Correspondence

CYP2C8 Status of Patients With Malaria Influences Selection of *Plasmodium falciparum pfmdr1* Alleles After Amodiaquine-Artesunate Treatment

TO THE EDITOR-We have read with great interest the article by Paganotti et al [1], in which evidence is presented about the possible influence of human pharmacogenetics on the development of chloroquine (CQ) resistance by Plasmodium falciparum. This prompted us to hypothesize that such influence would be more visible and significant when considering the structurally related amodiaquine (AQ). In fact, although CYP2C8 is a secondary player in the metabolism of CQ [2], it is essential for the cytochrome P450 (CYP) associated biotransformation of AQ to its therapeutically main active metabolite, desethylamodiaquine (DEAQ) [3].

The in vivo parasite response to AQ therapy is modulated by polymorphisms in the P. falciparum multidrug resistance 1 gene (pfmdr1), particularly those concerning the N86Y [4], Y184F, and D1246Y [5, 6] single-nucleotide polymorphisms (SNPs). Accordingly, we hypothesized that the CYP2C8 status of the target population would influence the intensity of posttreatment selection of these polymorphisms. Such events should be reflected in significant differences in the frequencies of these SNPs between parasites recurrently infecting patients carrying wild-type CYP2C8*1/*1 and those infecting subjects harboring minor CYP2C8 alleles coding for activitycompromised enzymes. In this context, the Zanzibari population offers the advantage of being an African native population with very high prevalence of the CYP2C8*3 allele, which codes for a verylow-activity enzyme [7].



Figure 1. Frequency of the carriers of $2C8^*1/*1$ and of one *3 allele among the different parasite *pfmdr1* SNPs/haplotypes. [#] YYY carrying infections, ie, including not only the pure, but also the MYY, YMY, YYM haplotype was considered. *p<0.05; **p<0.01; ***near significant trend, p = .056.

We retrospectively analyzed the CYP2C8 status of patients experiencing recurrent parasitemia in 2 similar trials on the efficacy of ASAQ for uncomplicated P. falciparum malaria [5, 8]. Both trials had a 42-day follow-up period and were performed at a time when malaria transmission was still high in Zanzibar. The CYP2C8*2 and 2C8*3 alleles were characterized using previously published PCR-RFLP methods [7]. The statistical analysis was done using the 2-tailed Fisher exact test (GraphPad QuickCalcs; available at: http://www.graphpad.com).

In total, 116 patients (age, <5 years) were analyzed for the main *CYP2C8* polymorphisms. These pharmacogenetics results were merged with previously published parasite genotype data on the *pfmdr1* N86Y, Y184F, and D1246Y SNPs [5, 8].

Here, we adopted an extreme discordant group analysis [9] through the comparison of a group of carriers with the 2*C8**3 allele, known to code for the significantly impaired enzyme encoded by *CYP2C8**3, with a group carrying the *1/*1 haplotype, which encode the fully functional enzyme. Carriers of *CYP2C8**3 had an increased frequency of infections harboring the *pfmdr1* 86Y in pure form (100% vs 74.6%; P < .05) and 1246Y (46.7% vs 13.6%; P < .01) alleles, compared with the reference homozygous wild-type group (Figure 1). The low level of polymorphisms at position 184 precluded a meaningful statistical analysis of this SNP in isolation.

The 86Y/184Y/1246Y haplotype is selected in vivo after AQ and ASAQ exposure [4–6]. For 105 of the analyzed infections, the complete parasite haplotype analysis was available. Consistent with the pattern observed with the individual SNPs, carriage of YYY-carrying parasites (ie, YYY, MYY, YMY, and YYM) tended to be more frequent among the 2C8*3 carriers, compared with the wild type

group (53.3% vs 24.6%; P = .056). After exclusion of parasites with the SNP at position 184, we observed that the pure haplotype 86Y/1246Y was more frequent among parasites in the 2*C*8*3 carriers (46.7% vs 10.7%; P < .01), suggesting the importance of these 2 SNPs and a minor role for the SNP at position 184 in this host pharmacogenetics/pathogen interaction.

In contrast with the observed with the 2C8*3 carrier subset, carriers of the relatively frequent homozygous 2C8*2/*2 haplotype, as well as the overall subset of 2C8*2 carriers, were not associated with any of the *pfmdr1* SNPs analyzed or the YYY and YY haplotypes.

The present data only allow a preliminarily discussion about the potential reasons for the observed frequency trends. The metabolism of AQ is complex, with several pathways expected to operate in parallel with the main AQ-to-DEAQ route [10]. It is conceivable that carriers of the CYP2C8*3 allele allow a longer AQ halflife. As a consequence, it is possible that a nonnegligible fraction of the AQ pool is deviated toward secondary metabolic pathways, leading to a decreased DEAQ exposure. This will particularly benefit parasites already prone to be tolerant to DEAQ, such as 86Y/1246Y haplotype carriers, which will be more readily selected.

In conclusion, consistent with the study by Paganotti et al [1], our work supports a potential influence of the host pharmacogenetic makeup on the long-term selection of parasite characteristics associated with antimalarial drug response. The precise nature of such pharmacogenetic-driven host-pathogen interactions is probably complex, but it is worth further explorations with specifically designed studies.

Notes

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