Influence of *CYP2C8*, *CYP3A4* and *CYP3A5* host genotypes on early recurrence of *Plasmodium vivax*

Anne C. G. Almeida[a,b], Maria C. B. Puça[a*], Erick F. G. Figueiredo[a,b], Laila R. Barbosa[a,b], Yanka E. A. R. Salazar[a], Emanuelle L. Silva[a], Marcelo A. M. Brito[a,b], André M. Siqueira[d], José L. F. Vieira[e], Marcus V. G. Lacerda[a,b,c], Wuelton M. Monteiro[a,b], Gisely C. Melo[a,b]

[a]Instituto de Pesquisa Clínica Carlos Borborema. Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil.

[b]Programa de Pós-graduação em Medicina Tropical. Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil.

[c]Instituto Leônidas & Maria Deane. Fundação Oswaldo Cruz, Manaus, Amazonas, Brazil.

[d]Fundação Oswaldo Cruz, Presidência da Fiocruz, Instituto de Pesquisa Clínica Evandro Chagas. Av Brasil 4365, Instituto Nacional de Infectologia Evandro Chagas – Fiocruz. Manguinhos, Rio de Janeiro, RJ – Brasil.

[e]Universidade Federal do Pará, Belem, Brazil.

Running Head: **Chloroquine CYPs on early recurrence of *P. vivax***

Corresponding author: Gisely Cardoso de Melo, Fundação de Medicina Tropical Dr. Heitor Vieira Dourado. Avenida Pedro Teixeira, N° 25, Dom Pedro. CEP: 69040-000. Manaus, Amazonas, Brazil. Email: cardosogisely@gmail.com

ABSTRACT

CYP450 enzymes are involved in biotransformation of chloroquine (CQ), but the role of the different metabolism profiles of this drug has not been properly investigated in relation to *P. vivax* recurrences. To investigate the influence of CYPs genotypes associated with CQ-metabolism on early recurrence rates of *P. vivax,* a case-control study was carried out. Cases included patients presenting an early recurrence (CQ-recurrent), defined as recurrence during the first 28 days after initial infection, plasma concentrations of CQ plus desethylchloroquine (DCQ, the major CQ metabolite) higher than 100 ng/mL. A control (CQ-responsive) with no parasite recurrence over the follow-up was also included. CQ and DCQ plasma levels were measured on Day 28. CQ CYPs (*CYP2C8, CYP3A4* and *CYP3A5)* genotypes were determined by real-time PCR. An *ex vivo* study was conducted to verify CQ and DCQ efficacy in *P. vivax* isolates. The frequency of alleles associated with normal and slow metabolism was similar between the cases and controls for *CYP2C8* (OR=1.45, 95% CI=0.51-4.14, p=0.570), *CYP3A4* (OR=2.38, 95% CI=0.92-6.19, p=0.105) and *CYP3A5* (OR=4.17, 95% CI=0.79-22.04, p=1.038) genes. DCQ levels were higher than CQ, regardless of the genotype. Regarding the DCQ/CQ rate, there was no difference between groups or between those patients who had a normal or mutant genotype. DCQ and CQ showed similar efficacy *ex vivo*. CYPs genotypes had no influence on early recurrence rates. Similar efficacy of CQ and DCQ *ex vivo* could explain the absence of therapeutic failure, despite presence of alleles associated with slow metabolism.

INTRODUCTION

Malaria remains an important public health problem worldwide. In 2018, there were 219 million cases, and 435,000 deaths were caused by malaria (1). In Brazil, the Amazon region is the main area of transmission and *Plasmodium vivax* and accounts for 88.4% of reported cases in the country (2). An important obstacle to *P. vivax* malaria elimination in *P. vivax* endemic areas stems from the frequent recurrences caused by this parasite. These recurrences are characterized as a relapse when they are caused by activation of hypnozoites in the liver; a reinfection if parasitemia returns due to a new infected mosquito bite; and a recrudescence when there is an early return of asexual parasitemia despite adequate levels of chloroquine (CQ) and the metabolite desethylchloroquine (DCQ) in host plasma, which often indicates the presence of drug resistant parasites leading to therapeutic failures (3).

CQ undergoes hepatic biotransformation through the N-dealkylation pathway into two main metabolites: DCQ and bisdesethylchloroquine (BDCQ). DCQ is the major CQ metabolite, with detected plasma concentrations from 20 to 50% of those of CQ (4). In contrast, BDCQ plasma or blood concentrations never reach more than 10 to 15% of CQ levels (5). The antimalarial action of DCQ, evaluated only for *Plasmodium falciparum*, was equally active against a CQ-sensitive strain, but significantly less active than the parent compound against CQ-resistant strain (6) For *P. vivax*, *in vivo* study to assess resistance to CQ, only CQ and DCQ levels are generally measured (7, 8), since they are the major CQ metabolites (4).

P. vivax resistance to CQ has been reported, with a high prevalence in Indonesia and Papua New Guinea (9). In the Brazilian Amazon, CQ resistance rates range from 5 to 11% *in vivo* (8, 10, 11) and 9.8% to 10.7% *ex vivo* (12, 13). Although the mechanisms that lead to *P. vivax* resistance to CQ are not well understood, some studies have shown an association between gene expression and variation in copy number of the *pvcrt-o* and *pvmdr-1* genes and resistance to CQ (7, 14). CQ remains at therapeutic levels against *P. vivax* until 35 days after starting treatment (3, 15). After this period, with decreasing plasma levels of CQ and DCQ, the return of parasitemia is due to reinfection or relapse (9). Several studies used D28 as the cutoff point to assess possible therapeutic failures by CQ (7, 8, 10, 11, 16), following the recommendation of the World Health Organization for monitoring effectiveness of antimalarials (17).

Recent studies have demonstrated the importance of host genetics in antimalarial treatment outcomes (18-21), based on single nucleotide polymorphisms (SNPs) detected in genes encoding drug metabolizing enzymes (22). The presence of certain genotypes related to these enzymes may be associated with an increase in drug metabolism rates, generating adverse effects, and an increase in the elimination rate, or a decrease in the metabolization rate could lead to therapeutic failure (20, 22, 23).

The biotransformation of primaquine (PQ) mediated by cytochrome P-450 enzymes is attributed to the *CYP2D6*, *CYP3A4*, *CYP1A2* and *CYP2C19* enzymes (22, 24). Therapeutic failures in the treatment of *P. vivax* with PQ are generally attributed to the presence of *CYP2D6* polymorphisms (19), a relationship also reported in Brazil (21, 25). *CYP2C8*, *CYP3A4* and *CYP3A5* were reported as the major enzymes involved in the formation of DCQ from CQ

(26). An effect of the *CYP2C8*2/*3/*4* gene on gametocyte clearance rate on patients undergoing CQ and PQ malaria treatment has been reported (27).

Pharmacogenetics has gained great importance over the last few years, since it can enable patients to received personalized drug therapy for various diseases (28). However, the frequency of CYP alleles associated with slow metabolism for CQ in individuals from the Brazilian Amazon has not been fully studied, in particular whether the presence of these alleles influences early recurrence. In addition, there is a paucity of studies to understand which molecule (CQ or DCQ) has the best antimalarial action on *P. vivax*, and whether a profile of low drug metabolism contributes to increased early recurrence rates.

This study aimed to investigate the frequency of genotypes associated with slow CQ metabolism for the main metabolizing CYPs in patients from the Brazilian Amazon and verify the influence of these alleles in early recurrence of *P. vivax* malaria.

RESULTS

Allele frequencies of CYPs associated with CQ metabolization

Twenty-six cases (CQ-recurrent) and 99 controls (CQ-responsive) were included. Clinical and laboratorial characterization of the patients included in this study are presented in Table 1. All individuals with CQ-recurrent *P. vivax* had positive qPCR for *P. vivax* and their mean blood levels of CQ plus DCQ were greater than 100 ng/mL at D28.

The allele frequencies of *CYP2C8* (p=0.3196), *CYP3A4* (p=0.0916) and *CYP3A5* (p=0.1064) were similar in cases and controls. Most individuals carried alleles associated with normal enzyme activity (**1* or **1A*). Alleles associated with slow enzyme activity were found in both cases and controls (Table 2).

The most frequent diplotype for *CYP2C8* was **1/*1* (76.2% in cases and 82.8% in controls) and **1/*3* (15.3% in cases and 10.1% in controls). For *CYP3A4*, the most frequent diplotypes were **1A/*1A* (65.3% in cases and 81.2% in controls) and **1A/*1B* (30.7% and 16.1%, respectively). For *CYP3A5*, **1/*1* (88.4% in cases and 96.9% in controls) was the most frequent diplotype; *1/*3* was found in cases (7.6%) and controls (2.0%); **1/*6* was found in cases (3.8%) and controls (1.0%). The frequency of diplotypes associated with normal and slow metabolism was similar between cases and controls for *CYP2C8* (OR=1.45, 95% CI=0.51-4.14, p=0.570), *CYP3A4* (OR=2.38, 95% CI=0.92-6.19, p=0.105) and *CYP3A5* (OR=4.17, 95% CI=0.79-22.04, p=1.038) genes.

Genotypes and *P. vivax* malaria recurrences

In addition to following up until D28, we also investigated the occurrence of vivax malaria recurrences in patients up to 1 year by passive case detection using the SIVEP-Malaria platform (The Brazilian official Malaria Epidemiological Surveillance Information System). When considering individuals with alleles associated with normal and slow metabolism for *CYP2C8*, *CYP3A4* and *CYP3A5,* no significant differences were found in the recurrence rates in cases and controls over the 1-year follow-up (Table 3).

144 **Drug levels and CQ CYPs genotypes**

145 When we compared CQ and DCQ concentrations between cases and
146 controls, no significant differences were found. Furthermore, no significant
147 differences were found between the mean concentration of CQ and DCQ in
148 individuals carrying alleles associated with slow metabolism (Table 3). DCQ
149 concentration was higher than CQ irrespective of genotype, indicating that most
150 individuals had drug biotransformation ability despite the mutated genotypes
151 found (for cases, *CYP2C8*1* [DCQ]=108.4nM (95% CI=89.7-127.1) and
152 *CYP2C8*2/*3/*4* [DCQ]=130.6nM (95% CI=57.8-203.4); for the controls,
153 *CYP2C8*1* [DCQ]=93.3nM (95% CI=68.2-118.4) and *CYP2C8*2/*3/*4* [DCQ]=
154 79.9nM (95% CI=40.2-119.6).

155 The possibility of genotype influencing metabolite/drug ratio was analyzed.
156 However, there was no significant difference between the DCQ/CQ ratio in
157 individuals carrying alleles associated with normal and slow metabolism of
158 *CYP2C8* and *CYP3A5*. The metabolite/drug ratio was higher in individuals
159 carrying alleles associated with normal *CYP3A4* metabolism than in individuals
160 with mutated genotype in the control group (Figure 1). For *CYP3A4*, significant
161 difference was observed, (cases: *CYP3A4*1A*, mean DCQ/CQ=1.76 and
162 *CYP3A4*1B*, mean DCQ/CQ=1.55, p=0.629; controls: *CYP3A4*1A*, mean
163 DCQ/CQ=1.89 and *CYP3A4*1B*, mean DCQ/CQ=1.21, p=0.0347). No
164 difference in CQ and DCQ levels (metabolite/drug ratio) was found between
165 cases and controls for *CYP2C8* and *CYP3A5* ($p>0.05$) (Figure 1).

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168 **Clearance of *P. vivax* parasitemia and CQ CYPs alleles**

169 Most individuals had clearance of asexual parasitemia on D2 (58/125;
170 46.4%) or D3 (39/125; 31.2%). Late clearance (from D4 to D7) was recorded for
171 23 patients (18.4%). Slow clearance of asexual parasitemia did not predict early
172 recurrences (OR=2.49, 95% CI=0.92-6.75, p=0.088). Genotypes associated
173 with normal and slow metabolism were found in these patients in a similar
174 frequency (Table 3), which suggests that the presence of a mutated genotype is
175 not associated with the clearance time for *P. vivax*. There was also no
176 difference regarding mean asexual parasitemia in D1, D2, D3 and D7 between
177 patients with alleles associated with normal activity and low activity in all the
178 enzymes studied (Figure 2). Only 1 patient belonging to the control group
179 (1.01%, 1/99), showed gametocyte clearance on D7. No association was
180 observed between late gametocyte clearance (D3 to D7) and genotypes
181 associated with slow metabolism of CYP in the cases (p=1.000) or controls
182 (p=0.259).

183

184 ***P. vivax ex vivo* assay**

185 Twenty-four malaria patients not included in the cases or controls were enrolled
186 in the *ex vivo* assays to evaluate the efficacy of CQ and DCQ against *P. vivax*.
187 None of these isolates was resistant to CQ. The mean IC50% of CQ was
188 11.67nM (95% CI=6.520-16.82) and mean IC50% of DCQ was 8.95nM (95%
189 CI=4.25-13.65) (Figure 3). None of these 24 patients presented early
190 recurrence.

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DISCUSSION

This is the first study to investigate the role of CYP genotypes in early recurrence of *P. vivax* malaria in samples from the Brazilian Amazon, and the first results regarding an evaluation of the sensitivity of CQ and DCQ in *P. vivax* isolates.

The frequency of allelic gene variants has already been used to predict the activity of drug metabolizing enzymes (29). In Brazil, the frequency of these variants for CYP450 enzymes has been described (30-33), but there is still little information regarding the influence of these genotypes on antimalarial therapy failure, including CQ. Studies of *P. falciparum* have demonstrated the importance of pharmacogenetics in the elimination of this parasite (34, 35).

For the *CYP2C8* gene, variant **2* is more frequent in Africans (11-17%) and *CYP2C8*3* is more frequent in Caucasians (15%)(36). The frequency of *CYP2C8*4* is higher in European populations (4-7%) (37). For *CYP3A4*, the frequency of the **1B* allele is variable, ranging from - 3.6% among white Americans to 76% in Africans (38). For *CYP3A5*, the highest frequencies of **3* are among Asians (60-75%) and Caucasians (85-95%); **6* is present in Africans (22%) and rare in Caucasians and Asians (22). The frequency of mutated alleles for *CYP2C8*, *CYP3A4* and *CYP3A5**6 found in our study is in agreement with other studies of Brazilian populations (27, 30, 33). Only the frequency found for variant *CYP3A5*3* was lower.

The *CYP2C8*2* variant is associated with a 50% reduction in metabolic activity, and *CYP2C8*3* with a 85% reduction in normal enzyme activity compared to the wild type allele, which was found in a study with paclitaxel and

araquidonic acid drugs, *in vitro* (36, 39). In Burkina Faso and Ghana, the role of *CYP2C8* variants in the response to amodiaquine and the correlation between *CYP2C8*2* and low levels of enzyme metabolism have been investigated, confirming their influence on drug metabolism (40, 41). However, they do not demonstrate the association between alleles associated with low enzyme activity and the treatment outcome.

Studies conducted in Papua New Guinea and Thailand described the risk of *P. vivax* recurrence in individuals with late parasitemia clearance (41, 42). In our study, the clearance of asexual parasitemia at D7, and gametocytemia after D3, were not predictors of early recurrence by *P. viva*x and were not associated with the presence of mutated CYP genotypes. A recent study in Brazil found an association between *CYP2C8*2*3/*4* and gametocyte clearance rate on patients undergoing CQ/PQ malaria treatment. From baseline to the first day of treatment, homozygous individuals for wild-type *CYP2C8* achieved greater gametocyte reduction (p=0.007) than individuals without this genotype (27). There was no difference between CQ and DCQ levels in patients with normal or mutated genotypes and in the frequency of these *CYP2C8* alleles in cases or in the control groups.

The *CYP3A5*3* variant is related to decreased enzyme activity and *CYP3A5*6* and **7* are null alleles that cause enzyme absence (29). Kim *et al* (26) demonstrated a role for *CYP3A5* in CQ metabolism, but another study showed a smaller role for this enzyme in the formation of DCQ *in vitro* (42). A recent study demonstrated that polymorphisms of *CYP3A5* and *CYP3A4* did not show any significant association with blood levels of hydroxychloroquine and desethylhydroxychloroquine in patients with Systemic Lupus Erythematosus

(43). In our study, the metabolite/drug ratio was higher in individuals carrying alleles associated with normal *CYP3A4* metabolism than in individuals with mutated genotypes in the control group. However, studies conducted so far have not clarified whether the occurrence of SNPs alters the metabolism of the drug (22).

In our study, we found no association between mutated genotypes and changes in CQ and DCQ levels. Although other studies report a role for the alleles associated with slow metabolism of *CYP2C8*, *CYP3A4* and *CYP3A5* in the levels of drugs for clinical use (37, 44, 45), the evidence of the influence of these alleles on enzyme phenotype is still limited (46). It is known that there may be a failure in the CYP450 genotype/phenotype correlation, with about 50% of errors in phenotype prediction being described. Factors such as gene splicing, transcriptional regulation, influence of microRNAs, in addition to other existing SNPs, would play a role in the final enzyme activity phenotype (47). These studies suggest that genotype information alone would not be sufficient to replace phenotype measurements, in this case, measurements of drug levels. In addition, the expression and activity of CYP enzymes can be affected by the inflammatory response triggered by the infection, as already demonstrated for *P. falciparum* malaria, with *CYP1A2* (48, 49) and *CYP3A* (50).

In vivo resistance to CQ signifies a persistence of *P. vivax* asexual stages, despite the presence of adequate levels of CQ plus DCQ (concentration higher than 100 ng/dL) (16). For *ex vivo* conditions, resistance occurs when IC50% values greater than 100nM of CQ are obtained after 42 hours of *P. vivax* culture (13, 51).

It is known that DCQ's action is similar to that of CQ and is effective against avian malaria, but little is known about its action in human malaria (52). According to Fu *et al*, 1986 (6), DCQ is as effective as CQ in sensitive isolates of *P. falciparum*, but its efficiency is significantly reduced in resistant isolates . *In vivo* sensitivity trials with CQ and DCQ showed that DCQ was more effective, and a combination of drugs had better potential when compared to monotherapy with just CQ. For resistant isolates, better effectiveness was reported using CQ monotherapy, and the combination of drugs was shown to be better than when used alone (53).

A limitation of this study was that recurrent parasitemia prior to D28 in the presence of normally lethal CQ blood levels could not be excluded, since there may be a relapse of the CQ-recurrent parasite after clearance of the original CQ-responsive parasitemia, however the frequency of this event is not expected to vary between different CYP genotypes. Unfortunately, the *ex vivo* and *in vivo* studies were not performed with the same samples of *P. vivax*. According to our experience, parasitemia on recrudescent day (DR) is often very low and the ring count is lower than 50% of parasites at the ring stage of development, making the experiment unfeasible on DR (54), but patients in the *ex vivo* study were followed-up for 28 days and none showed recurrence during this period. The sample size used in the *ex vivo* study is not adequate to confirm CQ resistance rates.

The study obtained a low sample size for CQ-recurrence, however, this frequency is in agreement with previous studies (8, 10, 11). No genetic marker analysis was performed for differentiating relapse, resistance or reinfection, as

some researchers have stated that there are no ideal genetic markers for these (55, 56).

Our results demonstrate that CQ and DCQ had similar efficacy against *P. vivax* isolates from the Amazon and could explain why alleles associated with low enzyme activity found in patients in the control group did not necessarily lead to failure in CQ treatment and early recurrence. The absence of drug metabolism problems, even in the occurrence of SNPs in CYP genes in the control group, indicates that these genetic host characteristics had little influence on early recurrence caused by *P. vivax*.

MATERIALS AND METHODS

Ethics statement

The study was approved by the Ethics Review Board of *Fundação de Medicina Tropical Dr Heitor Vieira Dourado* (FMT-HVD) (approval number 343/2009). Participants were instructed regarding the objectives of the study and signed an informed consent form. In the case of minors, the consent form was signed by the parents or legal guardians. Patients diagnosed with malaria were treated according to the Brazilian Ministry of Health guidelines (57).

Study site

The study was carried out between 2012 and 2014 at FMT-HVD, an infectious disease referral center located in Manaus, Western Brazilian Amazon. *Ex vivo* studies were carried out on samples obtained from 2017 to 2018.

312 **Selection of patients**

313 The study included patients of either sex who were infected with *P. vivax*
314 malaria, aged 6 months to 60 years, had bodyweight greater than 5 kg, and
315 blood parasite density from 250 to 100,000 parasites/mL and axillary
316 temperature of 37.5°C or history of fever in the last 48 hours. Exclusion criteria
317 were use of antimalarials in the previous 30 days, refusal to be followed up for
318 28 days and any clinical complication (58). All patients received supervised
319 treatment with 25 mg/kg of chloroquine phosphate over a 3-day period (10
320 mg/kg on days 1 and 7.5 mg/kg on days 2 and 3). PQ was prescribed for a 7-
321 day period, at the dosage of 0.5 mg/kg per day, starting only at the end of the
322 follow-up or on the day of recurrence. Patients who vomited the first dose within
323 30 minutes after drug ingestion were re-treated with the same dose. Clinical and
324 laboratory tests were performed, and interviews and sample collection done on
325 follow-up D1, D2, D3, D4, D7, D14 and D28. If there were any extra days of
326 follow-up, the same sample collection procedures were performed.

327 This study was conducted by using a convenience sampling from previous
328 patient follow-ups. The enrolled patients were divided into cases (CQ-recurrent
329 patients) when they presented *P. vivax* early recurrence, with parasites
330 returning during the first 28 days after initial infection, and plasma levels of CQ
331 plus DCQ higher than 100 ng/mL; and controls (CQ-responsive patients), a
332 group that consisted of patients with no parasitemia recurrence during 28-days
333 of follow-up.

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P. vivax malaria diagnosis

Asexual and sexual parasitemia at D1, as well as the clearance of asexual parasitemia were determined by microscopy by two experienced microscopists using parasite counts per 500 leukocytes. Patients were actively followed-up for 28 days. After day 28, recurrences were detected by passive case detection via the SIVEP-Malaria system, the official malaria epidemiological surveillance system in Brazil.

Genotyping of CQ CYPs

DNA was purified from the whole blood samples using QIAamp DNA mini Kit (Qiagen, USA). We genotyped three single-nucleotide polymorphisms (SNPs) in the *CYP2C8* (A805T [rs11572103]), (C792G [rs1058930]), (G416A [rs11572080]); one SNP in the *CYP3A4* (A392G ([rs2740574]) and two SNPs in the *CYP3A5* (G14690A [rs10264272]), (A6986G [rs776746]). Analyses were assessed using 7500 Fast Real-time PCR 2.3 version (Applied Biosystems) software. Amplification reactions and cycling parameters were determined according to the manufacturer's protocols.

CQ and DCQ levels

Three 100µL aliquots of sample collected on D28 of follow-up were used for measuring CQ and DCQ levels. Analysis was assessed by high-performance liquid chromatography (HPLC) as previously described (59).

P. vivax ex vivo culture

Plasmodium isolates were collected between August 2017 and June 2018 from FMT-HVD patients. Patients were recruited if they presented a

monoinfection with *P. vivax*, parasitemia ≥ 10,000 parasites/mL and a majority (>50%) of parasites at the ring stage of development. Four milliliters of whole blood were collected by venipuncture. After removal of leucocytes by using CF11 cellulose (Sigma-Aldrich), infected red blood cells (IRBC) were used for *ex vivo* drug susceptibility testing.

CQ and DCQ susceptibility of *P. vivax* isolates were measured using a protocol modified from the WHO microtest (60). Two hundred microliters of a 2% hematocrit blood media mixture (BMM), which consisted of McCoy's 5A medium plus 20% AB human serum was added to each well of pre-dosed drug plates containing 11 serial concentrations (2-fold dilutions) of the antimalarials (1.95-1000) ng/mL of CQ diphosphate and DCQ and each concentration of drug was tested in quadruplicate. A candle jar was used to mature the parasites at 37.5°C for 48 h. Incubation was stopped when ≥40% of ring-stage parasites had reached the mature schizont stage in the drug-free control well.

Thick-blood smears were made from each well, then stained with 5% Giemsa solution and examined microscopically. The number of schizonts per 200 asexual stage parasites was determined for each drug concentration and normalized to the control well.

Statistical analyses

Allele and genotype frequencies were estimated by gene counting and haplotype frequencies and linkage disequilibrium were estimated with Haploview. Fisher's exact test or χ^2 test was performed to compare the *CYP2C8*/*CYP3A4*/*CYP3A5* allele and the genotype frequencies between cases and controls. The odds ratios (OR) with their respective 95% confidence

intervals (95% CI) were determined to verify the risk of CQ- recurrent depending on the diplotypes found and to relate late clearance of parasitemia and cases. In the *ex vivo* essay, the percentage of schizont maturation at each drug concentration was estimated in 200 asexual parasites, and results were entered in the online *IC Estimator* software to calculate the IC50% of each sample by nonlinear regression analysis and t-test of averages comparison and graph construction was performed. A p-value <0.05 was considered significant in all analyses. Analysis was performed using software GraphPad Prism.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: GCM, WMM, MVGL, AMS. Sample processing: ACGA, MCBP, EFGF, YEARS, ELS. Performed the experiments: ACGA, MCBP, EFGF, LBR and JLFV. Data entry and analyses: ACGA and MCBP. Wrote the paper: ACGA, MCBP, GCM, YEARS, LBR and MAMB. All authors read and approved the final manuscript.

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FIGURE LEGENDS

Figure 1. Desethylchloroquine/chloroquine ratio between different genotype groups. No significant difference was found between the metabolite/drug ratios between different genotypes (associated with low and normal enzyme activity) in patients with *P. vivax* early recurrence (CQ-recurrent) and CQ-responsive for *CYP2C8* **(A)** and *CYP3A5* **(C)**. For *CYP3A4* **(B)**, the ratio was higher for genotype associated with normal metabolizing among CQ-responsive patients. Values obtained by unpaired t-test.

Figure 2. Asexual parasitemia clearance between low and normal activity genotypes. Parasitemia means at visits D1, D2, D3, D4 and D7 visits were evaluated. Between genotypes associated with low and normal enzyme activity, there was no significant difference in clearance day and asexual parasitemia, considering CQ-recurrent and CQ-responsive, for *CYP2C8* **(A)**, *CYP3A4* **(B)** and *CYP3A5* **(C)**. Values obtained by paired t-test.

Figure 3. *Ex vivo* efficacy of chloroquine and desethylchloroquine in *P. vivax* isolates. The drug and metabolite showed similar efficacy against isolates in patients from the Brazilian Amazon. Values obtained by paired t-test.

766 **TABLES**

767 Table 1. Demographic and parasitological baseline data of individuals involved

768 in this study.

Characteristics	**CQ- Recurrent**	**CQ-Responsive**	***p-value***[a]
N	26	99	
Age (mean, 95%CI)	34.69 (27.59-41.78)	35.21 (31.94-38.47)	0.8872
Gender; male (n,%)	19 (73.1)	73 (73.7)	1.0000
Parasitemia D0 (mean parasites/µL, 95%CI)	2618.82 (1359.20-3878.43)	2961.6 (2297.90-3625.29)	0.6368
Gametocytemia D0 (mean gametocytes/µL, 95%CI)	45.26 (24.89-65.62)	75.3 (39.12-111.47)	0.4060

Recurrent = new malaria

episode by D28.

Responsive = no malaria

episode by D28.

[a]χ^2or *t* test, Exact Fisher test.

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770 Table 2. Chloroquine CYPs allele frequency.

		CQ- Recurrent (52)		**CQ-Responsive** (198)		
Gene	Alleles	n	Frequency	n	Frequency	***p-value***[a]
CYP2C8	**1*	46	0.8846	179	0.9040	0.6137
	**2*	0	0.0000	6	0.0303	0.3493
	**3*	4	0.0769	11	0.0556	0.5225
	**4*	2	0.0385	2	0.0101	0.192
CYP3A4	**1A*	42	0.8077	178	0.8990	0.0916
	**1B*	10	0.1923	20	0.1010	
CYP3A5	**1A*	49	0.9423	195	0.9848	0.1064
	**3*	2	0.0385	2	0.0101	0.192
	**6*	1	0.0192	1	0.0051	0.3734

n = number of chromosomes

[a]χ^2 test

771 Table 3. Parasitemia clearance, drug levels and malaria recurrence patterns between different genotype groups.

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	n	Asexual parasitemia clearance day (n,%)[a]				CQ levels at D28 (mean, 95%CI)[b]	DCQ levels at D28 (mean, 95%CI)[b]	Malaria episodes up to 1 year (n,%)[a,c]		
		D1	D2	D3	D7			0	1	>1
CYP2C8										
CQ- Recurrent										
**1*	20	0 (0)	11 (55)	4 (20)	5 (25)	63.8 (49.33-78.26)	108.4 (89.68-127.11)	8 (40)	9 (45)	3 (15)
**2/*3/*4* allele carriers	6	1 (16.7)	2 (33.3)	0 (0)	3 (50)	42.2 (17.37-67.02)	130.6 (57.82-203.37)	2 (33.3)	3 (50)	1 (16.7)
p-value		0.2308	0.6447	0.5425	0.3301	0.1293	0.3266	1.0000	1.0000	1.0000
CQ-Responsive										
**1*	82	3 (3.7)	36 (43.9)	30 (36.6)	13 (15.8)	43.4 (37.85-48.94)	93.3 (68.17-118.42)	59 (71.9)	14 (17.1)	9 (11)
**2/*3/*4* allele carriers	17	1 (5.9)	9 (52.9)	5 (29.4)	2 (11.8)	64.1 (23.29-104.90)	79.9 (40.23-119.56)	10 (58.9)	4 (23.5)	3 (17.6)
p-value		0.5354	0.5956	0.7813	1.0000	0.0528	0.6459	0.3843	0.5047	0.4277
CYP3A4										
CQ- Recurrent										
**1A*	17	1 (5.9)	7 (41.2)	3 (17.6)	6 (35.3)	62.06 (46.61-77.50)	121.93 (94.73-149.12)	5 (29.4)	10 (58.8)	2 (11.8)
**1B* allele carriers	9	0 (0)	6 (66.7)	1 (11.1)	2 (22.2)	55 (30.04-79.95)	96.66 (75.71-117.60)	5 (55.6)	2 (22.2)	2 (22.2)
p-value		0.4375	0.411	1.0000	0.6673	0.5842	0.1948	0.2341	0.11	0.5906
CQ-Responsive										
**1A*	81	4 (4.9)	37 (45.7)	27 (33.3)	13 (16.1)	48.68 (39.82-57.53)	100.48 (74.65-126.30)	56 (69.2)	15 (18.5)	10 (12.3)
**1B* allele carriers	18	0 (0)	8 (44.4)	8 (44.4)	2 (11.1)	39.25 (18.81-59.68)	48.52 (29.23-67.80)	13 (72.2)	3 (16.7)	2 (11.1)
p-value		1.0000	1.0000	0.4195	0.732	0.3706	0.0662	1.0000	1.0000	1.0000
CYP3A5										
CQ- Recurrent										
**1A*	23	1 (4.4)	10 (43.5)	4 (17.4)	8 (34.7)	61.63 (47.95-75.30)	113 (91.80-134.19)	7 (30.4)	12 (52.2)	4 (17.4)
**3/*6* allele carriers	3	0 (0)	3 (100)	0 (0)	0 (0)	44 (6.91-81.08)	111.66 (53.18-170.13)	3 100	0 (0)	0 (0)

p-value		1.0000	0.22	1.0000	0.5292	0.3570	0.9637	0.0462	0.2246	1.0000
CQ-Responsive										
**1A*	96	4 (4.2)	42 (43.8)	35 (36.5)	15 (15.5)	47.5 (39.29-55.70)	92.46 (70.20-114.71)	67 (69.8)	17 (17.7)	12 (12.5)
**3/*6* allele carriers	3	0 (0)	3 (100)	0 (0)	0 (0)	29.92 (42.81-102.65)	45.25 (71.03-161.53)	2 (66.7)	1 (33.3)	0 (0)
p-value		1.0000	0.0905	0.5501	1.0000	0.4584	0.4615	1.0000	0.456	1.0000

[a] X^2 test

[b] *t* test or Exact Fisher test

[c] SIVEP-Malaria

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