Observational Study

Medicine

OPEN

The role of *CYP2B6* 516G>T polymorphism on efavirenz/nevirapine toxicity. Implications on treatment outcomes

Lessons from Botswana

Monkgomotsi J. Maseng, BSc[a,b], Leabaneng Tawe, PhD[a,b,c], Prisca K. Thami, PhD[b,d], Sikhulile Moyo, PhD[b,e], Ishmael Kasvosve, PhD[a], Vladimir Novitsky, PhD[b,e], Max Essex, PhD[b,e], Gianluca Russo, PhD[f], Simani Gaseitsiwe, PhD[b,e], Giacomo M. Paganotti, PhD[c,g,h,*]

Abstract

The two non-nucleoside reverse transcriptase inhibitors (NNRTIs), efavirenz (EFV) and nevirapine (NVP), are currently the core antiretroviral drugs for treatment of HIV in sub-Saharan Africa including Botswana. The drugs are metabolized by Cytochrome P450 2B6 (*CYP2B6*) liver enzyme. The *CYP2B6* gene that encodes for metabolism of these drugs is known to be highly polymorphic. One of the polymorphism in the *CYP2B6* gene, 516G>T, particularly the 516T allele, is known to confer poor metabolism of EFV and NVP. This may lead to high levels of plasma drug concentrations and development of treatment toxicities, like central nervous system toxicities, and cutaneous and hepatic toxicities, for EFV and NVP, respectively. The *CYP2B6* 516G allele on the other hand is associated with an extensive metabolism of the two NNRTIs drugs. We sought to establish association between possible developments of NNRTIs toxicities with *CYP2B6* 516G>T variation in Botswana.

A total of 316 peripheral blood mononuclear cells samples were used in a retrospective view. All the samples were from participants on EFV/NVP-containing regimen with known toxicity output. TaqMan Real-Time PCR approach was applied for assessing *CYP2B6* 516 allele variation in cases with treatment toxicity and those without. Analysis was performed by chi-square statistics and logistic regression analysis.

The rate of poor metabolizers among participants with toxicity and those without toxicity was 18.4% and 15.1%, respectively. The *CYP2B6* 516 genotype distribution comparisons between the participants with toxicity and those without were not statistically different (chi-square = .326; $P = .568$).

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. The results were discussed in the context of all the studies done in Botswana to date.

Editor: Jorddy Neves Cruz.

This work was supported through the Sub-Saharan African Network for TB/HIV Research Excellence (SANTHE), a DELTAS Africa Initiative [grant # DEL-15-006]. The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust [grant # 107752/Z/15/Z] and the UK government. The views expressed in this publication are those of the author(s) and not necessarily those of AAS, NEPAD Agency, Wellcome Trust or the UK government. The work was also supported by the Penn Center for AIDS Research [grant # P30 AI045008].

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] School of Allied Health Professions, Faculty of Health Sciences, University of Botswana, UB Campus, Block 246, Gaborone, Botswana, [b] Botswana-Harvard AIDS Institute Partnership, Plot 1836 North Ring Road, Gaborone, Botswana, [c] Botswana-University of Pennsylvania Partnership, UB Campus, Block 244G, Gaborone, Botswana, [d] Division of Human Genetics, Department of Pathology, Faculty of Health Sciences, University of Cape Town, Anzio Road Observatory, Cape Town, South Africa, [e] Department of Immunology and Infectious Diseases, Harvard School of Public Health, 677 Hungtinton Avenue, Boston, MA, [f] Department of Public Health and Infectious Disease, Faculty of Medicine, Sapienza University of Rome, Piazzale Aldo Moro 5, Rome, Italy, [g] Division of Infectious Diseases, Perelman School of Medicine, University of Pennsylvania, 3120-2740 Hamilton Walk, Philadelphia, PA, [h] Department of Biomedical Sciences, Faculty of Medicine, University of Botswana, UB Campus, Block 246, Gaborone, Botswana.

[*] Correspondence: Giacomo M. Paganotti, Botswana-University of Pennsylvania Partnership, UB Campus, Block 244G, Gaborone, Botswana (e-mail: paganottig@bup.org.bw).

How to cite this article: Maseng MJ, Tawe L, Thami PK, Moyo S, Kasvosve I, Novitsky V, Essex M, Russo G, Gaseitsiwe S, Paganotti GM. The role of CYP2B6 516G>T polymorphism on efavirenz/nevirapine toxicity. Implications on treatment outcomes: lessons from Botswana. Medicine 2022;101:17(e29066).

Received: 26 January 2022 / Received in final form: 24 February 2022 / Accepted: 25 February 2022

http://dx.doi.org/10.1097/MD.0000000000029066

Abbreviations: ART = antiretroviral therapy, BMI = body mass index, CNS = central nervous system, CYP2B6 = Cytochrome P450 2B6, EFV = efavirenz, NNRTI = non-nucleoside reverse transcriptase inhibitor, NVP = nevirapine, SNP = single nucleotide polymorphism.

Keywords: Botswana, *CYP2B6*, efavirenz, HIV, nevirapine, NNRTI toxicity, pharmacogenetics

1. Introduction

Botswana, with an overall HIV population prevalence of 19.9% (range: 18.2–21.0) among adults (15–49 years)[1] is one of the countries with the highest HIV prevalence in the world. The non-nucleoside reverse transcriptase inhibitors (NNRTIs), efavirenz (EFV), and nevirapine (NVP), still form an important part of Botswana's National HIV treatment program, despite the introduction of dolutegravir as first line antiretroviral therapy (ART) regimen since June 2016.[2] NNRTIs are still largely used for HIV treatment in African nations, including Botswana, with millions of people in Southern Africa still under NNRTI-based regimen.[3] ART efficacy largely depends on adequate drug exposure to suppress viral replication and allow immune system to recover. However, occurrence of drug toxicity, sub-optimal patient's compliance, sub-optimal virologic suppression, incomplete immune reconstitution, and/or emergence of drug resistance limit therapeutic outcomes. Among the factors that are capable of influencing EFV and NVP exposure is the variability in the gene that encodes the cytochrome P450 (CYP) 2B6 enzyme, that metabolizes both drugs. Studies have shown how Cytochrome P450 2B6 (*CYP2B6*) polymorphisms influence the rate of EFV/NVP clearance in plasma.[4–6] However, the influence of *CYP2B6* genotype on EFV/NVP exposure, and whether it contributes to prolonged detectable EFV/NVP concentrations, resistance, and toxicities, has not been investigated to a greater extent among Africans.

The *CYP2B6* gene is highly polymorphic, with 3 main single nucleotide polymorphisms (SNPs) (*CYP2B6* 516 G>T, *CYP2B6* 983 T>C, and *CYP3B6* 785 A>G) driving the prediction of individual metabolic status when combined in composite genotypes or haplotypes.[6,7] They have been recognized as the more important gene variations in studies concerning NNRTI pharmacogenetics. However, most of the predictions and associations are due to *CYP2B6* 516 that has been used as tag SNP for the *CYP2B6* gene in many studies.[4–6,8] Concerning NNRTI pharmacogenetics, *CYP2B6* 516G>T is known to confer poor metabolism of EFV, and it is associated with central nervous system (CNS) toxicity.[8–11] In fact, data from literature show that some patients taking EFV-containing ART regimen, especially non-Africans, may experience CNS toxicities (with or without virologic failure),[4,5,12,13] being lower in patients with *CYP2B6* 516GG genotype,[4] and higher among carriers of the *CYP2B6* 516T allele variant.[4,5,8,11] Nevertheless, several studies in African populations found no association of *CYP2B6* 516G>T polymorphism with CNS toxicities.[4,9,14,15] With regard to NVP, there is evidence that *CYP2B6* 516T containing genotypes (especially TT) are the genetic predictors of cutaneous or hepatic NVP toxicity[16,17]; in particular, subjects of African descent with *CYP2B6* 516TT genotype are more at risk of cutaneous toxicity to NVP than individuals of other ethnicities.[17]

To our knowledge, there are few studies to date that have been done in Botswana that looked at NNRTI metabolism (mainly EFV) and/or *CYP2B6* variation and/or treatment outcomes, including toxicity.[7,9,12,13,15,18] The prevalence of *CYP2B6* 516T allele among HIV-infected adults in Botswana has been first described by Gross et al,[18] being 36.6%. Although the high prevalence of slow metabolizers taking EFV-based ART, an unexpected inverse relationship between EFV metabolism and EFV-related adverse effects was observed in Botswana, with lower experience of CNS toxicity among slow EFV metabolizers,[15] similarly to what observed in Africans[14] and Afro-Americans,[4] but differently from Caucasian and Hispanic patients.[8,11] Another study from Botswana assessing the association between *CYP2B6* 516G>T polymorphism and CNS toxicity among HIV-infected individuals starting EFV-based ART regimen, showed that EFV extensive metabolizers (516GG) were reporting more CNS adverse events after 1 month of ART than slow metabolizers (516TT).[9] Thus, some authors suggest that the CNS toxicity of EFV in African population might not be the result of super-therapeutic parent EFV concentrations alone, but rather due to accumulation of 8-OH-EFV, the main metabolite of EFV generated by CYP2B6.[9,19] Furthermore, another study from Botswana showed that EFV-related adverse events may be transient.[19] According to this study, lower baseline lymphocyte T-CD4 count and depressive symptoms at baseline were associated to improved patient's experience of adverse effects over time (from month 1–month 6), whereas alcohol consumption was associated with adverse effects of EFV over time,[19] possibly because of the impairment of the drug's hepatic metabolism related to longitudinal alcohol consumption. A subsequent observational study in Botswana aiming to assess the association between *CYP2B6* polymorphisms and age to loss of care of patients taking EFV-based ART showed that, among poor metabolizers, older age was associated with higher risk of loss of care.[20] Moreover, poor metabolizer patients aged more than 50 years starting EFV-based ART regimen had a 4-fold higher risk of loss of care compared to intermediate metabolizers, but neurocognitive toxicity was not associated to this risk.[20] Finally, in patients from Botswana taking EFV-based regimen *CYP2B6* 516T allele conferred protection against late virologic failure in those with initial 6-month viral suppression,[13] and *CYP2B6* 516G allele was associated with a higher risk of NNRTI resistance mutations.[12]

Therefore, in these studies from Botswana surprisingly toxicity seems to be associated to the extensive metabolizer genotype as compared to poor metabolizer genotypes,[9,13,15] with *CYP2B6* 516 polymorphism being the main driver of metabolic status for NNRTIs.[6] Here we aimed to assess if and how the *CYP2B6* 516 G>T impacts NNRTI toxicity in a cohort of Botswana HIV patients where data on drug toxicity and *CYP2B6* 516 genotypes were available.[12,21] Understanding any possible relationship and/or association between individual genetic make-up and NNRTI toxicity may help to increase therapeutic efficacy and ultimately reduce the burden of drug resistance and deaths.

2. Methods

2.1. Ethics approval and informed consent

The retrospective study was conducted in accordance to the guidelines of the Declaration of Helsinki and was approved by the Ethics committee of Health Research Division Office (HRDC) of the Botswana Ministry of Health and Wellness. The approval was done in accordance with the amendments made to the initial permit of "The host genetics of HIV-1 subtype C infection progression and treatment in Africa/Gwas on determinants of HIV-1 subtype C infection" [Reference No: HPDME 13/18/1 X1 (163)].

Informed consent was obtained from all the study participants involved. In addition, Botswana-Harvard AIDS Institute Partnership, as the data-base owner authorized by HRDC, gave permission to use its data and samples for the current study.

2.2. Sample population and size

A total of 316 peripheral blood mononuclear cells samples were used from the original Tshepo study.[21] The Tshepo study was a 3-year randomized $3 \times 2 \times 2$ factorial design comparing tolerability and efficacy among 3 NRTI combinations (zidovudine + didanosine, zidovudine + lamivudine, and stavudine + lamivudine), 2 NNRTI combinations (EFV versus NVP), and 2 adherence strategies.[21] The study participants were HIV positive, >18 years ART naïve Botswana citizens. Samples were stored at –80°C after collection and processing. Treatment related toxicity was defined as any first incidence (after 5 weeks) of grade 3 or 4 adverse events.[22] In particular, for EFV they were measured: persistent CNS toxicity, convulsions, hepatotoxicity and cutaneous hypersensitivity reactions, when reaching grade 3 (severe) or grade 4 (potentially life threatening). For NVP, toxicity included: hepatotoxicity and cutaneous hypersensitivity reactions, when reaching grade 3 (severe) or grade 4 (potentially life threatening).

2.3. DNA extraction and CYP2B6 genotyping

Genomic DNA was extracted using QIAamp DNA Mini Kits (Qiagen, Hilden, Germany) according to the manufacturer's protocol (Qiagen, Hilden, Germany) from about 200 μL of peripheral blood mononuclear cell's. DNA concentration was quantified using a Nanodrop spectrophotometer (NanoDrop 1000, Thermo Scientific, MA). Real Time-PCR was performed using ABI 7500 RT-PCR machine (Applied Biosys-tems, CA). Predesigned TaqMan Drug Metabolism (DME) genotyping assays were used to genotype c.516G>T. The DME genotyping assays were ordered by part numbers C_7817765_60 (c.516G>T (SNP ID: rs3745274)) from Life Technologies (Pty) Ltd. Applied Biosystems (ABI; Applied Biosystems, CA), 96 microwell plates were filled with reaction mixture of 11.25 μL of diluted DNA extract, 1.25 μL of 20X SNP genotyping assay, and 12.5 μL of TaqMan Universal PCR Master Mix, No AmpErase UNG following the manufacturer's plate preparation instructions. Samples were run in duplicates and each run contained several negative controls (no template) and a reference sample.

2.4. Data analysis

Arlequin software (v3.5.2.2) was used to test for Hardy–Weinberg equilibrium. Chi-square was applied for comparing genotype distribution among cases with toxicity and cases without. Finally, Binary Logistic Regression analysis (run on IBM SPSS statistical package, version 20) was applied to find any association between the dependent variable "toxicity" with the independent variables (baseline $CD4^+$ T-cell count, baseline viral load, age, body mass index [BMI], and *CYP2B6* 516 genotype).

3. Results

3.1. Baseline population demographics

Out of 316 samples, 7 had gender information missing (but were genotyped) and all the other clinical data were available. The study population characteristics at baseline were as follows: mean age 34.4 years (range: 29.5–37.4); mean BMI 21.5 (range: 19.0–23.3); median $CD4^+$ T-cell count 188.8 (interquartile range: 142–231.5); and median baseline viral load of 3.16 $\log_{10}$ copies/mL (interquartile range: 0.71–5.19). Two hundred (200, 64.7%) participants were females and 109 (35.3%) were males. Table 1 summarizes the baseline characteristics of the study population.

3.2. CYP2B6 516 genotype, allele frequencies, and NNRTI toxicity

In a total of 316 patients, 38 (12.0%) developed treatment toxicity while 278 (88.0%) did not. Of the 38 who developed toxicities, 10 (26.3%) were EFV/NVP extensive metabolizers (516GG), 21 (55.3%) were intermediate metabolizers (516GT), and 7 (18.4%) poor metabolizers (516TT). The occurrence of adverse events was higher in individuals with 516GG versus 516TT genotype, but the difference was not statistically significant ($X^2 = .958$, $P = .328$). The rate of poor metabolizers on those with toxicity and those without was 18.4% (n = 7/38) and 15.1% (n = 42/278), respectively. The 516T allele frequency between cases who developed toxicity and those who did not was 46.1% (95% CI: 30.3–61.9) and 39.6% (95% CI: 33.9–45.3), respectively (Table 2). Finally, Binary Logistic Regression

Table 1

Baseline characteristics of the study population.

Characteristics	Total
Participants, n (%)	316 (100%)
Females*, n (%)	200 (64.7%)
Males*, n (%)	109 (35.3%)
Mean age (yrs), n (range)	34.4 (29.5–37.4)
Mean BMI (range)	21.5 (19.0–23.3)
Median CD4 T-cells/μL (IQR)	188.8 (142–231.5)
Median viral load, $\log_{10}$ copies/mL (IQR)	3.16 (0.71–5.19)

BMI = body mass index, IQR = interquartile range.
* Seven participants did not have gender assigned (309 instead were used for gender analysis).

Table 2

Genotype and allelic frequency of *CYP2B6* 516G>T SNP among study participants.

	516G>T			
Characteristics	GG (%)	GT (%)	TT (%)	*f*(T)
NNRTI toxicity (n = 38)	10 (26.3)	21 (55.3)	7 (18.4)	46.1
Non-NNRTI toxicity (n = 278)	100 (36.0)	136 (48.0)	42 (15.1)	39.6
Total (n = 316)	110 (34.8)	157 (49.6)	49 (15.5)	40.3

f(T) = allele frequency of the T allele.
NNRTI = non-nucleoside reverse transcriptase inhibitor, SNP = single nucleotide polymorphism.

Table 3

Binary Logistic Regression analysis on the dependent variable NNRTIs toxicity.

Factors/independent variables	OR (95% CI)	Binary Logistic Regression – *P* value
Age	1.02 (0.99–1.05)	.189
Baseline BMI	1.03 (0.93–1.13)	.611
Baseline_CD4 T-cells	1.00 (0.99–1.00)	.713
Baseline_RNA_log10	1.00 (1.00–1.00)	.298
*CYP2B6*_516	1.55 (0.89–2.71)	.125
Adherence	0.79 (0.39–1.61)	.517

BMI = body mass index, NNRTI = non-nucleoside reverse transcriptase inhibitor.

analysis revealed no effect of the independent variables tested on NNRTI toxicity (Table 3).

3.3. Hardy–Weinberg equilibrium test

CYP2B6 516 genotypes were in equilibrium in all the groups analyzed (EFV/NVP-toxicity, EFV/NVP-non-toxicity and both combined), (X^2 = .326, P = .568).

4. Discussions

It has been 2 decades since the effect of individuals' genetic profiling on the pharmacokinetics and clinical outcome to NNRTIs, especially EFV and NVP, was explored.[23–26] Studies done in Botswana have confirmed a high frequency of *CYP2B6* 516T allele (36.6%–38.1%) in the country.[7,9,12,13,15,18] The high prevalence of 516T allele has been reported in other sub-Saharan African settings like Ghana,[27] Malawi,[28] Mozambique,[29] South Africa,[30–34] and Zimbabwe.[35–38]

Our current analysis did not find any association of *CYP2B6* 516T allele with treatment toxicity in patients taking NNRTIs. Toxicity seems to be more associated with the extensive metabolizer genotypes (*CYP2B6* 516GG) compared to poor metabolizer genotypes (*CYP2B6* 516TT),[9] although our study did not find any statistical significance when different *CYP2B6* 516 genotypes (GG, GT, TT) were compared by toxicity. Other factors like age, low BMI, baseline low lymphocyte T-CD4 did not predict toxicity in this study (Table 3). Drug-to-drug interactions, depressive symptoms at baseline, longitudinal alcohol intake, EFV or NVP plasma exposure, and several uninvestigated factors, which occur with poor *CYP2B6* metabolism,[19,20] may contribute to the transient adverse effect phenomenon, but they were not analyzed in the current study.

Our findings agree with those findings from most studies conducted in the African region where no association has been found between poor NNRTI metabolizers (EFV and NVP) and toxicity, but are in conflict with studies involving Caucasian and Hispanic populations.[8,11] A potential explanation for these conflicting findings includes polymorphisms in genes other than CYP450 mitigating CNS toxicity in people of African origin.[27,39] It is also possible that there are other unidentified variants or polymorphisms that code for metabolizing enzymes unique to the population given the greater genetic variation in Africa.[40] For example, Radloff et al[40] found several polymorphisms at *CYP2B6* gene in Rwandese individuals which have not been reported elsewhere outside Africa but critical to be studied further. Finally, it is worth to note that Botswana population has a very high level of genetic admixture that leads to different phenotypic outcomes, that may be different from those experienced in other African settings.[7,41]

The main limitations of this study are as follows: the small sample size; the use of only *CYP2B6* 516G>T polymorphism as proxy of NNRTIs metabolism as many other studies, without considering the possible implication of other SNPs (i.e., *CYP2B6* 983T>C); the retrospective nature of the study without a control group; the lack of NNRTI plasma exposure measurements; the fact that we pooled together EFV and NVP therapies in the search for an association with *CYP2B6* 516G>T polymorphisms, not being able for all subject to retrieve the specific therapeutic regimen but knowing that they were however using NNRTIs.[21]

5. Conclusions

To summarize, it is apparent in all the studies on poor EFV metabolizers in Botswana, and in most studies done in the region,[4,16] that there is a consistent lack of significant correlation of the 516T allele with NNRTIs toxicities. There is need for prospective data to determine whether pre-treatment genotyping can improve therapeutic efficacy and/or reduce toxicity.[42] More studies are also needed in analyzing the *CYP2B6* variation to determine if it is necessary to switch poor metabolizers from NNRTI-based ART, balancing efficacy and toxicity, considering the transient nature of some adverse events observed. Furthermore, it is also important to better define the role of EFV/NVP metabolites in the appearance of NNRTI toxicity.[9]

Acknowledgments

We express gratitude to the University of Botswana, Faculty of Health Sciences, School of Allied Health Professions and Botswana-University of Pennsylvania Partnership laboratory staff for their help, assistance and continuous support to this study.

Author contributions

Conceptualization, G.M.P., S.G., M.E., and V.N.; methodology, G.M.P. and S.G.; validation, S.G. and I.K.; formal analysis, M.J.M. and G.M.P.; investigation, M.J.M., L.T., and P.K.T.; resources, G.M.P., S.G., M.E., V.N., and I.K.; data curation, M.J.M., L.T., and P.K.T., writing—original draft preparation, M.J.M.; writing—review and editing, G.M.P., S.G., I.K., S.M., L.T., P.K.T., G.R., M.E., and V.N.; visualization, G.M.P., L.T., and M.J.M.; supervision, G.M.P., S.G., and I.K.; project administration, G.M.P. and S.G. All authors have read and agreed to the published version of the manuscript.

Conceptualization: Vladimir Novitsky, Max Essex, Simani Gaseitsiwe, Giacomo Maria Paganotti.

Data curation: Monkgomotsi Maseng, Leabaneng Tawe, Prisca Thami.

Formal analysis: Monkgomotsi Maseng, Giacomo Maria Paganotti.

Investigation: Monkgomotsi Maseng, Leabaneng Tawe, Prisca Thami.

Methodology: Simani Gaseitsiwe, Giacomo Maria Paganotti.

Project administration: Simani Gaseitsiwe, Giacomo Maria Paganotti.

Resources: Ishmael Kasvosve, Max Essex, Simani Gaseitsiwe, Giacomo Maria Paganotti.
Supervision: Ishmael Kasvosve, Simani Gaseitsiwe, Giacomo Maria Paganotti.
Validation: Ishmael Kasvosve, Simani Gaseitsiwe.
Visualization: Monkgomotsi Maseng, Giacomo Maria Paganotti.
Writing – original draft: Monkgomotsi Maseng.
Writing – review & editing: Leabaneng Tawe, Prisca Thami, Sikhulile Moyo, Ishmael Kasvosve, Vladimir Novitsky, Max Essex, Gianluca Russo, Simani Gaseitsiwe, Giacomo Maria Paganotti.

References

[1] UNAIDS, 2021. Country Factsheets: Botswana. Available at: https://www.unaids.org/en/regionscountries/countries/botswana. Accessed January 23, 2022.
[2] Ministry of Health, Botswana; Masa; Treat All; et al. Handbook of the Botswana 2016 Integrated HIV Clinical Care Guidelines. 2016. Available at: http://www.moh.gov.bw/Publications/Handbook_HIV_treatment_guidelines.pdf. Accessed January 23, 2022.
[3] Riou J, Dupont C, Bertagnolio S, et al. Drivers of HIV-1 drug resistance to non-nucleoside reverse-transcriptase inhibitors (NNRTIs) in nine southern African countries: a modelling study [published correction appears in BMC Infect Dis. 2021 Oct 25;21(1):1098]. BMC Infect Dis 2021;21:1042.
[4] Ribaudo HJ, Liu H, Schwab M, et al. Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: an AIDS Clinical Trials Group study. J Infect Dis 2010;202:717–22.
[5] Gallien S, Journot V, Loriot MA, et al. Cytochrome 2B6 polymorphism and efavirenz-induced central nervous system symptoms: a substudy of the ANRS ALIZE trial. HIV Med 2017;18:537–45.
[6] Russo G, Paganotti GM, Soeria-Atmadja S, et al. Pharmacogenetics of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in resource-limited settings: influence on antiretroviral therapy response and concomitant anti-tubercular, antimalarial and contraceptive treatments. Infect Genet Evol 2016;37:192–207.
[7] Tawe L, Motshoge T, Ramatlho P, et al. Human cytochrome P450 2B6 genetic variability in Botswana: a case of haplotype diversity and convergent phenotypes. Sci Rep 2018;8:4912.
[8] Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. AIDS 2004;18:2391–400.
[9] Vujkovic M, Bellamy SL, Zuppa AF, et al. Polymorphisms in cytochrome P450 are associated with extensive efavirenz pharmacokinetics and CNS toxicities in an HIV cohort in Botswana. Pharmacogenomics J 2018;18:678–88.
[10] Robertson K, Liner J, Meeker RB. Antiretroviral neurotoxicity. J Neurovirol 2012;18:388–99.
[11] Rotger M, Colombo S, Furrer H, et al. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. Pharmacogenet Genomics 2005;15:1–5.
[12] Maseng MJ, Tawe L, Thami PK, et al. Association of CYP2B6 Genetic Variation with Efavirenz and Nevirapine Drug Resistance in HIV-1 Patients from Botswana [published correction appears in Pharmgenomics Pers Med. 2021 Mar 31;14:395]. Pharmgenomics Pers Med 2021;14:335–47.
[13] Vujkovic M, Bellamy SL, Zuppa AF, et al. Brief report: CYP2B6 516G>T minor allele protective of late virologic failure in efavirenz-treated HIV-infected patients in Botswana. J Acquir Immune Defic Syndr 2017;75:488–91.
[14] Mukonzo JK, Okwera A, Nakasujja N, et al. Influence of efavirenz pharmacokinetics and pharmacogenetics on neuropsychological disorders in Ugandan HIV-positive patients with or without tuberculosis: a prospective cohort study. BMC Infect Dis 2013;13:261.
[15] Gross R, Bellamy SL, Ratshaa B, et al. CYP2B6 genotypes and early efavirenz-based HIV treatment outcomes in Botswana. AIDS 2017; 31:2107–13.
[16] Streck EL, Scaini G, Rezin GT, Moreira J, Fochesato CM, Romão PR. Effects of the HIV treatment drugs nevirapine and efavirenz on brain creatine kinase activity. Metab Brain Dis 2008;23:485–92.
[17] Yuan J, Guo S, Hall D, et al. Toxicogenomics of nevirapine-associated cutaneous and hepatic adverse events among populations of African, Asian, and European descent. AIDS 2011;25:1271–80.
[18] Gross R, Aplenc R, Tenhave T, et al. Slow efavirenz metabolism genotype is common in Botswana. J Acquir Immune Defic Syndr 2008;49:336–7.
[19] Sonenthal PD, Ratshaa B, Chimbengo G, et al. Baseline predictors of efavirenz-containing antiretroviral regimen adverse experiences. Pharmacoepidemiol Drug Saf 2014;23:773–7.
[20] Torgersen J, Bellamy SL, Ratshaa B, et al. Impact of efavirenz metabolism on loss to care in older HIV+ Africans. Eur J Drug Metab Pharmacokinet 2019;44:179–87.
[21] Wester CW, Thomas AM, Bussmann H, et al. Non-nucleoside reverse transcriptase inhibitor outcomes among combination antiretroviral therapy-treated adults in Botswana. AIDS 2010;24(Suppl 1):S27–36.
[22] Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. [July 2017]. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Available at: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf.
[23] Erickson DA, Mather G, Trager WF, Levy RH, Keirns JJ. Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. Drug Metab Dispos 1999;27:1488–95.
[24] Riska P, Lamson M, MacGregor T, et al. Disposition and biotransformation of the antiretroviral drug nevirapine in humans. Drug Metab Dispos 1999;27:895–901.
[25] Chen H, Chen W, Gan LS, Mutlib AE. Metabolism of (S)-5,6-difluoro-4-cyclopropylethynyl-4-trifluoromethyl-3, 4-dihydro-2(1H)-quinazolinone, a non-nucleoside reverse transcriptase inhibitor, in human liver microsomes. Metabolic activation and enzyme kinetics. Drug Metab Dispos 2003;31:122–32.
[26] Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. J Pharmacol Exp Ther 2003;306:287–300.
[27] Kwara A, Lartey M, Sagoe KW, Rzek NL, Court MH. CYP2B6 (c.516G–>T) and CYP2A6 (*9B and/or *17) polymorphisms are independent predictors of efavirenz plasma concentrations in HIV-infected patients. Br J Clin Pharmacol 2009;67:427–36.
[28] Dickinson L, Chaponda M, Carr DF, et al. Population pharmacokinetic and pharmacogenetic analysis of nevirapine in hypersensitive and tolerant HIV-infected patients from Malawi. Antimicrob Agents Chemother 2014;58:706–12.
[29] Arnaldo P, Thompson RE, Lopes MQ, Suffys PN, Santos AR. Frequencies of cytochrome P450 2B6 and 2C8 allelic variants in the Mozambican population. Malays J Med Sci 2013;20:13–23.
[30] Cohen K, Grant A, Dandara C, et al. Effect of rifampicin-based antitubercular therapy and the cytochrome P450 2B6 516G>T polymorphism on efavirenz concentrations in adults in South Africa. Antivir Ther 2009;14:687–95.
[31] Swart M, Skelton M, Wonkam A, et al. CYP1A2, CYP2A6, CYP2B6, CYP3A4 and CYP3A5 polymorphisms in two bantu-speaking populations from Cameroon and South Africa: implications of global pharmacogenetics. Curr Pharmacogenomics Person Med 2012;10: 43–53.
[32] Swart M, Skelton M, Ren Y, Smith P, Takuva S, Dandara C. High predictive value of CYP2B6 SNPs for steady-state plasma efavirenz levels in South African HIV/AIDS patients. Pharmacogenet Genomics 2013;23:415–27.
[33] Sinxadi PZ, Leger PD, McIlleron HM, et al. Pharmacogenetics of plasma efavirenz exposure in HIV-infected adults and children in South Africa. Br J Clin Pharmacol 2015;80:146–56.
[34] Reay R, Dandara C, Viljoen M, Rheeders M. CYP2B6 haplotype predicts efavirenz plasma concentration in Black South African HIV-1-infected children: a longitudinal pediatric pharmacogenomic study. OMICS 2017;21:465–73.
[35] Nyakutira C, Röshammar D, Chigutsa E, et al. High prevalence of the CYP2B6 516G->T (*6) variant and effect on the population

pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. Eur J Clin Pharmacol 2008;64:357–65.
[36] Jamshidi Y, Moreton M, McKeown DA, et al. Tribal ethnicity and CYP2B6 genetics in Ugandan and Zimbabwean populations in the UK: implications for efavirenz dosing in HIV infection. J Antimicrob Chemother 2010;65:2614–9.
[37] Maimbo M, Kiyotani K, Mushiroda T, Masimirembwa C, Nakamura Y. CYP2B6 genotype is a strong predictor of systemic exposure to efavirenz in HIV-infected Zimbabweans. Eur J Clin Pharmacol 2012;68:267–71.
[38] Dhoro M, Zvada S, Ngara B, et al. CYP2B6*6, CYP2B6*18, Body weight and sex are predictors of efavirenz pharmacokinetics and treatment response: population pharmacokinetic modeling in an HIV/AIDS and TB cohort in Zimbabwe. BMC Pharmacol Toxicol 2015;16:4.
[39] Cummins NW, Neuhaus J, Chu H, et al. Investigation of efavirenz discontinuation in multiethnic populations of HIV-positive individuals by genetic analysis. EBioMedicine 2015;2:706–12.
[40] Radloff R, Gras A, Zanger UM, et al. Novel CYP2B6 enzyme variants in a Rwandese population: functional characterization and assessment of in silico prediction tools. Hum Mutat 2013;34:725–34.
[41] Thami PK, Chimusa ER. Population structure and implications on the genetic architecture of HIV-1 phenotypes within southern Africa. Front Genet 2019;10:905.
[42] Dandara C, Masimirembwa C, Haffani YZ, et al. African Pharmacogenomics Consortium: consolidating pharmacogenomics knowledge, capacity development and translation in Africa. AAS Open Res 2019;2:19.