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# Distribution of the cytochrome P450 *CYP2C8*2* allele in Brazzaville, Republic of Congo

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ABSTRACT

*Background:* Cytochrome P450 (CYP) enzymes are essential in the metabolism of most drugs used today. Single nucleotide polymorphism(s) occurring in CYP genes can adversely affect drug pharmacokinetics, efficacy, and safety. Individuals carrying the *CYP2C8*2 c.805A > T* (*CYP2C8*2*; rs11572103) allele have impaired amodiaquine metabolism, increased risk of amodiaquine-related adverse events, and may promote the selection of drug-resistant parasite strains. This study investigated the distribution of the *CYP2C8*2* allele in Brazzaville, Republic of Congo, where artesunate + amodiaquine is used as the second-line treatment for uncomplicated *Plasmodium falciparum* malaria.
*Methods:* A total of 285 febrile children visiting the Marien Ngouabi paediatric hospital were genotyped for *CYP2C8*2* using PCR-restriction fragment length polymorphism (PCR-RFLP). The allele frequencies and genotype distribution were determined.
*Results:* The *CYP2C8*2* allele was successfully genotyped in 75% (213/285) of the study participants. The *CYP2C8*2A* allele had a frequency of 63%, whereas the *CYP2C8*2T* allele had a frequency of 37%. Genotypes *CYP2C8*2AA* (rapid metabolizer), *CYP2C8*2AT* (intermediate metabolizer), and *CYP2C8*2TT* (poor metabolizer) were observed in 44%, 38%, and 18% of the investigated participants, respectively.
*Conclusions:* This study gives the first description of *CYP2C8*2* allele distribution in the Republic of Congo and highlights the potential risk of amodiaquine-related adverse events. Information from this study will be beneficial during pharmacovigilance investigations.


## Introduction

Malaria continues to be of great public heath importance despite a remarkable decline in prevalence between 2005 and 2015 (WHO, 2015a). In 2017, malaria accounted for approximately 219 million morbidities and 445 000 mortalities globally (WHO, 2018). The majority of these cases occurred in Sub-Saharan Africa, particularly in children under 5 years of age. Malaria transmission in the Republic of Congo is perennial with the annual entomological inoculation rate (EIR) ranging between 200 and 1000 infective bites/person (WHO & MHP, 2006). About 50% of outpatients and 18% of deaths reported in the Republic of Congo are due to malaria (NMCP, 2014). *Plasmodium falciparum*, the most lethal *Plasmodium* species, is predominant. The main strategies for reducing the malaria burden are mosquito vector control and prompt treatment of parasitologically confirmed cases of *P. falciparum* malaria with artemisinin-based combined therapy (ACT).

ACTs are a combination of a fast-acting artemisinin derivative (dihydroartemisinin, artesunate, or artemether) and a slow-acting drug(s) from different classes, namely lumefantrine, amodiaquine (AQ), mefloquine, sulfadoxine-pyrimethamine (SP), and piperaquine (WHO, 2015b). The ACT treatment for acute malaria comprises a 3-day regimen. In 2006, the national malaria control programme of the Republic of Congo adopted ACTs for the treatment of acute falciparum malaria in response to widespread

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parasite resistance to chloroquine and SP (NMCP, 2016; Nsimba et al., 2005; Koukouikila-Koussounda et al., 2017). Artesunate + amodiaquine (ASAQ) and artemether + lumefantrine (AL) were the initial first-line and second-line treatments, respectively, for uncomplicated *P. falciparum* malaria in this setting (MHP, 2014). However, the guidelines were amended in 2014 in favour of AL as the first-line drug, after ASAQ was associated with a higher number of drug-related adverse events than AL (MHP, 2014). ASAQ is presently the second-line drug treatment in Republic of Congo.

Drug-induced adverse events are modulated, in part, by inter-individual genetic variability in the genes encoding enzymes involved in the metabolism of drugs, such as cytochrome P450 enzymes (CYP) (Lynch and Price, 2007; Bains, 2013). CYP is a family of enzymes encoded by polymorphic genes mainly expressed in the liver. Of the 57 CYP identified in humans, seven are involved in the phase I metabolism of >90% of drugs used today (Lynch and Price, 2007; Guengerich, 2006). The CYP enzymes differ in substrate specificity. For example, CYP2C8 solely metabolizes the AQ prodrug to desethylamodiaquine (DEAQ) and plays a secondary role in the metabolism of chloroquine (Li et al., 2003; Totah and Rettie, 2005; Gil and Gil Berglund, 2007). The CYP2C8 gene (*CYP2C8*) is located on chromosome 10q24 (Totah and Rettie, 2005; Gray et al., 1995). Mutation at *CYP2C8 c.805A > T* (*CYP2C8*2*; rs11572103) impairs AQ metabolism and increases the risk of AQ-induced adverse events such as agranulocytosis and hepatotoxicity. The selection of *P. falciparum*-resistant strains may also occur because of parasite exposure to subtherapeutic DEAQ levels, as well as the long AQ half-life (Hombhanje et al., 2005; Hietala et al., 2007; Petersen et al., 2011; Paganotti et al., 2011).

The *CYP2C8*2* allele is most common in Africans (19%) and is rare (≤1%) in Europeans, Asians, and Americans (Backman et al., 2016). Studies conducted in Sub-Saharan Africa have shown a heterogeneous allele distribution between countries as well as among different ethnicities in a given geographical area (Paganotti et al., 2011; Rower et al., 2005; Paganotti et al., 2012; Motshoge et al., 2016; Mehlotra et al., 2006; Parikh et al., 2007). The high *CYP2C8*2* allele frequency in this region where malaria is also endemic clearly shows the increased risk of AQ-induced adverse events after ASAQ administration, particularly in intermediate (genotype *CYP2C8*2AT*) and poor (genotype *CYP2C8*2TT*) AQ metabolizers (Bains, 2013) It appears that the distribution of the *CYP2C8*2* allele has not been described in the Republic of Congo in spite of reports of ASAQ-induced adverse events (MHP, 2014). This study investigated the distribution of the *CYP2C8*2* allele in Brazzaville, Republic of Congo. Findings from this study will be relevant in the interpretation of ASAQ pharmacovigilance and efficacy data.

## Methods

### *Ethical approval and consent*

This study was approved by the Institutional Ethics Committee for Research in Health Sciences of the Republic of Congo. Written informed consent was obtained from the parents/guardians before samples were collected.

### *Study area and population*

Two hundred and eighty-five febrile (≥37 °C) children, aged between 1 and 10 years, recruited in a cross-sectional study conducted between September 2014 and February 2015 in the Marien Ngouabi Paediatric Hospital located in Brazzaville, were enrolled in this study (Figure 1). Brazzaville is the capital city of the Republic of Congo, with an estimated population of 1.8 million people. It is located along the Congo River. The majority of the ethnic groups in this area are Bantu (Worldatlas, 2018). The annual rainfall ranges from 1100 mm to 2000 mm (Samba et al., 2008). Malaria transmission is perennial with an annual entomological inoculation rate (EIR) of between 200 and 1000 infective bites/person (WHO & MHP, 2006). Malaria accounts for 48% of outpatients, 65% of hospital admissions, and 18% of deaths (NMCP, 2014).

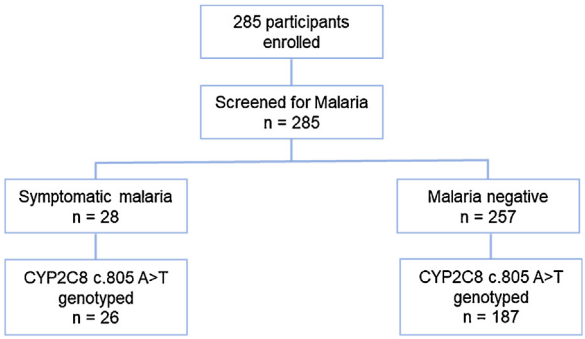


**Figure 1.** Baseline characteristics of the participants. Malaria infection tested by *Plasmodium falciparum* 18S ribosomal RNA (rRNA)-specific nested PCR.

### CYP2C8*2 *genotyping*

Genomic DNA was extracted from peripheral whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR-restriction fragment length polymorphism (PCR-RFLP) was used to genotype *CYP2C8*2 c.805A > T* (*CYP2C8*2*, rs11572103), as described previously (Marwa et al., 2014). Briefly, PCR reactions were conducted in a total volume of 50 μl containing 100 ng genomic DNA, 200 μM of dNTPs, 200 nM *CYP2B6*2* primers (forward primer: 5′-GAACAC-CAAGCATCACTGGA-3′ and reverse primer: 5′-GAAATCAAAATACT-GATCTGTTGC-3′), and 2.5 U Taq polymerase (Qiagen, Hilden, Germany) The thermocycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 38 cycles at 94 °C for 20 s, 55 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products (5 μl) were digested at 55 °C with 5 units of BcII in Buffer G (Thermo Scientific) for 3 h and resolved in a 2.5% agarose gel stained with SYBR Green. The restricted PCR fragments revealed three different genotypes. The *CYP2C8*2AA* (wild-type) showed two bands (50 bp and 57 bp), *CYP2C8*2TT* (homozygous mutant) showed one band (107 bp), and *CYP2C8*2AT* (heterozygous) showed three bands (50 bp, 57 bp, and 107 bp).

### *Data analysis*

Data were analysed using Stata v. 9.1 (StataCorp., College Station, TX, USA) and the level of significance was set at $p < 0.05$. Genotype frequencies were determined by simple gene counting and by using the expectation-maximum (EM) algorithm. The Chi-square test was used to determine the differences in allele and genotype distributions between the different subgroups of patients. The significance of deviation from Hardy–Weinberg equilibrium was tested using the random-permutation procedure as implemented in Arlequin v. 3.5.1.2 software (http://cmpg.unibe.ch/software/arlequin3/).

## Results

A total of 285 febrile children with a mean age of 3.2 ± 2.5 years were enrolled in this study (Table 1). Ten percent (28/285) of the participants were positive for *P. falciparum* infection (Figure 1). *CYP2C8*2* was successfully genotyped in 75% (213/285) of the

**Table 1**
Clinical and genetic characteristics according to malaria and non-malaria groups.

| Characteristics | All individuals ($n = 285$) | Symptomatic malaria ($n = 28$) | Non-malarial fever ($n = 257$) | $p$-Value |
|---|---|---|---|---|
| Age, years, mean ± SD | 3.2 ± 2.5 | 4.1 ± 2.8 | 3.1 ± 2.4 | 0.045 |
| Male/female ratio | 148/137 | 14/14 | 134/123 | 0.830 |
| CYP2C8 c.805 A > T genotyped[a] | $n = 213$ | $n = 26$ | $n = 187$ | |
| AA, $n$ (%) | 94 (44) | 14 (54) | 81 (43) | |
| AT, $n$ (%) | 81 (38) | 9 (35) | 72 (38) | |
| TT, $n$ (%) | 38 (18) | 3 (12) | 35 (19) | |
| Allele A, $n$ (%) | 271 (63) | 37 (71) | 234 (62) | 0.488 |
| Allele T, $n$ (%) | 157 (37) | 15 (29) | 142 (38) | |

[a] CYP2C8 c.805A > T genotypes: AA = rapid metabolizer; AT = intermediate metabolizer; TT = poor metabolizer.

participants; genotyping was not successful in the remainder (72 participants) and these patients were thus excluded from the analyses. Table 1 shows that distribution of alleles and genotypes in this study. The frequency of *CYP2C8*2* genotypes was 44% for AA (rapid metabolizer), 38% for AT (intermediate metabolizer), and 18% for TT (poor metabolizer). The *CYP2C8*2* genotype was in Hardy–Weinberg equilibrium ($p = 0.488$). The *CYP2C8*2T* allele was a minor allele (37%) in this Congolese population.

## Discussion

There are many data on cytochrome P450 gene (*CYP*) polymorphisms in many geographical regions because of the crucial role they play in drug metabolism (Lynch and Price, 2007); however, there are none from the Republic of Congo. Therefore, this study investigated the distribution of the *CYP2C8*2 c.805A > T* (*CYP2C8*2*; rs11572103) allele in Brazzaville, Republic of Congo.

Thirty-seven percent of participants in this study carried the *CYP2C8*2T* allele. This finding is higher than estimates reported previously (Backman et al., 2016). The *CYP2C8*2T* allele occurs mostly in people with a Sub-Saharan Africa ancestry (19%) and it is less frequent (≤1%) in individuals of European, Asian, or American origin (Backman et al., 2016). Furthermore, extensive allele frequency variation has been observed between and within African countries. For example, the reported prevalence of *CYP2C8*2* allele was 17% in Ghana (Rower et al., 2005) and ranged from 9% to 18% in Botswana (Motshoge et al., 2016) and from 10% to 24% in Burkina Faso (Paganotti et al., 2011). The mutant allele *CYP2C8*2T* was found in 11% in Uganda, 22% in Senegal, and 26% in Madagascar (Paganotti et al., 2012). This heterogeneity in allele frequency also occurs within countries and is primarily influenced by the genetic background of the human populations and ethnic groups (Mehlotra et al., 2006; Seripa et al., 2010; Dai et al., 2001; Guan et al., 2006; Daily and Aquilante, 2009; Hiratsuka et al., 2002). In Botswana, for instance, the frequency of the *CYP2C8*2* allele in the San was two-fold higher than that among the Bantu-related ethnic groups (Motshoge et al., 2016). Similarly, the *CYP2C8*2* allele was more frequent in the Mossi-Rimaibe ethnic groups (>2-fold) than in the Fulani ethnic group from Burkina Faso (Paganotti et al., 2011). Although the present study results may have been influenced by ethnicity, a stratified data analysis was not conducted due to the lack of information on the ethnic diversity of the study population. Nonetheless, most of the ethnic groups in the Republic of Congo are predominantly Bantu (Worldatlas, 2018). This may explain the high allele frequency in the present study.

The high frequency of the mutant allele and null homozygous genotype in this study draws attention to the pharmacological implication of *CYP2C8*2* on AQ-based ACT malaria treatment in the Republic of Congo, namely artesunate + amodiaquine (ASAQ). ASAQ was the first-line drug for uncomplicated *P. falciparum* malaria in the Republic of Congo from 2006 to 2014 (NMCP, 2016). In 2014, however, the national malaria control programme revised the malaria treatment guidelines, making ASAQ the second-line drug for uncomplicated malaria (MHP, 2014). This decision was informed by the occurrence of high numbers of ASAQ-induced adverse events in comparison to artemether–lumefantrine (AL) (MHP, 2014). Consequently, AL was adopted as the first-line drug treatment for uncomplicated malaria in the Republic of Congo. The study findings suggest that defective AQ metabolism by CYP2C8*2 is a likely explanation for the high frequency of ASAQ-induced adverse events in the Congolese population.

In vitro experiments have shown that the *CYP2C8*2* mutation reduces AQ metabolism and clearance (Parikh et al., 2007). Therefore, individuals carrying this allele have impaired AQ (which is a prodrug) enzymatic conversion to the active antimalarial compound, desethylamodiaquine (DEAQ) (Zhou et al., 2009). The alteration in AQ pharmacokinetics prolongs drug exposure and also increases the risk of drug-related adverse events such as agranulocytosis and hepatic toxicity (Paganotti et al., 2012; Marwa et al., 2014; Cavaco et al., 2005; Kerb et al., 2009; Maiteki-Sebuguzi et al., 2008). A study from Burkina Faso revealed that *CYP2C8*2A > T* heterozygotes and mutant homozygotes had significantly higher AQ-related adverse events than homozygote (wild-type) Burkinabes (Parikh et al., 2007). The pharmacological implication of being heterozygous or homozygous for CYP2C8*2 is not fully understood (Kerb et al., 2009). Data available thus far indicate that individuals carrying the heterozygous genotype exhibit an intermediate AQ metabolic phenotype (Bains, 2013). Thirty-eight percent of participants in this study were thus AQ intermediate metabolizers (heterozygous), whereas 18% were poor metabolizers (null homozygous). It is of note that the *CYP2C8*2* allele also adversely affects the metabolism of other drugs such as chloroquine, paclitaxel (anti-cancer drug), anti-diabetic drugs (e.g., troglitazone), opioids (e.g., morphine), and glucuronide drugs (Zhou et al., 2009).

Another potential consequence of the *CYP2C8*2* mutation is the emergence of AQ resistance. Slow CYP2C8*2 drug metabolism provides a fertile ground for the selection of *P. falciparum*-resistant strains, particularly because of prolonged parasite exposure to subtherapeutic levels and the long DEAQ half-life (7–12 days) (Hombhanje et al., 2005; Hietala et al., 2007; Petersen et al., 2011). This may be compounded by exposure of new *P. falciparum* infection to residual subtherapeutic drug concentrations after the treatment of a previous infection, especially in malaria-endemic settings. A study conducted in Burkina Faso demonstrated that *P. falciparum* chloroquine-resistant alleles, *Pfcrt 76T* and *Pfmdr1 86Y*, were associated with the *CYP2C8*2* allele (Paganotti et al., 2011). Other studies from East Africa have reported incongruent findings possibly due to high chloroquine-resistant allele frequency (Paganotti et al., 2014; Cavaco et al., 2013). In view of this, Paganotti et al. postulated that drug-resistant parasites may be selected based on *CYP2C8*2* at lower drug resistance levels (Paganotti et al., 2014). Since chloroquine metabolism is dependent on more than one CYP, it is difficult to investigate the role of *CYP2C8*2* in the emergence chloroquine-resistant parasites. However, AQ metabolism by CYP2C8 only raises the probability

of *CYP2C8*2* involvement in the emergence of AQ-resistant parasites.

In conclusion, this study found a high *CYP2C8*2* allele prevalence in Brazzaville, Republic of Congo. It also highlights the risk of AQ-induced adverse events which may compromise drug compliance. Information from this study will be used to promote personalized medicine and inform dose optimization as well as AQ-based ACT pharmacovigilance. More studies are needed to determine the distribution of the *CYP2C8*2* allele in other parts of the Republic of Congo, and to investigate the role of this allele in the pharmacokinetics of amodiaquine and treatment outcomes.

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## Conflict of interest

The authors have no conflict of interest to declare.

## Author contributions

FN supervised the overall study. SMP participated in the study design, performed the experiments, and wrote the manuscript. FKK supervised the molecular genetics study. CV and TPV analysed the data. DN and TPV reviewed and edited the manuscript. All authors participated in writing and approved the final manuscript.

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