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Synergism between Pfcrt and Pfmdr1 genes could account for the slow recovery of chloroquine sensitive *Plasmodium falciparum* strains in Ghana after chloroquine withdrawal

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Summary Unlike other countries, the chloroquine resistant marker Pfcrt T76 mutant has remained fairly stable in Ghana several years after official disuse of chloroquine. Certain mutations in Pfmdr1 may potentiate Pfcrt T76, offering a possible explanation for this observation. To understand the phenomenon, the co-existence of mutations in Pfmdr1 with Pfcrt T76 in Ghanaian *Plasmodium falciparum*

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isolates was studied. The reported presence of parasites with reduced sensitivity to amodiaquine and quinine in the country was also studied. Blood samples collected from confirmed malaria patients presenting at health facilities in two distinct ecological zones were analyzed. The prevalence of Pfcrt K76T and the five point mutations in Pfmdr1 were determined using nested PCR followed by RFLP analysis. The association between genes was determined by chi square analysis, and synergism between the two genes was ascertained using the Jonckheere—Terptra (J—T) test followed by Monte Carlo simulation (MCS). Nearly fifty-four percent (53.7%) of the *P. falciparum* isolates examined had the Pfcrt T76 gene, out of which 18.3% had both K76 and T76 alleles. Mutations at codon 86, 184, 1034, 1042 and 1246 of the Pfmdr1 gene were detected in 36.0%, 87.9%, 71.0%, 91.6% and 8.4% of the isolates, respectively. The haplotypes of Pfmdr1 present were NFCDD (43.46%), YFCDD (27.57%), NFSDD (7.48%), NYSNY (5.14%) and YFSDD (4.67%). Pfcrt T76 was significantly associated with a double mutation at codon 86 and 184 of Pfmdr1 (YF; $\chi^2 = 18.045$, $p = 0.006$). Associations were observed between Pfcrt K76T and Pfmdr1 triple mutation at codons 86, 184 and 1034 (NFC; $\chi^2 = 13.770$, $p = 0.032$ and YFC; $\chi^2 = 16.489$, $p = 0.011$). The J—T test showed significant synergism between Pfcrt 76 and Pfmdr1 polymorphisms ($p < 0.0001$), which was confirmed by MCS at 99% CI. Synergism between Pfcrt and Pfmdr1 mutant genes could account for the slow recovery of chloroquine sensitive *P. falciparum* in Ghana. The same phenomenon could explain resistance to amodiaquine and quinine. The outcomes of this study also indicated a possible emergence of artemether-lumefantrine resistance in Ghana.

Introduction

Several years after the replacement of chloroquine as a first line antimalarial drug in Malawi, Ethiopia and Tanzania, among others, chloroquine (CQ) sensitive *Plasmodium falciparum* has recovered [1—4]. The re-emergence of CQ-sensitive *P. falciparum* resulted from an increased dominance of wild-type parasites that have lysine at codon 76 of the Pfcrt gene [5—8]. Interestingly, the period of this recovery coincided with a reduction in point mutations of the Pfmdr1 genes, such as Asn86Tyr, Asn1042Asp and Asp1246Tyr [9]. This observation strongly suggests a possible link of Pfcrt with Pfmdr1 mutations in a modulation of the sensitivity of *P. falciparum* to CQ.

In our previous report, we showed the main CQ resistant marker K76T of the Pfcrt gene has remained fairly stable with a gradual decrease in certain parts of Ghana compared to Malawi [10,11]. Persistence of chloroquine resistant parasites in Ghana after the changes in anti-malarial drug policies has also been observed in other countries [12—14]. Though the prevalence of mutations in the Pfcrt gene on chromosome 7 and the Pfmdr1 gene on chromosome 5 has been determined individually in Ghana, the interplay between them has yet to be assessed and discussed in detail. Study is needed due to reports that indicate both Pfmdr1 gene duplication and mutations at codons 86, 184, 1034, 1042 and 1246 are associated with resistance to chloroquine, mefloquine, quinine and artemisinin derivatives [12,15,16]. Of particular interest is how different haplotypes of Pfmdr1 contribute to specific antimalarial drug resistance. Specifically, the combined effect of Y184F, N1042D and D1246Y (FDY) haplotype isolates from Africa, Asia and South America have been reported to be associated with CQ resistance phenotypes [17—19]. Additionally, study is needed for the S1034C, N1042D and D1246Y (CDY) isolates from South America that are associated with quinine resistance [20], as well as for the N86, F184 and D1246 (NFD) isolates from Tanzania and Mozambique that are associated with artemether lumefantrine recrudescence [21,22].

This study investigated the impact of the combined mutations in the Pfmdr1 and Pfcrt mutant genes on the delayed restoration of sensitivity of malarial parasites to CQ in Ghana. Additionally, the study sought an explanation for the reports of increasing levels of *P. falciparum* resistance to quinine and amodiaquine.

Methods

Study sites

This report describes a cross-sectional study conducted in Ghana health facilities located in areas