Large-Scale Surveillance of *Plasmodium falciparum crt(K76T)* in Northern Ghana[▽]

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Received 7 February 2007/Returned for modification 30 May 2007/Accepted 4 June 2007

Surveillance of *Plasmodium falciparum crt(K76T)* [Pfcrt(K76T)], a resistance marker of chloroquine and, limitedly, amodiaquine, in >4,000 children in northern Ghana revealed a prevalence of 79%. Pfcrt(K76T) was heterogeneously distributed and associated with chloroquine use, low parasitemia, and the dry season. Widespread chloroquine resistance challenges the regional life span of amodiaquine as a partner drug in artemisinin combination therapy.

Chloroquine (CQ) treatment has been the mainstay of malaria control in sub-Saharan Africa but now fails because of intensifying drug resistance (5, 11). Artemisinin-based combination therapy (ACT), e.g., amodiaquine-artesunate (AQ-AS), is being implemented as policy in many African countries. However, in practice, CQ will possibly continue to be widely used, particularly in home treatment (1, 7), since ACT is often not available or not affordable. CQ resistance is associated with a mutation in the *Plasmodium falciparum* CQ resistance transporter gene [Pfcrt(K76T)] (2). In northern Ghana, Pfcrt(K76T) confers a threefold-increased risk of CQ treatment failure occurring in 58% of patients within 2 weeks of follow-up (11).

Moreover, increasing evidence suggests that Pfcrt(K76T) is associated with AQ resistance, possibly to a lesser degree than with CQ resistance (3, 8, 12).

In 2002, i.e., 3 years before Ghana's official change from CQ to AQ-AS as a first-line antimalarial drug, we conducted two representative surveys comprising >4,000 children in 30 communities in the northern region of the country, where malaria is hyperendemic. We examined geographical patterns of Pfcrt(K76T) and blood CQ concentrations and analyzed associations with clinical and sociodemographic data.

The study design and sampling strategy are described elsewhere (6). Briefly, in 30 settlements in Tamale (urban) and

TABLE 1. Characteristics of the study population split by seasons

Patient parameter	Value for:			Difference between seasons (P value) ^a
	All patients (n = 4,227)	Patients in the dry season (n = 2,109)	Patients in the rainy season (n = 2,118)	
Median age in mo (range)	48 (6–108)	48 (6–108)	48 (6–108)	NS
No. (%) of female sex	2,131 (50.4)	1,077 (51.1)	1,054 (49.8)	NS
No. (%) with axillary temp ≥ 37.5°C	257 (6.1)	122 (5.8)	135 (6.4)	NS
No. (%) with microscopically detected parasitemia	2,478 (58.6)	1,171 (55.5)	1,307 (61.7)	<0.0001
No. (%) with PCR-detected parasitemia (no. tested, 4,226)	3,299 (78.1)	1,570 (74.5)	1,729 (81.6)	<0.0001
No. (%) with clinical malaria ^b (no. tested, 4,222)	162 (3.8)	58 (2.8)	104 (4.9)	0.0003
Mean Hb (g/dl) (SD)	10.2 (2.0)	10.5 (2.2)	10.0 (1.7)	<0.0001
No. (%) with anemia (Hb < 11 g/dl) (no. tested, 4,224)	2,707 (64.1)	1,249 (59.3)	1,458 (68.9)	<0.0001
No. (%) with residual CQ blood levels (no. tested, 4,216)	1,187 (28.2)	468 (22.2)	719 (34.1)	<0.0001
No. (%) with wild-type Pfcrt (no. tested, 3,182)	665 (20.9)	282 (18.8)	383 (22.7)	0.007
No. (%) with mixed mutant Pfcrt(K76T) and wild-type alleles (no. tested, 3,182)	1,045 (32.8)	476 (31.8)	569 (33.8)	NS
No. (%) with mutant Pfcrt(K76T) allele alone (no. tested, 3,182)	1,472 (46.3)	739 (49.4)	733 (43.5)	0.0009

^a NS, not significant.

^b Any patients with microscopically detected parasitemia plus fever.

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[▽] Published ahead of print on 11 June 2007.

FIG. 1. Distribution of residual CQ blood concentrations and of *Pfcr*(K76T) in the northern region of Ghana (dry season). Each symbol represents one settlement with 70 children aged 0.5 to 9 years.

surrounding districts (rural), 70 children aged 0.5 to 9 years were randomly recruited. Sampling was done twice, from January to April (dry season) and from July to October (rainy season). The study protocol was approved by the ethics committees of the National and Regional Ministries of Health, and parental written informed consent was obtained. Age, sex, and axillary temperature were documented, and a blood sample was collected into EDTA. Hemoglobin concentration (Hb) was measured using a HemoCue photometer (Ångelholm, Sweden), and anemia was defined as an Hb of <11 g/dl (6). Microscopy of Giemsa-stained thick blood films was performed and malaria defined as any parasitemia plus fever (axillary temperature of $\geq 37.5^{\circ}\text{C}$). Uncomplicated malaria and parasitemia of $>5,000$ parasites/ μl were treated with sulfadoxine-pyrimethamine; severe-malaria patients were transferred to a hospital. *Plasmodium* infection was confirmed by PCR and *Pfcr*(K76T) by PCR-restriction fragment length polymorphism analysis (2, 13). PCR results were available for 2,108 and 2,118 children in the dry and rainy seasons, respectively. CQ blood

concentrations were measured by enzyme-linked immunosorbent assay, with a detection limit of 31 nmol/liter (15). Continuous variables were compared by the Mann-Whitney *U* test and proportions by χ^2 tests. Factors independently associated with the presence of *Pfcr*(K76T) were identified by logistic regression analysis.

Characteristics of the study population are shown in Table 1. Regardless of the season, some three-quarters of the children were infected with *P. falciparum*. Blood CQ levels were observed in 22% of children during the dry season (geometric mean concentration, 104 nmol/liter; range, <31 to 2,500 nmol/liter) and in 34% during the rainy season (geometric mean concentration, 216 nmol/liter; range, <31 to 6,293 nmol/liter; $P < 0.0001$). In both the dry (Fig. 1A) and rainy (data not shown) seasons, the prevalences of CQ in blood differed between the 30 communities surveyed (P was <0.0001 for each). The presence of CQ was increased in children of urban residence (odds ratio [OR], 1.43; 95% confidence interval [CI], 1.24 to 1.65; $P < 0.0001$), with anemia (OR, 1.28; 95% CI, 1.11

to 1.48; $P = 0.0007$), and of <5 years of age (OR, 1.73; 95% CI, 1.50 to 1.99; $P < 0.0001$).

Nearly 80% of infected children harbored parasites with the resistant *Pfcr(K76T)* genotype, irrespective of the season (Table 1). The distributions of resistant genotypes differed significantly between communities (Fig. 1B) ($P < 0.0001$). Factors associated with the prevalence of *Pfcr(K76T)* were CQ in blood (OR, 2.35; 95% CI, 1.84 to 3.01; $P < 0.0001$), low parasitemia ($\leq 10,000$ parasites/ μ l) (OR, 1.79; 95% CI, 1.34 to 2.38; $P < 0.0001$), asymptomatic infection (OR, 1.45; 95% CI, 1.02 to 2.04; $P = 0.03$), and the dry season (OR, 1.27; 95% CI, 1.06 to 1.51; $P = 0.007$). No geographical pattern (e.g., distances to the next river, road, or larger city and differences between the north and south) predicted *Pfcr(K76T)*. However, a trend for a higher prevalence of *Pfcr(K76T)* with increasing district population size ($\chi^2_{\text{trend}} = 10.1$; $P = 0.001$) was observed. The same trend was seen for the prevalence of CQ in blood ($\chi^2_{\text{trend}} = 57.8$; $P < 0.0001$). Multivariate analysis adjusted for the respective communities confirmed residual CQ to be the strongest factor associated with the presence of *Pfcr(K76T)* (adjusted OR [OR_{adj}], 2.46; 95% CI, 1.93 to 3.15; $P < 0.0001$), followed by low parasitemia (OR_{adj}, 1.69; 95% CI, 1.26 to 2.27; $P = 0.0004$) and the dry season (OR_{adj}, 1.30; 95% CI, 1.10 to 1.60; $P = 0.006$).

This largest antimalarial surveillance study in this region demonstrates that CQ concentrations and *Pfcr(K76T)* are common in a representative sample of children in one area in sub-Saharan Africa. Our results accord with the selection of resistance by residual CQ but also highlight the pronounced geographical heterogeneity of both parameters. Current malaria control in sub-Saharan Africa relies predominately on early treatment, and CQ resistance is still a key problem in this context. In much of sub-Saharan Africa, first-line antimalarial drug policies have been changed to sulfadoxine-pyrimethamine or ACT. In northern Ghana, CQ treatment fails in $>50\%$ of patients (5), and in 2005, AQ-AS was officially implemented in this country. Unfortunately, only sparse data on its efficacy are available (10). Recent data suggest that *Pfcr(K76T)* is associated with AQ resistance (3, 8, 12). For Ghana, no association between AQ efficacy and *Pfcr(K76T)* has been established so far. In neighboring Burkina Faso, the presence of *Pfcr(K76T)* (prevalence, 62%) increased the risk of recrudescence after AQ treatment approximately sixfold (3). In the present study, *Pfcr(K76T)* parasites were found predominantly among asymptomatic children with low parasitemia and in the dry season. Bearing the limitations of a cross-sectional study in mind, these children are rather unlikely to be treated for malaria and, thus, constitute a reservoir for resistant parasites to “hibernate” and be further transmitted. The overrepresentation of *Pfcr(K76T)* parasites in areas of low-density infections might suggest reduced fitness of resistant parasites; however, in the context of ongoing selection, recombinations, and potential compensatory mechanisms, this hypothesis cannot be confirmed in a cross-sectional study (14). Efficacy and, equally important, the useful therapeutic life span of any ACT critically depend on the choice of the partner drug and preexisting resistance patterns (4, 9). The high prevalence of *Pfcr(K76T)*

parasites in northern Ghana and the possibility of even further distribution could produce a frightening scenario: given that the abundance of the *Pfcr(K76T)* mutation is confirmed to translate into impaired AQ efficacy (study in progress), the useful life span of AQ-AS may be shortened considerably. Caution and continued monitoring of the efficacy of AQ-AS is warranted, especially in highly populated areas, to prevent this ACT from failing and artemisinin resistance from emerging in the near future.

(This research forms part of the doctoral thesis of S. Kaiser.)

We thank the families who participated in this study and the members of the Northern Region Malaria Project (NORMAP) for recruitment. Melanie Mues kindly performed the artwork.

This study was supported by Charité (grant 2002-677).

The authors do not have a commercial or other association that might pose a conflict of interest.

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