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Distribution of human *CYP2C8*2* allele in three different African populations

Giacomo M Paganotti*, Silvia Gramolelli, Francesca Tabacchi, Gianluca Russo, David Modiano, Mario Coluzzi and Rita Romano

Abstract

Background: The aim of this study was to investigate cytochrome P450 *2C8*2* (*CYP2C8*2*) distribution and allele frequency in three populations from West and East Africa exposed to *Plasmodium falciparum* malaria. *CYP2C8* enzyme is involved in the metabolism of the anti-malarials amodiaquine and chloroquine. The presence of the *CYP2C8*2* defective allele has been recently associated to higher rate of chloroquine-resistant malaria parasites.

Methods: A total of 503 young subjects were genotyped for the single nucleotide polymorphism rs11572103 (A/T). Eighty-eight were from southern Senegal, 262 from eastern Uganda and 153 from southern Madagascar. The PCR-RFLP technique was used to discriminate the wild-type (A) from the defective allele (T).

Results: A *CYP2C8*2* (T) allele frequency of 0.222 ± 0.044 was detected in Senegal, 0.105 ± 0.019 in Uganda and 0.150 ± 0.029 in Madagascar.

Conclusions: This study demonstrated that *CYP2C8*2* allele is widespread in Africa. This allele occurs at different frequency in West and East Africa, being higher in Senegal than in Uganda and Madagascar. These data indicate that an important fraction of the populations analysed has a decreased enzymatic activity, thus being at higher risk for drug accumulation with two possible consequences: i) an exacerbation of drug-associated adverse side effects; ii) an increase of drug-resistance selection pressure on *P. falciparum* parasites.

Keywords: *CYP2C8* enzyme, *CYP2C8*2* allele, *Plasmodium falciparum* malaria, Africa, Poor metabolizers, Chloroquine, Amodiaquine, Drug-resistance

Background

Plasmodium falciparum malaria is one of the most important infectious diseases in the developing world, representing a priority in public health mainly in sub-Saharan Africa. Nowadays, anti-malarial strategies include: the development of a vaccine, the vector control, as well as drug treatment, which remains the most effective remedy to clear the infection. However, the spread of anti-malarial drug-resistance affects the outcome of treatments [1], since *P. falciparum* has selected resistant strains for the majority of the molecules used in anti-malarial therapy [2]. As recently demonstrated, host genetic variation in drug metabolizing enzymes influences the selection of *P. falciparum* drug-resistance in Burkina Faso [3]. In particular, the cytochrome P450

2C8 (*CYP2C8*), a polymorphic enzyme that mainly contributes to the hepatic metabolism of amodiaquine (AQ) and chloroquine (CQ), shows a genetic variant (*CYP2C8*2*) that is associated with higher rate of drug-resistant parasites in the infected host (*pfprt-76Y* and *pfmdr1-86Y* *P. falciparum* alleles) [3].

CYP2C8 is a member of the human *CYP2C* enzyme family, which also includes *CYP2C9*, *CYP2C18* and *CYP2C19*, whose genes are located on the chromosome 10q24 [4]. Human *CYP2C8* is involved in the metabolism of a variety of clinically important drugs, including the anti-malarials AQ, CQ and, to a lesser extent, dapsone (DDS) [4]. *CYP2C8* is the only enzyme involved in the biotransformation of AQ [4], whereas for CQ it plays a major role [5,6], the secondary routes for CQ metabolism being limited by genetic and inhibitory factors in Africans [3,5-11]. *CYP2C8* gene is known to be polymorphic, and the distribution of variant alleles

* Correspondence: giacomo.paganotti@uniroma1.it
Department of Public Health and Infectious Diseases, Sapienza University, P. le Aldo Moro 5, 00185 Rome, Italy

differs among ethnic populations [4]. *CYP2C8*2*, the variant most common in Africans, is related to a poor metabolizer phenotype (PM) in subjects carrying at least one copy of the defective allele [4,9]. Subjects who are poor metabolizers experience a longer drug half-life [12] and have increased adverse side effects. In particular, *CYP2C8*2* shows six fold lower intrinsic clearance of AQ than wild type [12]. On the other hand, no evidence is yet available from the literature about the role of *CYP2C8* genetic variance in CQ pharmacokinetics [13], although there is indirect evidence of lower CQ metabolism in *CYP2C8*2*-carriers shown through the association between the allele and rates of CQ-resistant *P. falciparum* parasites [3]. In humans, CQ concentrations decline multi-exponentially and elimination at its initial anti-malarial concentrations is relatively rapid. This means that it usually persists for only a few days at concentrations sufficient to select resistant over sensitive parasites (its window of selection). The poor metabolizers obviously greatly extend these windows. A slower metabolism of an anti-malarial drug leads to a longer time of parasite exposure to a sub-therapeutic level of the molecule, therefore acting as a further co-factor in drug-resistance selection. Few studies had described the *CYP2C8*2* allele frequency in Africa, and the actual knowledge of its distribution is incomplete all over the continent. The prevalence of this allele is reported to be 13.9% in Zanzibar [5], whereas in Ghana it ranges between 16.8% and 17.9% [14-16]. Two studies had been conducted in Burkina Faso, the former showed a *CYP2C8*2* prevalence of 11.5% in the south of the country [12]. In the second study, sympatric ethnic groups living in the central area of Burkina Faso were analysed: the Fulani showed a prevalence of 9.9% and the Mossi-Rimaibè group 23.7% [3]. Moreover, Dai et al. [17] reported a value of 18% in African-Americans. *CYP2C8*2* is virtually absent in non-African populations [17], as in Caucasians, where instead the poor metabolizer allele is represented by *CYP2C8*3*, which is absent or found at very low frequency in Africa [17].

Actually, the official policies for the treatment of uncomplicated forms of *P. falciparum* malaria are based on artemisinin combination therapy (ACT), including the association of artesunate with AQ. However, CQ is still used as anti-malarials in several African countries [18-22]. Moreover, the CQ tablets are often of poor quality [23,24] and the compliance to the therapy is low and then the selective effects on parasite are improved.

The aim of the study was to describe the distribution of *CYP2C8*2* in Senegal, Uganda and Madagascar, areas that were not investigated by previous studies and characterized by different levels of *P. falciparum* malaria transmission intensity.

Methods

Study area and subjects

The samples analysed in the present study were collected during cross-sectional surveys performed during 2007 in Senegal (Thionck Essyl health centre - Casamance region); Uganda (Namalu and Rupa health centres, Kakoliye and Nadunget primary schools - Karamoja region; Makindiye children centre - Kampala region); and Madagascar (Amboasary, Antsanomaro, Masihanaka and Fort Dauphin health centres - Anosy region) (Figure 1). A total of 503 children and adolescents had been enrolled: 88 were from Senegal (mean age in years \pm SD = 10.4 \pm 3.5); 262 from Uganda (mean age 7.8 \pm 3.0) and 153 from Madagascar (mean age 6.0 \pm 4.1). For the purpose of the study were considered only unrelated individuals according to different family names and interviews. The same protocol for enrollment was followed in all sites [25,26]. Signed informed consent for multiple genetic and epidemiologic surveys was obtained from the subjects or their parents/caregivers [25,26]. This study was conducted with the approval of the ethics committee and research committee of the Sapienza University of Rome. Fingert-pick blood samples were spotted on Whatman grade 1 filter papers at the time of the field survey and then air dried before being separately stored in sealed plastic containers.

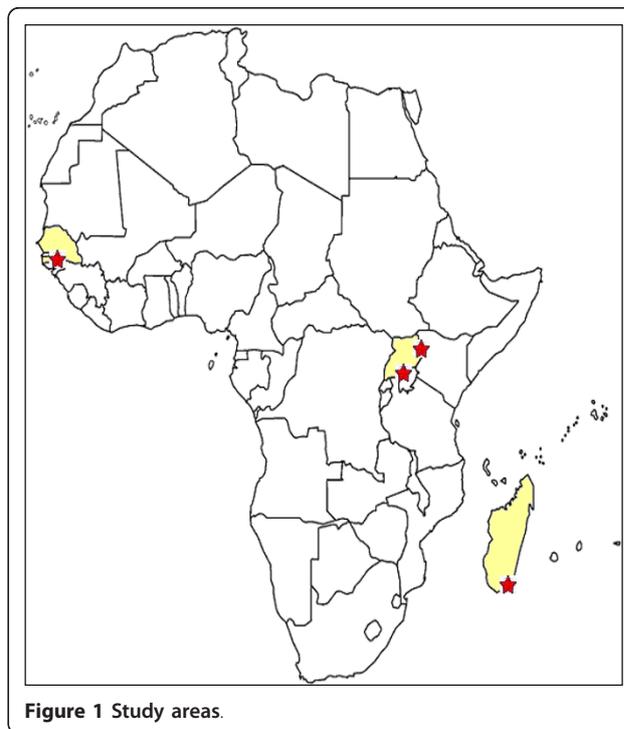


Figure 1 Study areas.

Genotyping

Human DNA was extracted with Chelex-100 resin (Bio-Rad). *CYP2C8*2* (rs11572103, A > T) detection was carried out using the PCR-RFLP technique. Two µl of DNA template were used to amplify by PCR a 107 bp fragment of the *CYP2C8* gene (forward primer: 5'-GAA-CACCAAGCATCACTGGA-3'; reverse primer: 5'-GAAATCAAAATACTGATCTGTTGC-3'); the PCR product was then incubated with *BclI* enzyme that cuts the wild-type allele only (A); undigested products represent the variant allele (T). To detect the size polymorphisms, the samples were ran on a Metaphor 3% gel. Controls for human genotyping were utilized after sequencing of the PCR product obtained from each different genotype. Moreover, to avoid genotyping error, the analysis was repeated for all the heterozygous samples.

Statistical analysis

Inter-populations comparisons were obtained by Yates-corrected χ^2 test and by Mantel-Haenszel χ^2 test (M-H). Odds ratios (ORs) were calculated with 95% confidence intervals (CIs). The analyses were performed with epi info 6 statistical package. GenePop software was used for the evaluation of Hardy-Weinberg equilibrium [27].

Results

The frequencies of the *CYP2C8*2* allele for each country included in the present study are reported in Table 1. Genotype frequencies were in Hardy-Weinberg equilibrium both for Uganda and Madagascar ($\chi^2 = 1.52$, $P = 0.221$ and $\chi^2 = 2.61$, $P = 0.106$, respectively), while an excess of AT heterozygotes was found for Senegal ($\chi^2 = 7.11$, $P = 0.008$), probably because of the small sample size ($N = 88$). Comparing the allele frequency among the populations studied, a statistically significant difference was found between Senegal and Uganda (OR = 2.43, 95% CI 1.51-3.91; Yates-corrected $\chi^2 = 14.43$, $P = 0.0001$; M-H $\chi^2 = 15.49$, $P < 0.0001$) whereas a lower difference was revealed in the other comparisons (Senegal vs Madagascar: OR = 1.85, 95% CI 1.11-3.10; Yates-corrected $\chi^2 = 5.62$, $P = 0.0177$; M-H $\chi^2 = 6.23$, $P = 0.0126$; Madagascar vs Uganda: OR = 0.76, 95% CI 0.48-1.20; Yates-corrected $\chi^2 = 1.24$, $P = 0.2654$; M-H $\chi^2 =$

1.50, $P = 0.2201$). Furthermore, the percentage of subjects carrying at least one copy of the *CYP2C8*2* allele (T) was 44.3%, 20.6% and 26.1% for Senegal, Uganda and Madagascar, respectively.

Discussion

The discovery of functional variability in genes encoding drug metabolizing enzymes has contributed significantly to the understanding of the inter-individual variability in dose-concentration relationships and drug response. Knowledge of polymorphisms in genes encoding enzymes that metabolize anti-malarial drugs, as well as their associations with clinical and parasitological outcomes, can be useful in designing dosage regimens and modulating drug therapy that is safe, effective and therefore less likely to select for pathogen's drug resistance.

This work describes the distribution of the African defective allele *CYP2C8*2*, whose corresponding enzyme is an important player in the metabolism of two widespread anti-malarial compounds, CQ and AQ. The allele frequencies of *CYP2C8*2* observed in the three different populations are partially in agreement with other data available in the literature. In particular, the allele frequency found in Uganda (10.5%) is in line with that reported from Zanzibar (13.9%), to date the only other study conducted in East Africa [9]. In Uganda, the Karamoja region and the suburban districts of the capital Kampala are areas of hyper-endemic malaria transmission [28], where anti-malarial treatment is based on ACT, mainly a combination of artemether and lumefantrine (AL) and, as an alternative, artesunate (AS) plus AQ [28]. Consequently, the presence of the *CYP2C8*2* allele may be a potential co-factor in the onset of adverse side effects associated with AQ administration. Furthermore, as there is evidence that both *pfprt* and *pfmdr1* parasite resistant alleles play a role in AQ-resistance [29], it could emerge the risk related to the presence of *CYP2C8*2* in selecting AQ-resistant strains, since the interplay between host and parasite genetic variation could be similar to that of CQ.

No data of *CYP2C8* polymorphisms in Madagascar are available in the literature. Here it is reported a non-negligible frequency of 26.1% of *CYP2C8*2*-carriers in an area of low malaria transmission, where CQ was still

Table 1 *CYP2C8* genotypes and T allele frequency in three African populations

Countries	<i>CYP2C8</i> (rs11572103, A > T) frequencies				
		Relative and (absolute) genotype frequencies			Allele frequency ± SE
Sample	N	AA	AT	TT	T
Senegal	88	0.557 (49)	0.443 (39)	0.000 (0)	0.222 ± 0.044
Uganda	262	0.797 (208)	0.199 (53)	0.004 (1)	0.105 ± 0.019
Madagascar	153	0.739 (113)	0.222 (34)	0.039 (6)	0.150 ± 0.029

used for therapy and prophylaxis although it has been replaced by AS plus AQ combination therapy as the first-line treatment for uncomplicated cases since 2005 [30]. In Madagascar, *P. falciparum* CQ-resistance is mainly based on *pfmdr1*-86Y rather than *pfcr1*-76Y alleles [30,31]. Intriguingly, this setting is similar to that previously reported from Burkina Faso, where the observation of CQ-resistance was mainly based on *pfmdr1* polymorphism [3].

Concerning Senegal, the excess of AT heterozygotes could be attributable to a stochastic effect due to the sampling, despite the efforts to avoid the enrolment of related subjects. Nevertheless, a deviation of the genotype frequencies from the expected Hardy-Weinberg proportions could be expected in discrete populations [32]. The *CYP2C8**2 allele frequency found in the Senegalese subjects (22.2%) is similar to that of Mossi-Rimaibè from Burkina Faso (23.7%) [3] and, in general, the frequency of this allele is higher in West than in East Africa, two-fold higher in Senegal than in Uganda, as reported in this study.

Conclusions

Although malaria infection in Africa is associated with a perverse cycle of disease and poverty that hampers most eradication and control efforts, the study of pharmacogenetics of anti-malarial drugs in human populations exposed to *P. falciparum* suggests that an early identification of PM individuals could lead to alternative therapy of selected individuals/groups in order to minimize the adverse side effects (and therefore the compliance to therapy) as well as delay the spread of drug-resistance.

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Authors' contributions

GMP and RR conceived the study. GMP, FT, DM, MC and RR coordinated the study. GMP, SG and FT carried out the molecular genetic study, performed database and statistical analyses. GR carried out field surveys. GMP, SG, FT and RR drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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