treatment.

keywords malaria, *Plasmodium falciparum*, drug resistance, amodiaquine, *Pfcrt*, *Pfmdr-1*

Although chloroquine (CQ) and amodiaquine (AQ) are chemically similar; AQ might still be effective where CQ resistance is high (Gorissen *et al.* 2000; Staedke *et al.* 2001; Tinto *et al.* 2006). Nevertheless, several *in vitro* studies have shown cross-resistance between CQ and AQ or monodeshethyl-AQ, the active metabolite of AQ (Gonzalez *et al.* 2003; Tinto *et al.* 2006). This suggests that molecular markers linked to CQ resistance, such as *Pfcrt* T76 and *Pfmdr-1* Y86, might be useful for monitoring AQ resistance (Ochong *et al.* 2003). We investigated the relationship between the two main CQ resistance markers (*Pfcrt* T76 and *Pfmdr-1* Y86) and the clinical efficacy of AQ in children aged 6 months to 15 years.

The study was conducted in Burkina Faso in two sites with differing intensity of malaria transmission; the north with an entomological inoculation rate (EIR) estimated at around 15–20 infecting bites/man/year and the centre with an EIR estimated to be around 50 to 60 infecting bites/man/year (T. Baldet, personal communication). Children were treated and followed up according to the World Health Organization (WHO) 28-day *in vivo* test (WHO 2003). Outcomes were defined according to the WHO classification for monitoring antimalarial drug resistance (World Health Organization 2003). Blood samples for the molecular analysis were collected on filter paper (Whatman 3; Maidstone, England) at day 0 before treatment and at the time of recurrent parasitaemia. DNA was extracted using the Chelex-100 method (Plowe *et al.* 1995). Detection of *Pfcrt* T76 and *Pfmdr-1* Y86 mutations was done by using PCR followed by sequence-specific restriction enzyme digestion (Djimdé *et al.* 2001a). Nested PCR (Ranford-Cartwright *et al.* 1997) was adopted for the analysis of *Msp1* and *Msp2* to distinguish between recrudescence and new infection.

Outcome at day 28 was known for most patients enrolled (90%, 195/217). The PCR-corrected total treatment failure (TTF) was 6% (12/195) with no difference between the two sites ($P = 0.6$) nor age groups [<5 years *vs.* >5 years ($P = 0.11$)].

Before treatment, the prevalence of the *Pfcrt* T76, *Pfmdr-1* Y86 or both mutations in the same infection was significantly higher among the TTF than among the adequate clinical and parasitological response (ACPR) samples ($P = 0.03$; $P = 0.007$; and $P = 0.001$, respectively) (Table 1). The prevalence of molecular markers did not differ between sites or age groups with the exception of *Pfmdr-1* Y86, whose prevalence was significantly higher in children <5 years old (40%) than in the older group (21%) ($P = 0.03$].

Table 1 Treatment outcome and *Pfcrt* T76/*Pfmdr-1* Y86 mutations before treatment % (*n*/N)

	Pfcrt (*n* = 195)		*Pfmdr-1* (*n* = 195)		*Pfcrt*/*Pfmdr-1* (*n* = 195)			
	Wild (K76)	Mutant (T76)	Wild (N86)	Mutant (Y86)	Wild/wild (K76/N86)	Wild/mutant (K76/Y86)	Mutant/wild (T76/N86)	Mutant/Mutant (T76/Y86)
TTF (*n* = 12)	8.3 (1/12)	91.7 (11/12)	33.3 (4/12)	66.7 (8/12)	8.3 (1/12)	0.0 (0/12)	25 (3/12)	66.7 (8/12)
New infection (*n* = 14)	35.7 (5/14)	64.3 (9/14)	71.4 (10/14)	28.6 (4/14)	35.7 (5/14)	0.0 (0/14)	35.7 (5/14)	28.6 (4/14)
ACPR (*n* = 169)	40.8 (69/169)	59.2 (100/169)	72.8 (123/169)	27.2 (46/169)	33.1 (56/169)	7.7 (13/169)	38.5 (65/169)	20.7 (35/169)

TTF, total treatment failures; ACPR, adequate clinical and parasitological response; K, lysine; T, threonine; N, asparagine; Y, tyrosine.

The *Pfcrt* T76 mutation is the main determinant for CQ resistance (Djimdé *et al.* 2001a; Dorsey *et al.* 2001; Tinto *et al.* 2003). In our study, the *Pfcrt* T76 mutation was strongly associated with AQ resistance, a result consistent with earlier studies in Burkina Faso (Dokomajilar *et al.* 2006) and in other African countries (Ochong *et al.* 2003; Holmgren *et al.* 2006), confirming its primary role in determining aminoquinolones resistance. A study in Nigeria reported that none of the sample collected at the time of failure carried the wild-type *Pfcrt* K76 allele, indicating the critical role of the *Pfcrt* T76 mutation in determining AQ resistance (Happi *et al.* 2006).

The prevalence of *Pfmdr-1* mutation was also significantly high in TTF than in ACPR samples, even if the relation was not as strong as for *Pfcrt* T76, a result consistent with earlier studies in Kenya and Nigeria, where AQ resistance is substantially higher than in Burkina Faso (Happi *et al.* 2006; Holmgren *et al.* 2006). However, a study in Sudan was unable to find an association (Ochong *et al.* 2003). In our study, the prevalence of both mutations *Pfcrt* T76 and *Pfmdr-1* Y86 was higher in post-treatment than in pre-treatment samples, even if the difference was not statistically significant, possibly because of low AQ resistance. Indeed, in an earlier study carried out in South-Western Burkina Faso, where AQ resistance is slightly higher (9%), this difference was statistically significant (Dokomajilar *et al.* 2006).

From the results obtained in this study, the definition of an AQ Genotype Failure Index, similar to that established for CQ (Djimdé *et al.* 2001b; Tinto *et al.* 2005) would be extremely useful in countries where AQ is used in combination with artesunate as the first- or second-line treatment. However, we were unable to define that, possibly because of the limited number of failures we detected.

In conclusion, the two molecular markers of CQ resistance seem to be linked to AQ resistance as well and could be used for surveillance purposes. However, larger studies in areas of higher AQ resistance should be conducted for this purpose.

Acknowledgements

We thank the parents of the children included in this study for their participation. We would also like to thank the health staff of the health centres where the study was conducted for their collaboration. We thank the WHO and United Nations Development Programme (UNDP) offices in Burkina Faso, National Malaria Control Programme and International Atomic Energy Agency (IAEA), for their financial and technical support.

References

Djimdé A, Doumbo OK, Cortese JF *et al.* (2001a) A molecular marker for chloroquine-resistant Falciparum malaria. *New England Journal of Medicine* **344**, 257–263.

Djimdé A, Doumbo OK, Steketee RW & Plowe CV (2001b) Application of a molecular marker for surveillance of chloroquine-resistant falciparum malaria. *Lancet* **15**, 890–891.

Dokomajilar C, Lankoande ZM, Dorsey G, Zongo I, Ouedraogo JB & Rosenthal PJ (2006) Roles of specific *Plasmodium falciparum* mutations in resistance to amodiaquine and sulfadoxine-pyrimethamine in Burkina Faso. *American Journal of Tropical Medicine and Hygiene* **75**, 162–165.

Dorsey G, Kamya MR, Singh A & Rosenthal PJ (2001) Polymorphisms in the *Plasmodium falciparum* pfcrt and pfmdr-1 genes and clinical response to chloroquine in Kampala, Uganda. *Journal of Infectious Diseases* **183**, 1417–1420.

Gonzalez IJ, Varela RE, Murillo C *et al.* (2003) Polymorphisms in cg2 and pfcrt genes and resistance to chloroquine and other antimalarials in vitro in *Plasmodium falciparum* isolates from Colombia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **97**, 318–324.

Gorissen E, Ashruf G, Lamboo M *et al.* (2000) In vivo efficacy study of amodiaquine and sulfadoxine/pyrimethamine in Kibwezi, Kenya and Kigoma, Tanzania. *Tropical Medicine and International Health* **5**, 459–463.

Happi CT, Gbotosho GO, Folarin OA *et al.* (2006) Association between mutations in *Plasmodium falciparum* chloroquine resistance transporter and *P. falciparum* multidrug resistance 1 genes and in vivo amodiaquine resistance in *P. falciparum* malaria-infected children in Nigeria. *American Journal of Tropical Medicine and Hygiene* **75**, 155–161.

Holmgren G, Gil JP, Ferreira PM, Veiga MI, Obonyo CO & Bjorkman A (2006) Amodiaquine resistant *Plasmodium falciparum* malaria in vivo is associated with selection of pfcrt 76T and pfmdr1 86Y. *Infection, Genetics and Evolution* **6**, 309–314.

Ochong EO, van den Broek IV, Keus K & Nzila A (2003) Short report: association between chloroquine and amodiaquine resistance and allelic variation in the *Plasmodium falciparum* multiple drug resistance 1 gene and the chloroquine resistance transporter gene in isolates from the upper Nile in southern Sudan. *American Journal of Tropical Medicine and Hygiene* **69**, 184–187.

Plowe CV, Djimdé A, Bouare M, Doumbo O & Wellems TE (1995) Pyrimethamine and proguanil resistance-confering mutations in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. *American Journal of Tropical Medicine and Hygiene* **52**, 565–568.

Ranford-Cartwright LC, Taylor J, Umasunthar T *et al.* (1997) Molecular analysis of recrudescent parasite in a *Plasmodium falciparum* drug efficacy trial in Gabon. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 719–724.

Staedke SG, Kamya MR, Dorsey G *et al.* (2001) Amodiaquine, sulfadoxine/pyrimethamine, and combination therapy for treatment of uncomplicated falciparum malaria in Kampala, Uganda: a randomised trial. *Lancet* **358**, 368–374.

Tinto H, Ouedraogo JB, Erhart A *et al.* (2003) Relationship between the Pfcrt T76 and the Pfmdr-1 Y86 mutations in *Plasmodium falciparum* and in vitro/in vivo chloroquine resistance in Burkina Faso, West Africa. *Infection, Genetics and Evolution* **3**, 287–292.

Tinto H, Sanou B, Dujardin JC *et al.* (2005) Usefulness of the *Plasmodium falciparum* chloroquine resistance transporter T76 genotype failure index for the estimation of in vivo chloroquine resistance in Burkina Faso. *American Journal of Tropical Medicine and Hygiene* **73**, 171–173.

Tinto H, Rwagacondo C, Karema C *et al.* (2006) In-vitro susceptibility of *Plasmodium falciparum* to monodesethyl-amodiaquine, dihydroartemisinin and quinine in an area of high chloroquine resistance in Rwanda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **100**, 509–514.

World Health Organization (2003) Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria WHO/HTM/RBM/2003.50.

Corresponding Author **Halidou Tinto**, Institut de Recherche en Sciences de la Santé/Centre Muraz 01 BP 390, Tel.: 00226 20 98 48 43; Fax: 00226 20 98 48 43; E-mail: tintohalidou@yahoo.fr