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The *CYP2B6* G516T polymorphism influences CD4$^+$ T-cell counts in HIV-positive patients receiving antiretroviral therapy in an ethnically diverse region of the Amazon

Maria Alice Freitas Queiroz [a], Rogério Valois Laurentino [a], Ednelza da Silva Graça Amoras [a], Mauro Sérgio Moura de Araújo [b], Samara Tatielle Monteiro Gomes [a], Sandra Souza Lima [a], Antonio Carlos Rosário Vallinoto [a], Marluísa de Oliveira Guimarães Ishak [a], Ricardo Ishak [a], Luiz Fernando Almeida Machado [a,*]

[a] *Laboratório de Virologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Rua Augusto Correa s/no., Guamá, CEP 66.075-110, Belém, Pará, Brazil*
[b] *Hospital Universitário João de Barros Barreto, Universidade Federal do Pará, Belém, Pará, Brazil*

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SUMMARY

Objectives: Cytochrome P450 (CYP) enzyme polymorphisms seem to significantly influence the variability of the responses to certain antiretroviral drugs and their toxicity levels. The objective of this study was to evaluate the influence of the *CYP2B6* G516T polymorphism on hepatic, renal, immunological, and viral marker changes in HIV-1-positive patients receiving treatment in an ethnically diverse region of the Amazon.

Methods: *CYP2B6* G516T genotyping was performed by real-time PCR (RT-PCR) in samples from 185 patients. Urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), CD4$^+$/CD8$^+$ T-cell counts, and HIV-1 plasma viral load were measured.

Results: The polymorphic *CYP2B6* G516T allele frequency was 0.36, which is different from the frequencies in other ethnic groups. The polymorphic genotype was associated with changes in the urea and ALT levels, although the median values were within the normal range. The TT genotype was also associated with significantly lower CD4$^+$ T-cell counts in patients using efavirenz.

Conclusions: The *CYP2B6* G516T polymorphism seems to affect the response to efavirenz treatment by reducing CD4$^+$ T-cell counts in patients with a high degree of miscegenation who use this antiretroviral agent.

1. Introduction

Antiretroviral therapy (ART) in HIV-1-positive patients promotes a reduction in the viral genomic RNA to levels below the detection limit of standard assays,[1] resulting in significant improvements in the clinical course of the infection and consequently in the patient's quality of life.[2] However, the administration of standard doses of most antiretroviral agents may result in drug-associated toxicity symptoms depending on the plasma concentrations found in different individuals.[3]

Genetic differences among HIV-1-infected patients seem to partially explain variations in the responses to antiretroviral drugs and their toxicity.[4] This variability is caused in part by polymorphisms in the genes involved in the enzymatic metabolism of the drugs.[5] As such, some cytochrome P450 (CYP) polymorphisms are able to directly interfere in the bioavailability of antiretroviral drugs, resulting in an increased toxicity and decreased efficacy.[6,7]

The standard antiretroviral treatment schedule consists of the administration of two nucleoside reverse transcriptase inhibitors (NRTIs) combined with a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI).[8] The most frequently used NNRTIs are efavirenz (EFV), which is used in first-line schedules, and nevirapine (NVP). EFV is widely used in the initial antiretroviral treatment of HIV-1 infection; however, the administration of the standard dose of this drug is associated with a variable response and adverse effects in the central nervous system (CNS) in a substantial number of patients.[8–10]

* Corresponding author. Tel.: +55 91 99368-2845.
E-mail address: lfam@ufpa.br (L.F.A. Machado).

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The main CYP enzyme involved in EFV metabolism is CYP2B6, which is responsible for the oxidative hydroxylation and clearance of most of the drug (approximately 90%). Variations in the gene encoding this enzyme seem to affect EFV activity and its circulating concentration.[11–14]

The *CYP2B6* G516T polymorphism is the most significant allelic variant of the *CYP2B6* gene, in which the TT genotype is related with a slower metabolism of EFV and a natural increase in the plasma concentration of the drug.[15] This polymorphism occurs due to a switch from nucleotide G to T at position 516 of exon 4 of the *CYP2B6* gene, which causes the replacement of the amino acid glycine (Gly) with histidine (His) at position 172.[11] This variation is the most common among the other *CYP2B6* alleles, and its frequency varies between different ethnic groups.[13,16,17] The presence of the *CYP2B6* G516T polymorphism promotes a reduction of up to 75% in EFV clearance and has been associated with increased EFV and NVP plasma levels. High plasma concentrations of these drugs may lead to adverse reactions, such as CNS and liver toxicity.[14,15,18–20] Although the *CYP2B6* G516T polymorphism seems to influence the metabolism and the plasma concentration of EFV, it does not seem to affect the $CD4^+$ T-cell counts or plasma HIV-1 viral load among persons with different genotypes of the polymorphism.[21–23]

The Amazon region of Brazil is frequently characterized as an area that shows a variable and sometimes high degree of miscegenation among the Europeans, Africans, and native Indians,[24] a situation that clearly favors the variety of gene expression and the resistance or susceptibility to infectious agents and their associated diseases.[25]

The presence of the *CYP2B6* G516T polymorphism in HIV-1-infected patients may promote the development of adverse reactions associated with antiretroviral agents. These reactions may compromise the treatment protocol in these individuals and cause an increase in the viral load and the selection of viruses with resistance mutations, thus limiting ART options. The present study assessed the frequency of this polymorphism in HIV-1 carriers from a miscegenated population in the Amazon region of Brazil and determined whether the presence of the polymorphism was related to kidney and liver toxicity and changes in $CD4^+$ and $CD8^+$ T-cell counts and the plasma HIV-1 viral load.

2. Materials and methods

2.1. Study design and population examined

An observational vertical study was conducted. Peripheral blood samples (5.0 ml) were collected in two tubes containing ethylenediaminetetraacetic acid (EDTA) using a Vacutainer system from 185 HIV-1-infected persons receiving ART attending the Specialist Infectious and Parasitic Diseases Reference Unit (Unidade de Referência Especializada em Doenças Infecciosas e Parasitárias, URE-DIPE) located in the city of Belém, state of Pará, Brazil, from October 2010 to February 2011. Blood samples were transported to the Virus Laboratory within 24 h in order to determine $CD4^+$/$CD8^+$ T lymphocyte counts and the plasma HIV-1 viral load, following biosafety procedures recommended by the Brazilian Ministry of Health. Samples were also centrifuged at 5000 rpm for 10 min to separate plasma and cells and stored at −70 °C.

HIV-1 patients aged 18 years or older and receiving treatment with antiretroviral agents of the NRTI pharmacological class combined with a PI or NNRTI were included in the study. Patients with a diagnosis of hepatitis or with chronic kidney conditions were excluded. The study was approved by the research ethics committee and all of the participants signed an informed consent form. All of the technical procedures were performed in NB2 or NB3 facilities within the Virus Laboratory, following strict procedures for biosafety and viral containment.

2.2. CYP2B6 *G516T (rs3745274) genotyping*

DNA was extracted from peripheral blood mononuclear cells (PBMC) using the Puregene kit (Puregene, Gentra Systems, Inc., USA) according to the manufacturer's protocol; this included the steps of cell lysis, precipitation of proteins, and precipitation and hydration of DNA. The nucleic acid was eluted and stored at −20 °C until use.

Genotyping was performed on extracted DNA using a real-time PCR (RT-PCR) and premade TaqMan SNP Genotyping Assay system (Applied Biosystems, Foster City, CA, USA) with identification C_7817765_60 according to the manufacturer's instructions. Reactions were performed in a Step One PLUS Real-Time PCR System (Applied Biosystems), using 1 × TaqMan Universal PCR Master Mix (2 ×), 1 × TaqMan Assay (20 ×), and 20 ng DNA in a final volume of 20 μl. A total of 40 cycles were used (10 min at 95 °C, 15 s at 95 °C, and 1 min at 60 °C).

2.3. Biochemical assays

Quantification of urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) was performed with Architect 8000 equipment (Abbott Diagnostics, Illinois, USA) and the enzymatic/automated method using DiaSys kits (Diagnostic Systems, Holzheim, Germany), according to the manufacturer's instructions.

2.4. $CD4^+$ and $CD8^+$ T-cell counts

T-cells were counted by flow cytometry (FacsCalibur, Becton & Dickinson, USA) using BD Trucount tubes and BD Multitest kits (Becton Dickinson, USA); the procedures recommended by the Brazilian National Network for $CD4^+$ and $CD8^+$ T-cell Counts of the Ministry of Health were followed.

2.5. Plasma HIV-1 viral load quantification

The plasma viral load was determined by branched DNA (bDNA) method using a Versant HIV-1 RNA 3.0 Assay bDNA kit (Bayer Corporation, MA, USA) and a System 340 bDNA Analyzer scanner (Siemens, Deerfield, MI, USA), according to the procedures recommended by the Brazilian National Network for HIV-1 Plasma Viral Load Quantification of the Ministry of Health.

2.6. Statistical analysis

Hardy–Weinberg equilibrium was calculated to evaluate the genotype frequency distribution. The Shapiro–Wilk test was performed to look for normality. The association of the *CYP2B6* G516T genotype with variations in serum levels of laboratory variables was determined using the non-parametric Kruskal–Wallis test and Mann–Whitney test. The *G* test was used to evaluate viral loads among the different individual genotypes. All calculations were performed using BioEstat 5.3 software,[26] and the associations were considered significant at $p < 0.05$.

3. Results

The individuals included were mostly male (56.7%). The mean age of the subjects was 41.7 years. All of them were on ART with two NRTIs; 58.4% were taking an NNRTI (55.2% EFV and 3.2% NVP) and 41.6% were taking a PI as the third drug in the standard treatment schedule. The treatment duration ranged from 4 months to 18 years.

Creatinine levels ranged from 0.2 to 1.8 mg/dl (median 1 mg/dl) and urea from 0.9 to 79 mg/dl (median 30 mg/dl). AST values