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Cytochrome P450 2B6 6 distribution among Burkinabè population

Raoul KARFO[1,2], Fabienne Marie SOUDRE[1], Alice KIBA[1], Arnaud KOURAOGO[4], Elie KABRE[1,3] and Jean SAKANDE[1,3]

[1] Training and Research Unit in Health Sciences, University Joseph Ki-Zerbo, Ouagadougou, Burkina Faso.
[2] Biology Laboratory Clinic, Camp General Aboubacar Sangoule Lamizana Medical Center, Ouagadougou, Burkina Faso.
[3] Laboratory of Biochemistry, University Hospital Yalgado Ouedraogo, Ouagadougou, Burkina Faso.
[4] Institute of Health sciences, University Nazi Boni, Bobo Dioulasso, Burkina Faso.
[*]Corresponding author; E-mail: rkarf006@yahoo.fr.

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ABSTRACT

Mutations affect the genes that encode certain cytochromes P450 (CYP450) isoenzymes that are responsible for metabolism of drugs. The aim of this study was to determine the frequency of CYP2B6 (516G>T) SNP in the Burkinabè population. Genomic DNA extraction from blood was performed by GenJET® Genomic DNA according to the manufacturer's instructions. Genotyping for the cytochrome P450 variant alleles was performed using predesigned primers. The amplification was carried out by PCR while the differentiation between the alleles was carried out after digestion with restriction enzymes by electrophoresis. The CYP2B6 c.516G>T was successfully analyzed in 92,38% (291/315) of the study participants. Among the investigated individuals, the distribution of the major allele CYP2B6*G was 56% and the minor CYP2B6*T allele was 44%. The results obtained showed a prevalence of 56% for the G allele and 44% for the T allele. This was distributed as 31% for the GG genotype (reduced dosage required), 51% for the GT genotype (normal dosage range), and 18% for the TT genotype (high drug toxicity). The presence of the rapid and poor metabolizer phenotypes in the studied sample shows the need for additional studies to better assess their impact on Burkinabe people.

Keywords: CYP 450, CYP 450 2B6, HIV, Malaria, Burkina Faso.

INTRODUCTION

The function of cytochrome P450 (CYP) enzymes, one of the major catalysts in drug metabolism, is significantly influenced by genetic polymorphisms leading to substantial inter-individual variability in drug response and/ or adverse reactions activity (Adzu et al., 2014; Eziah et al., 2016; Peko et al., 2019; Oseni et al., 2019; Hashemi-Soteh et al. , 2021; Mango et al., 2022). Although human CYP2B6 constitutes only 1–4% of hepatic CYP protein content, it is responsible for the metabolism of some clinically important drugs, including the antidepressant bupropion, the antiretroviral efavirenz, the anticancer cyclophosphamide, the analgesic ketamine and methadone1–4. N-Demethylation of the anticonvulsant S-mephenytoin to nirvanol and hydroxylation of bupropion as well as the oxidative hydroxylation of efavirenz are used as probe substrates and selective reactions for in vitro characterization of CYP2B6 activity (Hashemi-Soteh et al., 2021; Mango et al., 2022). The frequency of the CYP2B6 Single

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Nucleotide Polymorphism (SNPs) varies also across different ethnicities (Arnaldo et al., 2013; Zhou et al., 2017). The CYP2B6*6 allele, characterized by two amino acid changes, Gln172His and Lys262Arg, occurs at a considerably greater frequency in people of African ancestry compared to Asians and Caucasians (klein et al., 2005). In a review of several African genomic studies, the reported frequency of the CYP2B6*6 allele ranged from 17% to as high as 60% in various African population (Alessandrini et al., 2013). In Nigeria, the CYP2B6*6 allelic frequency of 38%, 42%, and 42% was reported among Ibos, Hausas, and Yorubas (Matimba, 2008). The 516G>T SNP causes abnormal splicing, decreased expression and activity of the CYP2B6*6 (Hofmann et al., 2008). The CYP2B6 516G>T polymorphism was shown to modulate the TB therapeutic response (Fernandes et al., 2015). There is a scarcity of data on the distribution of CYP2B6 516G>T especially in West African population. In this context, this study aimed at determining the CYP2B6 516G>T of allele frequencies and genotypes in Burkinabe volunteers.

MATERIALS AND METHODS

Materials

A total of 315 participants were enrolled in this study. 300 men and 5 women. The recruited volunteers underwent an HIV screening test. The study was approved by the ethics committee for health research, deliberation N° 2019-3-037, which is under the dual supervision of the ministry of higher education, scientific research and innovation and the ministry of health. Authorization for data collection was obtained from the Ministry of Defense and Veterans Affairs. All patients in the study gave their free and informed consent to participate in the study. Confidentiality of data was maintained throughout the study.

Methods

HIV testing was performed according to a standard procedure recommended by national HIV testing guidelines that used two rapid diagnostic tests (RDTs) in a sequential algorithm. The samples had been tested using a first TDR (Determine Alere ® HIV1/2). The samples which proved to be reactive with this first RDT were evaluated by a second immuno-chromatographic test (SD Bioline® HIV1/2 3.0) which made it possible to specify the type of HIV (HIV1, HIV2, HIV1 and 2).

CYP2B6*6 genotyping

Venous blood (5-7 ml) was collected into sterile EDTA containing tubes from participants from the biomedical laboratory of the Medical Center of Camp General Aboubacar Sangoule Lamizana (CMCGASL). The collected blood was stored at -40°C until genomic DNA extraction was done. Genomic DNA extraction was performed with Gen JET Genomic DNA Purification (Thermo Scientific® Lithuania Kit). The DNA concentration extracted by this technique was determined with spectrophotometer (NanoDrop®). We made a working solution of DNA preferably in a volume of 50 µL with a concentration of 50 ng/µL. We made primer working solutions of 10 µM from stock solutions of 100 µM (in a total volume of 100 µL) for the following primers: CYP2B6-4F: 5'-GGTCTGCCCATCTATAAAC-3' and CYP2B6-4R: 5'-CTGATTCTTCACATGTCTGCG-3'. We added 2 µL of 50 ng/µL DNA to each PCR tube separately and change pipette tips at each stage. We prepared the PCR reaction MIX in a total volume of 25 µL as follows Table 1. Mix properly by tapping or pulse mix. Add 23 µL of the PCR reaction mix to each PCR tube which already contains 2 µL of DNA. PCR amplify using the "T100 Thermal Cycler from Bio-Rad" with the following PCR cycling conditions: denaturation at 94°C for 3min, denaturation at 94°C for 30s, annealing at 54°C for 30s, extension at 72°C for 40s, final extension at 72°C for 10min. Repeat steps denaturation at 94°C for 30s annealing at 54°C for 30s, extension at 72°C for 40 s for 35 cycles (Table1). After completion of PCR, we proceeded with agarose gel electrophoresis by loading 5 µL of PCR product on a 1.5% agarose gel stained with GRGreen (100 mins, 100V, 5 µL GeneRuler™ 100bp DNA Ladder Plus). Expected PCR product: 526bp DNA

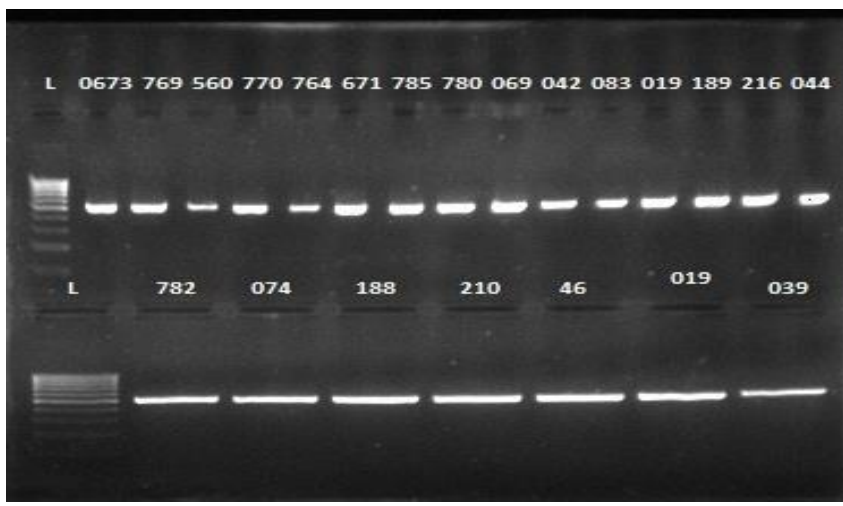
fragment. Check for quality of the PCR product by visualization on a UV-doc system (Figure 1). If successful proceed to add 10 µL of each PCR product to a separate eppendorf tube marked with the identity of each sample for digestion below (RFLP). This technique is also used for the identification of bacteria (Azokpota et al., 2007). Prepare the digestion Mix in a total volume of 30 µL as follows Tab 3. Add 20 µL of digestion mix to each tube with individual PCR products. Incubate for overnight at 60°C and inactivate the enzyme for 5min at 80°C. Proceed with agarose gel electrophoresis by loading 30 µL of the digestion product on a 2.5% agarose gel stained with GRGreen (60-180 mins, 80V, 5 ul GeneRuler^{TM} 100bp DNA Ladder Plus) (Table 2). Expected banding pattern: Wildtype- 17bp, 241bp, 268bp DNA fragments. Mutant- 17bp, 509bp DNA fragments. Heterozygote- 17bp, 241bp, 268bp, 509bp DNA fragments (Figure 2).

Statistical analysis

All study data were entered in Excel and analyzed using Stata version 13.0. The Student's T-test was used to compare the means between the different groups. Statistical tests were considered significant when p was less than 0.05.

Table 1: The PCR reaction MIX in a total volume of 25 µL.

Reagents	Volume used in a single reaction (in µL)
5X GoTaq poly reaction buffer	5
5mM dNTPs	0.625
10µM CYP2B6-4F	1
10µM CYP2B6-4R	1
5U/µL GoTaq Flexi DNA pol	0.2
Sterile distilled water (sdH_2O)	17.175 (Make up to 25 µL)
Total reaction volume	25

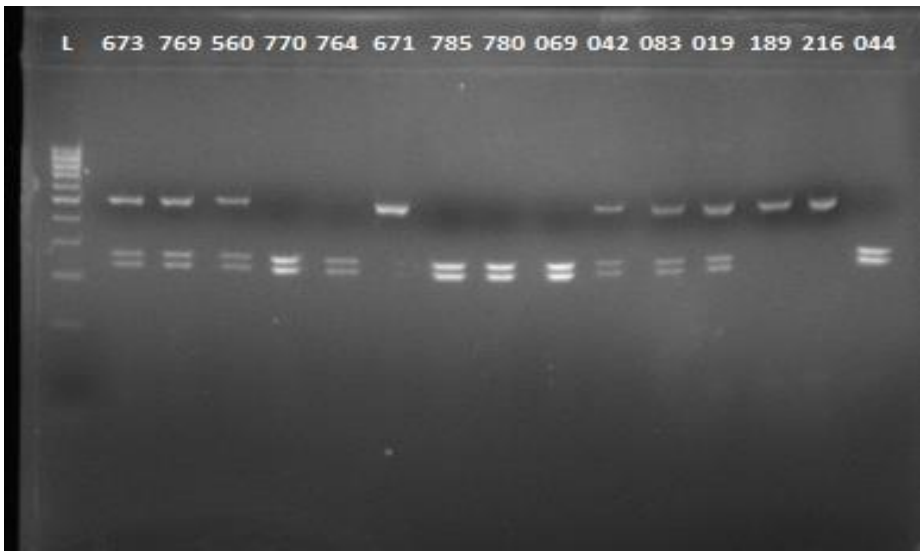


►**526pb**
►**526pb**

Figure 1: PCR product on a 1.5% agarose gel stained with GRGreen (100min, 100V, 5µL GeneRulerTM 100bp DNA Ladder Plus) for quality control by visualization on a UV-doc system.

Table 2: The digestion Mix in a total volume of 30µL.

Reagents	Volume used in a single reaction (µL)
PCR product	10
10X Buffer B	2
10U/µL BSeNI (or BsrI)	0.3
Sterile distilled water (sdH_2O)	17.7(Make up to 30 µL)
Total reaction volume	30



►509pb
►268pb
►241pb

Figure 2: Genotyping of CYP2B6 variants by PCR-RFLP analysed with 2,5% agarose gel electrophoresis: (a) show the fragments after digestion with BSeNI (or BsrI) of the PCR product for identification of CYP2B6 G 516 T variant.

RESULTS

This study shows that Burkinabé volunteers carry the CYP 2B6 genotype which is involved in the metabolism of commonly prescribed drugs. The CYP2B6 516G>T was successfully genotyped among 291/315 (92,38%) of the enrolled study subjects (Figure 1 and 2). After checking the quality of our DNA samples, we remark that the quality was very low for some of this. In Genetics, the quality of the starting material which is either DNA or RNA is very important. The frequency of the observed SNP variant CYP2B6 516G>T in our study revealed a higher proportion of G variant in the Burkinabe population compared to that of the T allele (CYP2B6 516G = 0.56 and CYP2B6 516T = 0.44) (Table 3). The Burkinabe individuals had a high frequency of genotypes that contribute to intermediate and rapid response to treatment (CYP2B6 516GT: 51% and CYP2B6 516 GG: 31% respectively). The genotype distribution was also similar among individuals, when stratified for different infections. The CYP2B6 516G allele remains a major allele in the Burkinabe population.

Table 3: CYP2B6 516G>T allele and genotype distribution among Burkinabe volunteers.

CYP2B6 c.516G>T	All individuals (n = 291)	HIV(n = 5)
Male/female ratio	286/5	5/0
GG/rapid (%)	89 (31)	1 (20)
GT/intermediate (%)	150 (51)	4 (80)
TT/poor (%)	52 (18)	0
Allele G (%)	328 (56)	6 (60)
Allele T (%)	254 (44)	4 (40)

DISCUSSION

The frequencies of different CYP2B6 polymorphisms have been studied in different patient populations, showing a highly variable distribution specifically for the 516G>T polymorphism. In Nigeria the genotype frequencies shows that the GG, GT, and TT genotypes of CYP2B6: 516G >T variant alleles were present in 35.9%, 46.6%, and 17.6% of participants, respectively (Isaac et al., 2020). In Europe, the distribution is G: 76.8%, T: 23.2%, in the United States of America it is G: 63.8%, T: 36.2% and in Africa, which has the highest prevalence of the polymorphism, it is G: 65%, T: 35% (Scibona et al., 2015). The prevalence of TT genotype of Burkinabe population was 18%, which is different of its frequency in congolese population (28%) (Peko et al., 2019). The South African study revealed the prevalence of the allelic variant CYP2B6 TT (poor metabolisers) to be 23% amongst their study population (Gounden et al., 2010). The prevalence of TT genotype of Argentinians population was 10.8%, which is double than its frequency in European populations (4.2%) and similar to the frequency found in Native Americans and persons of African descent (13.3 and 13%, respectively) (Scibona et al., 2015). The global distribution for CYP2B6∗6 in irian study was reported 73% and 26% for the G and T alleles, respectively (Hashemi-Soteh et al., 2021). The frequency of CYP2B6 minor allele (T) is estimated about 21.5% in East Asian, 38.1% in South Asian and 33.8% in Pakistan population (Hashemi-Soteh et al., 2021). A previous study made up of adult HIV-positive patients on antiretroviral treatment, initiated and followed up at the CMCGACL dermatology department, included 94 patients with an average age of 44 years with extremes of 16 and 81 years. HIV type 1 accounted for the majority of infection with 80.9% of cases. The therapeutic regimen combining 2 Nucleotide Reverse Transcriptase Inhibitors (NRTIs) and 1 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) was the most used (74.5%) (Karfo et al., 2018). Efavirenz is NNRTI which is one of the molecules used for the management of certain patients with HIV type 1. The HIV prevalence rate in Burkina is 0.7%. In the current study due to worldwide selection of the study population we recorded only 5 (1.71%) HIV+ patients. The prevalence of TT genotype of this population was 0%. CYP2B6 516G>T is considered to be an independent predictor of EFV plasma concentrations in HIV-infected patients. EFV plasma concentrations 4 mg/l are associated with an increased risk for central nervous system (CNS) impairment and liver toxicity (Kwara et al., 2009; Zakeri et al., 2014; Peko et al., 2019). CYP2B6 genotyping may be useful for predicting efavirenz plasma concentrations (Kwara et al., 2009). Previous reports show that CNS side effects occur within the first week of EFV treatment following EFV treatment with the recommended dose (600 mg/per day) and may persist for a long period of time (Dhoro et al., 2015; Vo et al., 2016). Genotyping of CYP2B6 516G>T serves as a valuable tool for identifying HIV positive individuals who are most likely to develop CNS side effects following EFV treatment (Swart et al., 2015). However, some studies have not been able to establish this correlation.

A study in Botswana found that CYP2B6 516 variation was not associated with NNRTI toxicity. No other factors were associated with toxicity when considering age, baseline body mass index, baseline CD4, baseline HIV viral load and adherence (Maseng et al., 2022). Artemisinin-based combination therapies are recommended by the World Health Organization as the first- and second-line treatment for uncomplicated Plasmodium falciparum malaria and chloroquine-resistant *Plasmodium vivax* malaria. Artemether is rapidly and extensively demethylated (both in vitro and in humans) to the biologically active main metabolite dihydroartemisinin, predominantly through CYP3A4/5. However, a previous study has shown that CYP2B6 has a more important role in the demethylation of artemether than CYP3A4/5 (Abdullahi et al., 2020). Malaria is a public health concern in Burkina Faso. Some drug like artesunate–amodiaquine and artemether–lumefantrine were used for the treatment of acute uncomplicated malaria. The human CYP2B6 gene is also involved in the metabolism of artemisinin and its derivatives (Simonsson et al., 2003), The CYP2B6*6 allele is associated with increased plasma concentrations of artemisinin and artemether (Kerb et al., 2009). The important frequency of the CYP2B6*6 homozygous mutant allele (18%) indicates that a large proportion of individuals in our study might be poor metabolizers of artesunate, resulting in an increased risk to toxicity of the above anti-malarial drugs. Individuals with a CYP2B6 reduced metabolic phenotype may have an increased risk of malaria treatment failure when treated with artemisinin derivatives (Tawe et al., 2018).

Conclusion

The results obtained provide new information on the prevalence of polymorphisms in key enzymes involved in the metabolism of several drugs used by the Burkinabe population. The intermediate metabolizer genotype (CYP2B6 516 G/T) was more common in our studied population and comparable to other African populations. The presence of the rapid and poor metabolizer phenotypes in our studied sample shows the need for additional studies to better assess their impact on Burkinabe who have HIV and who are on efavirenz treatment and also on certain antimalarials for optimizing drugs effectiveness, while minimizing toxicity.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Conceptualization, Data processing, formal analysis and software: RK, EK. Survey, methodology and project administration: RK and EK. Supervision, validation and visualization: EK and JS. Writing - original version: RK. Drafting - revision and editing: RK, AK, AK, EK and JS.

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