



EFAVIRENZ PHARMACOGENETICS AND METABOLIC TOXICITY IN BLACK SOUTH AFRICANS

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DECLARATION

I, Lesiba Meshack Makgai, do hereby declare that this minor dissertation entitled **“Efavirenz pharmacogenetics and metabolic toxicity in black South Africans”** is submitted by me to the University of Cape Town, Faculty of Health Sciences in partial fulfilment of the requirements for the award of the degree of Master of Philosophy (MPhil) in Clinical Pharmacology (coursework and dissertation) in the Division of Clinical Pharmacology, Department of Medicine. I further declare that to the best of my knowledge the work reported in this project has not been submitted and will not be submitted, either in part or in full, for the award of any other degree or diploma in this institute.

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NOTICE

This minor dissertation is submitted in partial fulfilment of the requirements for the degree of MPhil in Clinical Pharmacology and is presented following the guidelines set out by the University of Cape Town. Therefore, it contains the following:

1. Title page.
2. Minor dissertation body, which is structured in four chapters:

Chapter 1: Introduction.

Chapter 2: Literature Review.

Chapter 3: The results of the study presented in a manuscript format for a journal article (**British Journal of Clinical Pharmacology**), meeting all the authors' instructions required.

Chapter 4: Supporting documents.

LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine transaminase
ART	Antiretroviral therapy
CYP2B6	Cytochrome P450 isoenzyme 2B6
EFV	Efavirenz
HIV	Human Immunodeficiency Virus
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
UNAIDS	United Nations HIV/AIDS
PLHIV	People Living with HIV
PXR	Pregnane X receptor
SNP	Single Nucleotide Polymorphism
SSA	Sub-Saharan Africa
WHO	World Health Organization

CHAPTER 1
INTRODUCTION

INTRODUCTION

Human immunodeficiency virus (HIV) is an infectious disease that weakens the human immune system and interferes with the body's ability to fight off infections (1). Without early and adequate treatment, this virus can progress into acquired immunodeficiency syndrome (AIDS), which is a more severe stage of infection with increased chances of death (1). HIV is one of the leading causes of mortality in the world and one of the top 10 leading causes of mortality in low-income countries (2). The virus was first isolated in 1983 (3), and has since become a global epidemic. Currently, there are 37.6 million people living with HIV (PLHIV) globally, and HIV has claimed more than 34.7 million lives to date (4, 5). In the year 2020 alone, HIV caused 1.5 million new infections and has claimed 690 000 lives (4, 5).

Sub-Saharan Africa (SSA) bears the brunt of the epidemic and accounts for 68% (25.7 million) of PLHIV globally (6). South Africa is the most affected country in the world and accounts for a third of the HIV infections in the Southern African countries (7, 8). Despite many scientific advances since its discovery, HIV/AIDS still has no effective cure, and it is currently treated with antiretroviral therapy (ART) regimens. ART has drastically reduced HIV-associated morbidity and mortality (1). However, cumulative use of ART is associated with the development of side effects (9). The side effects affect the treatment adherence and sometimes lead to discontinuation (10). Therefore, finding effective treatment regimens with minimal side effects is critical for improving the health and quality of life of PLHIV. As the benefits of taking ART far outweigh the risks of developing side effects, understanding how these side effects develop is important. This will aid in stratifying risks when choosing which regimen to prescribe for any given patient. This study focuses on a compound known as efavirenz, which is part of the fixed-dose antiretroviral therapy (ART) used for the treatment of HIV.

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) known to suppress HIV type-1 replication (9, 11). Efavirenz-based ART was widely prescribed in combination with nucleoside reverse transcriptase inhibitors (NRTIs) tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC), as the first-line regimen across the world (11-13). Recently, it has largely been replaced by dolutegravir in first-line regimens as dolutegravir has less toxicity, fewer drug–drug interactions and better tolerability (11-14). However, efavirenz is still recommended for women planning to

conceive and in early pregnancy (≤ 6 weeks gestation), people with tuberculosis co-infection and children due to its relative safety and effectiveness (14). Therefore, research investigating mechanisms of efavirenz toxicity is relevant.

Efavirenz is associated with the development of neuropsychiatric side effects (9, 15), central nervous system (CNS) toxicity, hepatotoxicity and metabolic toxicities such as dyslipidaemia and dysglycaemia (9, 15-17). Some toxicities lead to treatment discontinuation (10). These adverse effects are concentration-dependent, with efavirenz concentrations predicted by the highly polymorphic *CYP2B6* gene, which encodes the cytochrome P450 2B6 (CYP2B6) enzyme primarily responsible for efavirenz metabolism (18, 19). Genetic studies have identified three main *CYP2B6* single nucleotide polymorphisms (SNPs) that determine the status of *CYP2B6* metabolisers (extensive, intermediate and slow), namely: *CYP2B6* 516G \rightarrow T (rs3745274), *CYP2B6* 983T \rightarrow C (rs28399499) and *CYP2B6* 15582C \rightarrow T (rs4803419) (11, 19, 20). The frequency of *CYP2B6* slow metaboliser genotypes is higher in African populations compared with other populations (18). However, data on the associations between genetic polymorphisms and metabolic toxicity in African populations are lacking.

My study, which utilises data from the efavirenz arm of the ADVANCE randomised clinical trial (NCT03122262) characterised associations between the *CYP2B6* composite metaboliser genotypes and metabolic parameters (which include glucose, lipids and alanine transaminase (ALT)). We hypothesised that *CYP2B6* slow metabolisers will be associated with higher lipids, glucose and ALT concentrations.

The thesis will comprise the narrative literature review and the manuscript prepared according to the British Journal of Clinical Pharmacology (BJCP) author guidelines and appendices (Ethics approval letter, protocol and BJCP author guidelines).

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CHAPTER 2
LITERATURE REVIEW

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1. Title of the review

Are the *CYP2B6* metaboliser genotypes associated with dyslipidaemia, dysglycaemia and hepatotoxicity in people living with HIV (PLHIV) on efavirenz-based antiretroviral therapy (ART)?

2. Outline of the review

This review will cover the following topics: current HIV epidemic, review of efavirenz and its current role, efavirenz toxicity (subheadings: lipids, glucose and hepatotoxicity), pharmacogenetics of efavirenz pharmacokinetics, and pharmacogenetics of efavirenz-associated metabolic toxicity.

2.1. Current HIV epidemic

The World Health Organization (WHO) and United Nations HIV/AIDS programme (UNAIDS) global HIV statistics indicate that 77.5 million people have been infected with HIV globally since the beginning of this epidemic (1, 2). Currently, there are 37.6 million PLHIV, with 1.5 million new infections diagnosed in 2020 (1, 2). Recent death rate statistics indicate that 690 000 people died from HIV-related illnesses in 2020 (1, 2). Globally, ART coverage was 73%, with 27.4 million receiving treatment in 2020 (1, 2). Young women (aged 15 to 24) accounted for 50% of new HIV infections in 2020, with 5 000 women becoming infected every week and twice as likely to be infected than men (2). The UNAIDS programme focuses mostly on these HIV/AIDS projects and statistics globally.

The UNAIDS initial target was to achieve 90:90:90 by 2020 and 95:95:95 by 2030, which means to ensure that 90% of PLHIV are aware of their status, 90% of HIV-positive people are on treatment, and 90% of the people on ART achieved viral suppression by 2020 and 95% of each category by 2030 (3). However, of the PLHIV in the world by 2020, 84% were reported to know their status, 73% were on ART, and 66% had achieved viral suppression (2). What is noteworthy among PLHIV who knew their status is that 87% had access to treatment and 90% of those on treatment were virally suppressed (2). This clearly shows the effectiveness of ART in controlling the

virus in patients receiving and adhering to the treatment. This HIV epidemic also appears to have different prevalence in different regions of the world.

As mentioned, the HIV epidemic is more prevalent in the Sub-Saharan Africa (SSA) regions, where about 68% (25.7 million) of PLHIV are found (4), out of the 37.6 million in the world (2). However, many people in the SSA are still undiagnosed or diagnosed late with HIV (5). The East and Southern Africa sub-regions are severely concentrated with HIV infection, with over 54% (20.6 million) of PLHIV residing within these regions (6). Out of the 15% of young women (aged 15 to 24) living with HIV globally, 80% are found in the SSA regions and HIV is also more prevalent in women than in men in East and Southern Africa (6). This study will specifically focus on PLHIV found in South Africa.

South Africa has the highest prevalence of HIV in the world, with 7.5 million South Africans living with HIV, and the country accounting for a third of HIV infections in Southern African countries (7, 8). The country has an HIV prevalence of 20.4%, which amounts to one person out of every five PLHIV globally (8, 9). The highest prevalence is found in KwaZulu-Natal (24%) and the lowest in Western Cape (12.6%) (8, 10). South Africa experienced a death rate of 74 000 people with AIDS-related illnesses by 2019, with a reported high death rate in men as compared to women (11). South Africa has made good progress towards the 2020 UNAIDS 90:90:90 target, with 92% of PLHIV diagnosed and made aware of their status, 75% of them on ART, and 92% of those on ART virally suppressed (8, 11).

2.2. Current role of efavirenz in the modern ART era

Efavirenz was first introduced in 1995 as a non-nucleoside reverse transcriptase inhibitor (NNRTI) (12), which is known to suppress HIV type-1 replication by blocking enzyme reverse transcriptase from replicating viral RNA into its complementary DNA (shown in **Figure 1** below) (13, 14). It has been widely prescribed, together with a dual nucleos(t)ide reverse transcriptase inhibitor (NRTI) backbone, as the first-line regimen globally, but is now relegated to the second-line treatment regimen (13, 15, 16). Efavirenz is now recommended for women planning to conceive and in early pregnancy (≤ 6 weeks gestation), people with tuberculosis and children due to its safety and effectiveness in these groups, as compared to alternative regimens (17).

2.3. Efavirenz toxicity

Efavirenz has been associated with adverse neuropsychiatric side effects, including confusion, dizziness, insomnia, abnormal dreams, anxiety, depression, agitation, hallucination, and delusional, psychotic, paranoid and manic behaviours (12). Efavirenz also alters adipocyte function and is associated with the development of metabolic complications over a prolonged treatment period (18, 19). It is further associated with the development of steatosis (accumulation of fat in the liver), which is a risk factor for obesity and type-2 diabetes (18, 20). In this review, the focus is on the following toxicities: dyslipidaemia, dysglycaemia and hepatotoxicity.

2.3.1. Efavirenz and dyslipidaemia

Dyslipidaemia is characterised by an elevation in concentrations of total cholesterol (>5.2 mmol/L), LDL cholesterol (>2.5 mmol/L), triglycerides (>1.7 mmol/L) and reduced HDL cholesterol (<1.0 mmol/L) concentrations, which are associated with increased risk for cardiovascular complications (21-23). The development of dyslipidaemia can be influenced by personal factors (which include familial background, age and sex), environmental factors (which include diet, alcohol, smoking and weight), diseases (including diabetes, cardiovascular diseases, cholestasis, hypothyroidism and nephrotic syndrome), genetic factors, and use of drugs such as ART-related regimens (18). PLHIV in Western countries are found to be at a higher risk of myocardial infarction due to a high incidence of pre-existing risk factors such as smoking and high baseline lipids.(24). The prevalence of dyslipidaemia is relatively high as PLHIV get older and is significantly higher in HIV-infected people when compared to uninfected people (18, 24). This develops over a prolonged ART treatment period.

The chronic treatment with several HIV-based ART regimens including efavirenz is known to be associated with the development of cardiovascular complications resulting from dyslipidaemia (25-28). However, the SMART study showed increased chances of developing cardiovascular complications upon stopping antiretrovirals (29). The efavirenz concentrations above the upper therapeutic index are reported to result in elevated lipid concentrations (30). In efavirenz pharmacogenetics studies, the *CYP2B6* slow metaboliser genotypes are mostly associated with increased

concentrations of total cholesterol, LDL cholesterol and HDL cholesterol in individuals on efavirenz treatment (18, 30, 31). Elevated triglycerides are also reported as a causal pathway for myocardial infarction (32), and efavirenz plasma concentrations are reported to be associated with triglycerides concentrations (18, 25). Efavirenz-based ART demonstrated higher changes in serum lipids after 48 weeks, especially in HIV-naïve patients (25, 33).

2.3.2. Efavirenz and dysglycaemia

Dysglycaemia is characterised by an abnormality in fasting blood glucose levels outside the 5.6 mmol/L and 6.9 mmol/L range, with fasting glucose below 5.6 mmol/L indicating hypoglycaemia and above 6.9 mmol/L indicating hyperglycaemia, which is a risk factor for type-2 diabetes mellitus (21). A study by Dave *et al.* indicated that dysglycaemia is more prevalent in the older age group, and more likely in males and in patients with higher CD4 T-cell count, especially those on an efavirenz-based ART regimen (34). Patients with dysglycaemia are known to have high proportions of insulin resistance as compared to normoglycemic patients, which indicates that dysglycaemic patients are at higher risk of developing type-2 diabetes mellitus (34, 35). The risk for diabetes mellitus is higher in HIV-positive patients on ART treatment than in HIV-negative patients (36-38), with efavirenz reported to be associated with the development of diabetes mellitus (39), elevated fasting plasma glucose (40), and significantly reduced fasting serum insulin levels (41). The elevated efavirenz concentrations are reported to be associated with higher glucose concentrations (30).

2.3.3. Efavirenz and hepatotoxicity

Drug-induced hepatotoxicity (DIH) is caused by medications (prescription or over the counter), herbal and dietary supplements, or other xenobiotics that result in abnormalities in liver tests or in hepatic dysfunction that cannot be explained by other causes. It is characterised by elevated levels of serum liver enzymes with or without bilirubin (42). ALT is mostly used as a marker for hepatocyte injury or inflammation (43, 44). Elevated ALT levels signal ischemia–reperfusion injury or other forms of liver tissue damage, as it indicates the occurrence of ALT leakage from the liver tissue into the systemic circulation (44). Hepatotoxicity occurs more frequently with nevirapine

(1.4 – 17% of patients) than with efavirenz (1.1 – 8%) (45). In South Africa, efavirenz-induced hepatotoxicity, which developed a few months after the efavirenz-based ART was introduced, was initially been reported in case reports (46, 47). Subsequently, efavirenz was reportedly associated with nonspecific hepatitis and necrosis in a South African cohort (48). However, in a case series including 81 patients on efavirenz, a more severe clinico-pathological pattern of drug-induced liver injury was described (49). Elevation of ALT has also been reported elsewhere, including a Thai longitudinal cohort, where older age, baseline ALT and CD4 count >350 cells/ μ L were reported as predictors of elevated ALT at week 48 (50). In the 2NN study, higher efavirenz trough concentrations were associated with elevated liver enzymes (51). Therefore, understanding whether efavirenz-associated hepatotoxicity is also predicted by genetic factors is important.

2.4. Pharmacogenetics and efavirenz pharmacokinetics

The standard dose of efavirenz is 600 mg/d, with an expected therapeutic index ranging from 1 to 4 mg/L, is adequate to achieve viral suppression (52). Efavirenz is mainly metabolised by the enzyme cytochrome P450 2B6 (CYP2B6) into an inactive metabolite 8-hydroxy-efavirenz, and alternatively metabolised by enzymes CYP2A6 and uridine 5'-diphospho-glucuronosyltransferase 2B7 (UGT2B7), as shown in **Figure 1** below.

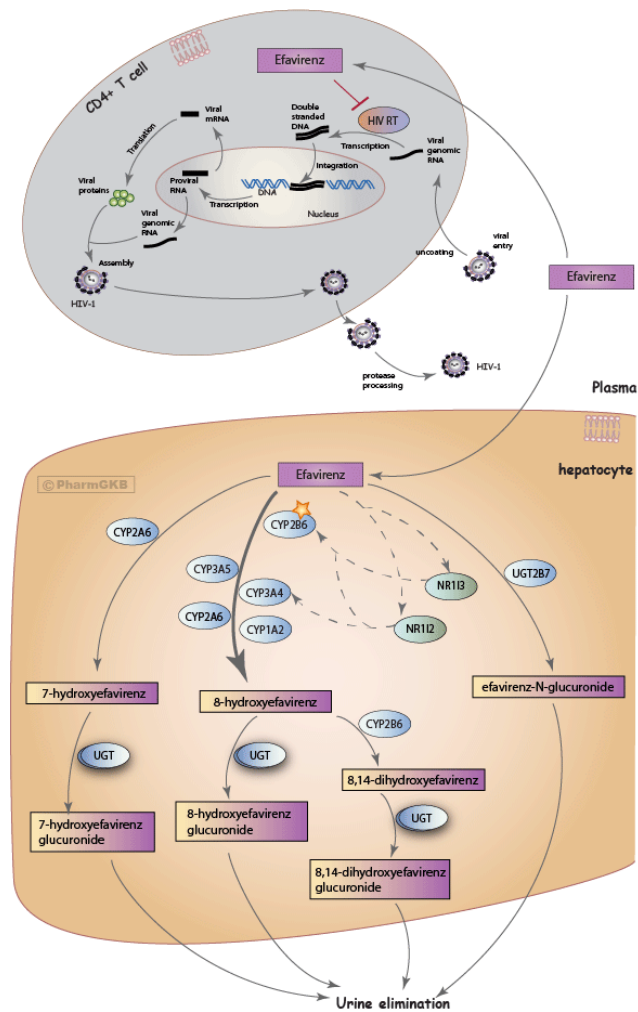


Figure 1. Efavirenz function and metabolic pathway. The function and metabolic pathway of efavirenz, also showing various genetic enzymes involved in the efavirenz disposition, are indicated above. The purpose of Efavirenz is shown in the CD4 T-cell (blue oval cell), where it blocks the activity of enzyme reverse transcriptase (HIV-RT) from reverse transcribing HIV RNA into its complementary DNA that is to be incorporated into the human genome. The metabolism of Efavirenz is shown in the hepatocyte (orange cell), where multiple enzymes are responsible for metabolising efavirenz into multiple metabolites as shown. URL: <https://www.pharmgkb.org/pathway/PA166123135>.

The *CYP2B6* gene is known to be highly polymorphic, and its loss of function polymorphisms, namely *CYP2B6* 516G→T (rs3745274), *CYP2B6* 983T→C (rs28399499) and *CYP2B6* 15582C→T (rs4803419) are well described and associated with reduced plasma efavirenz clearance (13, 53, 54). In European descendants, the *CYP2B6* 516G→T, 983T→C and 15582C→T single-nucleotide polymorphisms (SNPs), are reported to have observed minor allele frequency (MAF) of 23.6%, 0.0% and 32.0%, respectively. In contrast, the observed MAF for the same SNPs in African descendants is 34.7%, 8.2% and 8.2%, respectively (55).

The association between the loss of function SNP, *CYP2B6* 516G→T, and higher plasma efavirenz exposure is well established, and has been replicated across different populations (13, 56). In African populations where the less common *CYP2B6* 983T→C SNP with a higher effect is found, the efavirenz concentrations are higher (57, 58). The combined effect of the *CYP2B6* 516G→T and *CYP2B6* 983T→C has been associated with even higher efavirenz concentrations and similar results have been reported in various African populations, such as the South African (54), Ugandan and Zimbabwean populations (59). As mentioned previously, the *CYP2B6* 983T→C SNP is monomorphic in European populations (60), and the observed MAF differences between populations explain the variability in efavirenz concentrations seen between the populations (61). The genetic differences stress the need for including African populations in genetic studies as they are, in general, currently underrepresented. These differences have important dosing implications, as virological suppression is achieved using lower doses (400 or 200 mg/d) in poor metabolisers (13).

2.5. Pharmacogenetics of efavirenz toxicity

The *CYP2B6* 516G→T and 983T→C with or without 15582C→T are reported to be associated with the development of CNS toxicity (55, 58, 60). These effects have an early onset and are usually self-limiting, even in slow metabolisers (62). However, they can lead to treatment discontinuation (63, 64). Both efavirenz and nevirapine are significantly associated with hepatotoxicity in *CYP2B6* 516G→T carriers (65). Hepatotoxicity was shown to be associated with efavirenz concentration above 4mg/L in *CYP2B6* slow metabolisers in a Zimbabwean population (66). In a subset of participants (n=57) enrolled in a cross-sectional South African study evaluating associations between efavirenz concentrations and lipids and glucose, *CYP2B6* 516G→T was associated with total cholesterol. No associations were found between other metabolic parameters and individual polymorphisms or the composite (516/983/15582) metaboliser genotypes (30). This was one of the first studies done on the association between the *CYP2B6* composite metaboliser genotypes and metabolic parameters. Additionally, the phenome-wide association study report association of *CYP2B6* SNPs with high lipids phenotypes (32). More studies

investigating genetic predictors of antiretroviral associated metabolic toxicity are needed.

3. Summary of interpretation of literature

A narrative literature review was conducted to evaluate what is known about the efavirenz pharmacogenetics and metabolic toxicity in patients living with HIV. Efavirenz pharmacogenetics are best described using three important polymorphisms namely: *CYPB6* SNPs 516G→T, 983T→C and 15582C→T, to classify participants into extensive, intermediate and slow metabolisers. The *CYPB6* slow metaboliser genotypes are common in Africans. The association between the *CYPB6* SNPs 516G→T, 983T→C and/or 15582C→T polymorphisms and efavirenz concentrations is well established. There are limited data on the associations between *CYPB6* slow metabolisers and increased neuropsychiatric effects and treatment discontinuation in patients on efavirenz-based ART. Importantly, associations between *CYPB6* slow metabolisers and metabolic toxicity including hepatotoxicity are reported only in small studies or case reports and have not been replicated in larger studies.

Knowledge gaps identified

The following gaps have been identified:

1. There is paucity of data on the associations between efavirenz concentrations and metabolic toxicity in African populations.
2. The data characterising associations between *CYPB6* metaboliser genotypes and lipids and glucose are limited
3. The data characterising associations between *CYPB6* metaboliser genotypes and hepatotoxicity are limited.

Our study aimed to characterise relationships between *CYP2B6* metaboliser genotypes and metabolic toxicity associated with efavirenz use. The data generated will contribute towards filling the gaps identified above.

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CHAPTER 3
RESULTS: MANUSCRIPT

EFAVIRENZ PHARMACOGENETICS AND METABOLIC TOXICITY IN BLACK SOUTH AFRICANS.

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WHAT IS ALREADY KNOWN ABOUT THE SUBJECT

- Cumulative efavirenz use is associated with higher prevalence of dyslipidaemia and dysglycaemia.
- The prevalence of *CYP2B6* slow metabolisers is relatively higher in populations of African ancestry.
- The *CYP2B6* slow metaboliser genotype is associated with higher plasma efavirenz concentrations.
- Higher efavirenz concentrations are associated with elevated concentrations of total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol.

WHAT THIS STUDY ADDS

- *CYP2B6* slow metabolisers are associated with higher concentrations of total cholesterol, HDL cholesterol and LDL cholesterol in the Black HIV-positive South African population.
- There is no association between *CYP2B6* genotypes and glucose, triglycerides or alanine transaminase (ALT) concentrations.

ABSTRACT

Background: Efavirenz is associated with hepatotoxicity, dyslipidaemia and dysglycaemia. We aimed to determine if *CYP2B6* composite metaboliser genotypes influence lipids, glucose and ALT concentrations.

Methods: Data and DNA from South African antiretroviral therapy (ART)-naïve participants initiating efavirenz with emtricitabine plus tenofovir disoproxil fumarate (TDF) were used to characterise associations between *CYP2B6* metaboliser genotypes and the percentage difference in metabolic parameters from baseline to week 48 using univariate and multivariate linear regression models.

Results: A total of 171 participants were successfully genotyped. Median baseline age was 32 years, CD4 count was 292 cells/mm³ and log₁₀ viral load was 4.42 copies/ml. Univariate analyses showed significant associations between *CYP2B6* slow metaboliser genotype and total cholesterol ($\beta = 13.78$, $p = 0.003$), LDL cholesterol ($\beta = 15.89$, $p = 0.008$) and HDL cholesterol ($\beta = 24.78$, $p = 0.002$). These associations remained significant in multivariate analyses adjusting for age, sex, weight, baseline CD4 cell count and viral load [total cholesterol ($\beta = 14.93$, $p < 0.001$), LDL cholesterol ($\beta = 15.57$, $p = 0.014$), and HDL cholesterol ($\beta = 24.22$, $p < 0.001$)]. No associations were found between *CYP2B6* metaboliser genotype and triglycerides, glucose or ALT.

Conclusion: Among Black South African participants on efavirenz-based ART, *CYP2B6* slow metaboliser genotype was associated with high cholesterol concentrations. In an African population with high prevalence of *CYP2B6* slow metabolisers, close monitoring of lipids is needed.

INTRODUCTION

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) known to suppress HIV type-1 replication (1, 2). It has been widely prescribed, together with a dual nucleos(t)ide reverse transcriptase inhibitor (NRTI) backbone, as the first-line regimen globally (1, 3, 4). Efavirenz is still recommended for women planning to conceive and in early pregnancy (≤ 6 weeks gestation), people with tuberculosis and children (5).

Efavirenz is predominantly metabolised by cytochrome P450 2B6 (CYP2B6) enzyme (1), encoded for by the highly polymorphic *CYP2B6* gene. The *CYP2B6* single nucleotide polymorphisms (SNPs), *CYP2B6* 516G \rightarrow T (rs3745274), 983T \rightarrow C (rs28399499) and 15582C \rightarrow T (rs4803419) are known to be associated with efavirenz exposure, with slow metabolisers associated with high efavirenz concentrations (6). The *CYP2B6* 516G \rightarrow T and 983T \rightarrow C with or without 15582C \rightarrow T are reported to be associated with the development of CNS toxicity (7-9). These effects have an early onset and are usually self-limiting, even in slow metabolisers (10).

On the other hand, cumulative use of efavirenz has been associated with hepatotoxicity (11-13), dyslipidaemia (14-16), dysglycaemia (17, 18) and overt diabetes (19). Efavirenz-induced hepatotoxicity is rare but is a treatment-limiting toxicity and can be fatal. Dyslipidaemia and dysglycaemia is characterised by abnormal concentration levels of both lipids and glucose, respectively, and can lead to development of cardiovascular complications in the long-term (20, 21).

Understanding the pathogenesis of efavirenz-associated metabolic toxicity is important as efavirenz is still used in some settings. It is not well established if all cumulative use is associated with higher concentrations or, that higher concentrations are associated with these metabolic toxicities. In *CYP2B6* 516G \rightarrow T carriers, efavirenz was reported to be associated with hepatotoxicity in patients with efavirenz concentrations above 4 mg/L (13, 22). A South African cross-sectional study reported associations between higher efavirenz concentrations and higher cholesterol and glucose concentrations (17). In a subset of participants (n = 57) with genotype data, *CYP2B6* 516G \rightarrow T was associated with total cholesterol. No associations were found between other metabolic parameters and individual polymorphisms or the composite (516/983/15582) metaboliser genotypes. These data have not been replicated in

larger studies. We investigated the association of *CYP2B6* composite metaboliser genotypes with metabolic parameters (lipids, glucose and ALT) in HIV-positive Black South Africans on efavirenz-based ART. We hypothesised that *CYP2B6* slow metaboliser genotype will be associated with high lipids, glucose and ALT concentrations.

METHODS

Study Design and Participants

We conducted a pharmacogenetics sub-study of the ADVANCE study, which is reported elsewhere (23). Briefly, ADVANCE was a 96-week randomised, phase 3 non-inferiority clinical trial of 1 053 South African HIV-positive, ART-naïve individuals who were randomly assigned to one of three ART treatment arms. Inclusion criteria for this pharmacogenetics sub-study were ADVANCE participants randomised to the efavirenz arm (together with tenofovir disoproxil fumarate (TDF) and emtricitabine) who consented to genetic testing. Exclusion criteria included those who were receiving treatment with any other investigational drugs or drugs known to interact with efavirenz, and those with active tuberculosis coinfection. The study was approved by the University of Cape Town and University of the Witwatersrand Human Research Ethics Committees.

Characterisation of Genetic Polymorphisms

The extraction of DNA was done using the salting-out method. Genotyping was done using the MassARRAY® single nucleotide polymorphism (SNP) genotyping system (Agena Bioscience, San Diego, California, USA) at Inqaba Biotechnical Industries, Pretoria, South Africa. Genotyping performed included SNPs *CYP2B6* 516G→T (rs3745274), *CYP2B6* 983T→C (rs28399499) and *CYP2B6* 15582C→T (rs4803419). Composite genotypes of *CYP2B6* 15582/516/983 were assigned as extensive, intermediate and slow metabolisers as follows: 15582CC-516GG-983TT or 15582CT-516GG-983TT as extensive metaboliser genotypes; 15582TT-516GG-983TT, 15582CC-516GT-983TT, 15582CC-516GG-983CT, 15582CT-516GT-983TT or 15582CT-516GG-983CT as intermediate metaboliser genotypes; and 15582CC-

516GT-983CT, 15582CC-516GG-983CC or 15582CC-516TT-983TT as slow metaboliser genotypes (6).

STATISTICAL ANALYSIS

The demographic characteristics of participants in the pharmacogenetic sub-study were described using medians (interquartile ranges) and proportions for continuous and categorical data, respectively. The data was analysed using R-Software (24). Boxplots, Kruskal-Wallis test, univariate and multivariate linear regression analyses were used to determine the relationships between the *CYP2B6* composite metaboliser genotypes and the percentage difference (which is the difference or change from baseline to week 48) of the following metabolic parameters: total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, fasting glucose and ALT. Participants with *CYP2B6* extensive metaboliser genotypes were used as a reference group to compare with the intermediate and slow metaboliser groups. Univariate linear regression determined the association between the *CYP2B6* composite metaboliser genotypes and metabolic parameters. In multivariate linear regression, we adjusted for age, sex, weight, CD4 cell count at baseline and viral load at baseline.

RESULTS

Participant characteristics

A total of 351 participants enrolled in the efavirenz arm of the ADVANCE study, 171 of whom consented to a pharmacogenetics sub-study. All participants were Black, and all were on isoniazid prophylactic therapy in the first year of enrolment. Baseline characteristics of included participants are shown in **Table 1**.

Genetic polymorphisms

The three SNPs were successfully genotyped. All the SNPs were in Hardy Weinberg equilibrium. The minor allele frequency of the *CYP2B6* single nucleotide polymorphisms were 0.39, 0.10 and 0.05 for 516G→T, 983T→C and 15582C→T, respectively. The *CYP2B6* 516G→T had 69 (40.35%) homozygous for GG, 72 (42.11%) heterozygous for GT and 30 (17.54%) homozygous for TT; 983T→C had

140 (81.87%) homozygous for TT, 29 (16.96%) heterozygous for TC and 2 (1.71%) homozygous for CC; and 15582C→T had 154 (90.06%) homozygous for CC, 16 (8.77%) heterozygous for CT and 1 (0.58%) homozygous for TT. The frequency of *CYP2B6* composite metaboliser genotypes was 0.32, 0.46 and 0.29 for extensive, intermediate and slow metabolisers, respectively.

Genetic associations

We investigated associations between *CYP2B6* composite metaboliser genotypes and percentage change from baseline to 48 weeks of lipids, glucose and ALT concentrations. **Figure 1** shows the boxplots of the effect of *CYP2B6* composite metaboliser genotypes on the percentage difference of metabolic parameters, with Kruskal-Wallis test p-values representing their statistical associations. In **Figure 1(A-C)**, there were statistically significant differences in percentage changes of total cholesterol ($p < 0.001$), LDL cholesterol ($p = 0.012$) and HDL cholesterol ($p < 0.001$) concentrations between *CYP2B6* metaboliser genotypes. In **Figure 1(D-F)**, there were no statistically significant differences in percentage changes of triglycerides, fasting glucose and ALT concentrations between *CYP2B6* metaboliser genotypes. In **Figure 1F**, six extreme outliers with percentage changes from baseline to week 48 of ALT concentration at over 500% were observed, the highest being 1 206%. Sensitivity analyses removing the extreme outliers still showed no significant differences ($p = 0.091$) among the *CYP2B6* metaboliser genotypes.

We conducted univariate and multivariate linear regression models with *CYP2B6* extensive metaboliser genotype as the reference group and the results are outlined in **Table 2**. In univariate models, *CYP2B6* slow metaboliser genotype was significantly associated with higher percentage change from baseline to week 48 of total cholesterol ($\beta = 13.77$, $p = 0.003$), LDL cholesterol ($\beta = 15.89$, $p = 0.008$) and HDL cholesterol ($\beta = 24.78$, $p = 0.002$), and these associations remained significant after adjusting for age, sex, weight, baseline CD4 cell count and baseline viral load. There were no significant associations between *CYP2B6* composite metaboliser genotypes and triglycerides, fasting glucose and ALT in all analyses. Sensitivity analyses were done on both univariate and multivariate linear regression models of ALT after removing participants with percentage difference above 500% and there were no significant associations found (data not shown). There were no independent

associations between the metabolic parameters and the covariates adjusted for in multivariate analyses (age, sex, weight, baseline CD4 cell count and baseline viral load). In all the regression analyses, there were no significant differences observed between extensive and intermediate metaboliser genotypes.

Discussion

We investigated associations between the *CYP2B6* composite metaboliser genotypes and change in metabolic parameters in ART-naïve participants starting efavirenz-based ART. We found significant positive associations between *CYP2B6* slow metaboliser genotype and total cholesterol, HDL cholesterol and LDL cholesterol concentrations in the univariate and multivariate analyses. No associations were found between *CYP2B6* slow metaboliser genotype and triglycerides, fasting glucose and ALT concentrations.

Our findings support a previously reported association between *CYP2B6* composite metaboliser genotypes and cholesterol (total, LDL and HDL) in South African participants enrolled in an observational study (17); significant associations were also reported between higher plasma efavirenz concentrations and elevated cholesterol (total, LDL and HDL) concentrations (17). An increase in total and LDL cholesterol is associated with increased risk of cardiovascular events, whereas an increase in HDL cholesterol is protective (25). In addition to the association with *CYP2B6* 516G→T, elevated HDL cholesterol has been associated with *ABCB1* 3435C→T (26). Pregnane X receptor (PXR) is involved in regulating lipids and cholesterol metabolism and modifies the risk of dyslipidaemia (27). Efavirenz-associated HDL cholesterol increase is mediated by the induction of PXR in a dose-dependent manner (28). Therefore, it is biologically plausible that *CYP2B6*-mediated plasma efavirenz concentration increase causes an increase in cholesterol.

We found no associations between *CYP2B6* slow metaboliser genotype and triglycerides or glucose concentrations and our results confirm those observed in a similar population in the South African observational study (17). In a genome-wide association study looking at multiple phenotypes, *CYP2B6* SNPs were not associated with elevated triglycerides (29). Efavirenz association with triglycerides concentrations has been widely reported (17, 30, 31). As the association between efavirenz and triglycerides is well established, it is possible that there are other predictors that are

more important than *CYP2B6*, such as apolipoprotein genes (32). The association between dysglycaemia and cumulative efavirenz use is also well reported (18, 19). Several mechanisms for efavirenz-associated dysglycaemia have been postulated, including mitochondrial toxicity and its ability to reduce secretion of adiponectin (an insulin-sensitising, antidiabetic adipokine) in adipocytes (19). However, the effect sizes in percentage change from baseline to week 48 were small (<5%), and a larger sample size is likely needed to ascertain whether there is an association between the *CYP2B6* slow metaboliser genotype and glucose concentrations.

We also found no associations between *CYP2B6* slow metaboliser genotype and percentage change in ALT concentrations. This is in contrast with findings from studies that have discovered associations between *CYP2B6* 516G→T (33) and NNRTI (both efavirenz and nevirapine)-associated hepatotoxicity, or *CYP2B6* 983T→C and nevirapine-associated elevation of aminotransferases (22). In an Ethiopian case-control study investigating pharmacogenetic predictors of drug-induced liver injury (DILI), *CYP2B6* 516G→T was reported to be significantly associated with efavirenz-DILI (34). However, this was not replicated in the GWAS study investigating the ART or antituberculosis DILI (35). In the 2NN study, high plasma efavirenz trough concentrations were shown to be associated with elevated transaminases (36), and the lack of association between *CYP2B6* slow metaboliser genotype and elevated transaminases observed in our study could not be explained. We posit that other possible mechanisms for hepatotoxicity, such as HLA type, could be more important and cannot be ascertained in a small sample size such as ours.

Our study contributes to further understanding of efavirenz pharmacogenetics in African populations. Where efavirenz is still used, identification of slow metabolisers will assist in determining the correct dose to use, as adverse events are dose related, with low-dose efavirenz considered to have more tolerability while able to maintain adequate virological control (37). This may delay or prevent the development of efavirenz-associated toxicities and subsequent cardiovascular complications.

Our study had limitations. First, among the 351 efavirenz recipients, only 171 (49%) of those participants consented to this pharmacogenetic sub-study. This reduced our power for detecting small percentages from baseline to week 48 between *CYP2B6* composite metaboliser genotypes. However, this sample was larger than the 57

participants in the pharmacogenetic study reported by Sinxadi *et al.*, which examined the association between *CYP2B6* composite metaboliser genotypes with dyslipidaemia and dysglycaemia (17). Second, we did not include efavirenz concentrations as a covariate. Third, we did not look at the effects of efavirenz-based ART on mitochondrial toxicity and epigenetics.

In conclusion, among the ART-naïve HIV-infected Black South Africans, efavirenz-based ART is significantly associated with high cholesterol (total, HDL and LDL) concentrations in *CYP2B6* slow metaboliser individuals over prolonged treatment (48 weeks). No association was found between *CYP2B6* composite metaboliser genotypes and glucose, triglycerides and ALT. In African populations, where there is high prevalence of *CYP2B6* slow metabolisers, larger and adequately powered studies are needed to establish associations between *CYP2B6* and metabolic toxicities.

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Conflict of interest statement

There is no conflict of interest.

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Table 1: Baseline characteristics

Variable	Total (N=171)	CYP2B6 composite metaboliser genotypes		
		Extensive (N=51)	Intermediate (N=74)	Slow (N=46)
Age (years)	32 (28 to 37)	31 (26 to 37)	31 (27 to 36)	32 (29 to 37)
Sex				
Male	74 (43.27)	24 (47.06)	27 (36.49)	23 (50.00)
Female	97 (56.73)	27 (52.94)	47 (63.51)	23 (50.00)
Weight (kg)	66.40 (59.05 to 79.20)	63.10 (57.85 to 70.10)	68.75 (61.10 to 81.10)	69.45 (58.92 to 79.10)
Height (m)	1.69 (1.63 to 1.76)	1.70 (1.64 to 1.76)	1.69 (1.63 to 1.76)	1.68 (1.63 to 1.76)
BMI (kg/m²)	23.71 (20.24 to 27.46)	21.36 (19.70 to 25.33)	24.41 (21.49 to 28.30)	24.11 (20.49 to 27.44)
CD4 count (cells/mm³)	292.0 (173.0 to 405.5)	290.0 (182.0 to 390.5)	303.0 (181.2 to 416.2)	267.5 (160.0 to 383.0)
Log viral load (log₁₀ copies/ml)	4.42 (3.76 to 5.00)	4.42 (3.87 to 4.80)	4.27 (3.50 to 5.05)	4.56 (4.03 to 5.07)
Metabolic parameters (mmol/L)				
Total cholesterol	3.78 (3.24 to 4.32)	3.82 (3.22 to 4.34)	3.78 (3.11 to 4.43)	3.74 (3.39 to 4.15)
LDL cholesterol	2.38 (1.89 to 2.82)	2.28 (1.95 to 2.88)	2.53 (1.93 to 2.86)	2.24 (1.75 to 2.65)
HDL cholesterol	1.08 (0.90 to 1.36)	1.17 (0.94 to 1.47)	1.06 (0.91 to 1.29)	1.04 (0.84 to 1.25)
Triglycerides	0.91 (0.72 to 1.21)	0.84 (0.63 to 1.12)	0.89 (0.72 to 1.19)	1.06 (0.75 to 1.46)
Fasting glucose	4.30 (3.90 to 4.60)	4.20 (3.90 to 4.60)	4.20 (3.90 to 4.56)	4.40 (4.10 to 4.80)
ALT	21.50 (14.00 to 31.25)	20.00 (15.00 to 29.00)	22.00 (14.50 to 30.50)	23.00 (13.00 to 47.75)

Data represented in median (IQR) and n (%). ALT = Alanine transaminase, LDL = Low-density lipoprotein, HDL = High-density lipoprotein, BMI = body mass index, n = number of participants and IQR = interquartile range.

Table 2: Univariate and multivariate linear regression for percentage difference from baseline to week 48 of metabolic parameters, with CYP2B6 extensive metaboliser serving as the reference group.

Variable	Univariate β -coefficient (95%CI)	P-value	Multivariate ^c β -coefficient (95%CI)	P-value
Total cholesterol^a				
Intermediate metaboliser	5.66 (-1.87 to 13.19)	0.138	6.29 (-1.04 to 13.63)	0.115
Slow metaboliser	13.78 (5.46 to 22.10)	0.003	14.93 (6.99 to 22.86)	<0.001
LDL cholesterol^a				
Intermediate metaboliser	6.22 (-3.69 to 16.12)	0.208	5.99 (-4.14 to 16.13)	0.253
Slow metaboliser	15.89 (4.94 to 26.83)	0.008	15.57 (4.60 to 26.54)	0.014
HDL cholesterol^a				
Inter metaboliser	10.01 (-5.18 to 25.20)	0.217	9.72 (-4.33 to 23.77)	0.306
Slow metaboliser	24.78 (7.99 to 41.57)	0.002	24.22 (9.01 to 39.44)	<0.001
Triglycerides^a				
Intermediate metaboliser	-10.79 (-29.58 to 8.00)	0.316	-9.07 (-28.94 to 10.80)	0.448
Slow metaboliser	-17.01 (-37.77 to 3.75)	0.094	-15.00 (-36.51 to 6.52)	0.152
Fasting glucose^a				
Intermediate metaboliser	5.31 (-3.86 to 14.47)	0.246	5.80 (-3.85 to 15.44)	0.229
Slow metaboliser	3.88 (-6.25 to 14.01)	0.325	4.39 (-6.07 to 14.85)	0.298
Alanine transaminase (ALT)^b				
Intermediate metaboliser	-11.45 (-78.00 to 55.09)	0.723	-16.81 (-86.96 to 53.35)	0.660
Slow metaboliser	36.69 (-36.75 to 110.14)	0.295	30.63 (-45.59 to 106.85)	0.370

^a N = 160 (11 observations deleted due to missingness), ^b N = 159 (12 observations deleted due to missingness), ^c Adjusted for age, sex, weight, CD4 cell count at baseline and log viral load at baseline; LDL = Low-density lipoprotein, HDL = High-density lipoprotein; and 95%CI = 95% confidence interval.

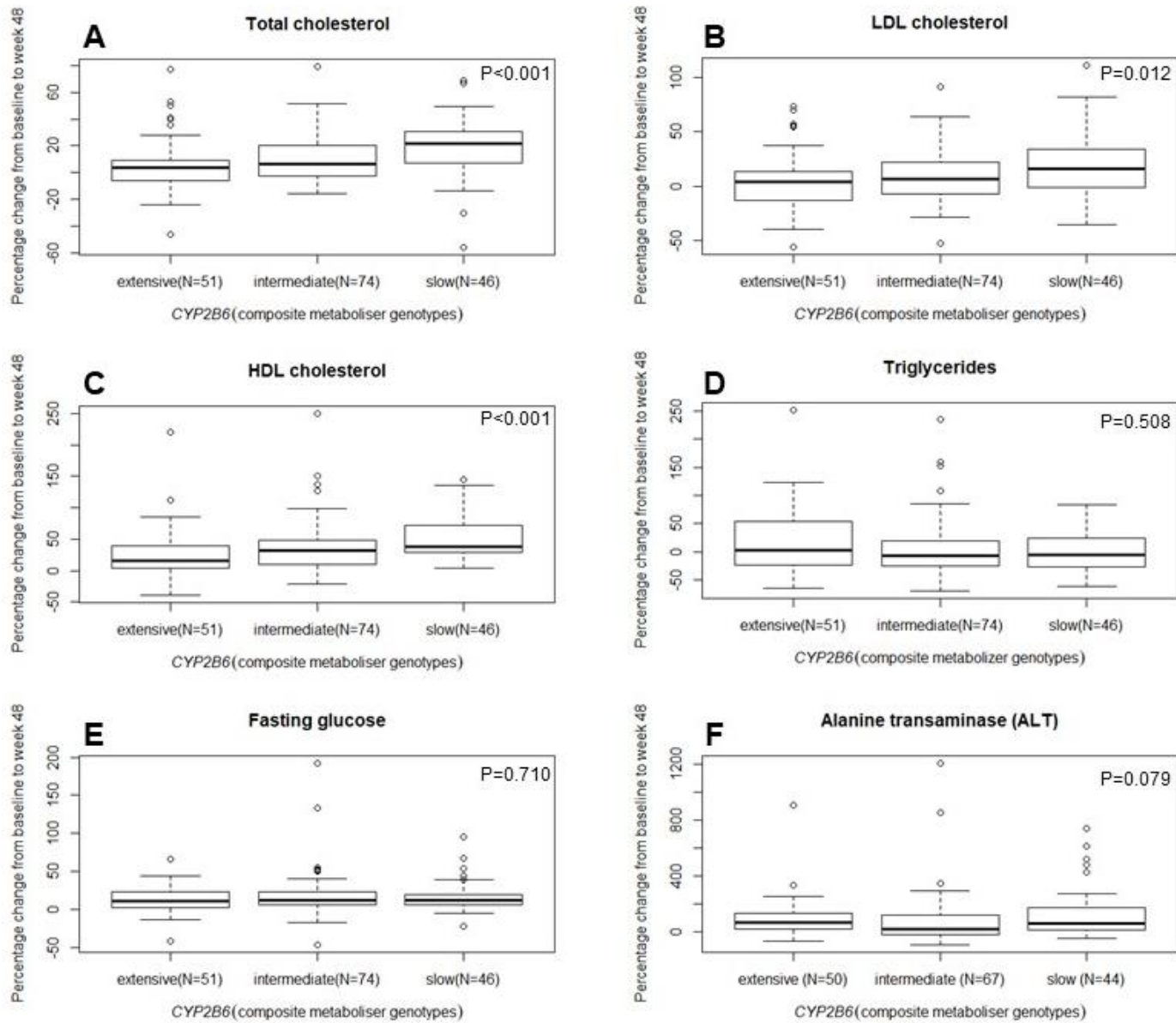


FIGURE 1. Boxplots for percentage change from baseline to week 48 of metabolic parameters by *CYP2B6* composite metaboliser genotypes. The x-axis represents the *CYP2B6* composite metaboliser genotypes (extensive, intermediate and slow) and the y-axis represents the percentage change from baseline to week 48 of each metabolic parameter: (A) Total cholesterol, (B) LDL cholesterol, (C) HDL cholesterol, (D) Triglycerides, (E) Fasting glucose and (F) Alanine transaminase. Boxes indicate median (IQR), whiskers indicate the minimum and maximum values, and the circles are the outliers. LDL = Low-density lipoprotein and HDL = High-density lipoprotein. Kruskal-Wallis test is represented by P-values.

CHAPTER 4
SUPPORTING DOCUMENTS

Appendices:

1. Ethics approval letter
2. Study protocol
3. British Journal of Clinical Pharmacology (BJCP) Author Guidelines

Appendix 1
Ethics approval letter



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee



Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za

Website: www.health.uct.ac.za/fhs/research/humanethics/forms

13 July 2020

HREC REF: 393/2020

Prof P Sinxadi

Division of Clinical Pharmacology
K-45.47, OMB

Email: Phumla.sinxadi@uct.ac.za

Student: mkgles018@myuct.ac.za

Dear Prof Sinxadi

PROJECT TITLE: PHARMACOKINETIC INTERACTIONS BETWEEN EFAVIRENZ AND ISONIAZID AND THE EFFECT OF CYP2B6 AND NAT2 METABOLIZER GENOTYPES ON EFAVIRENZ-RELATED TOXICITIES IN PATIENTS INITIATING ISONIAZID PREVENTIVE THERAPY MPHIL CANDIDATE-MR LESIBA MESHACK MAKGAI

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020.

Approval is granted for one year until the 30 July 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Mr Lesiba M Makgai will also be Involved in this study.

Please quote the HREC REF In all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal Investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely



PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix 2
Study protocol

STUDY PROTOCOL

Title:

EFAVIRENZ PHARMACOGENETICS AND METABOLIC TOXICITY IN BLACK SOUTH AFRICANS

AIM:

To determine how *CYP2B6* composite metaboliser genotypes influence the lipids, glucose, and ALT concentrations.

Objectives:

Primary objective:

- To determine the association between efavirenz pharmacogenetics and dyslipidaemia, dysglycaemia and hepatotoxicity.

Endpoints:

- Association between *CYP2B6* composite metaboliser genotypes and lipids (HDL cholesterol, LDL cholesterol, total cholesterol, and triglycerides).
- Association between *CYP2B6* composite metaboliser genotypes and fasting glucose.
- Association between *CYP2B6* composite metaboliser genotypes and alanine transaminase (ALT).

Methods:

Study Design

Prospective cohort study (pharmacogenetics sub-study).

Setting

We conducted a pharmacogenetics sub-study of the ADVANCE study, which is reported elsewhere (1). Briefly, ADVANCE study which was a 96-week randomized, phase 3 non-inferiority clinical trial in which 1053 HIV-positive, antiretroviral therapy (ART)-naïve individuals randomly assigned to one of three treatment arms, with each arm comprising a three-drug regimen. Participants received either: 1) dolutegravir, tenofovir alafenamide fumarate (TAF), and emtricitabine (FTC); 2) dolutegravir,

tenofovir disoproxil fumarate (TDF), and FTC; or 3) efavirenz (EFV), TDF, and FTC. The study was conducted at Witwatersrand Reproductive Health and HIV Institution (WRHI), South Africa.

Participants

Among 351 efavirenz recipients, only 171 (49%) consented to Pharmacogenetic sub-study in the efavirenz-arm (arm no.3) and their blood samples were subjected to genetic testing.

Inclusion criteria

Inclusion criteria for this pharmacogenetics sub-study were ADVANCE participants randomised to the efavirenz arm (together with tenofovir disoproxil fumarate (TDF) and emtricitabine) who consented to genetic testing.

Exclusion Criteria

Exclusion criteria included those who were receiving treatment with any other investigational drugs or drugs known to interact with efavirenz, and those with active tuberculosis coinfection.

Ethical consideration

The study was approved by the University of Cape Town and University of the Witwatersrand Human Research Ethics Committees.

Characterization of Genetic Polymorphisms

The extraction of DNA was done using the salting-out method. Genotyping was done using the MassARRAY® single nucleotide polymorphism (SNP) genotyping system (Agena Bioscience, San Diego, California, USA) at Inqaba Biotechnical Industries, Pretoria, South Africa. Genotyping performed included SNPs *CYP2B6* 516G→T (rs3745274), *CYP2B6* 983T→C (rs28399499) and *CYP2B6* 15582C→T (rs4803419). Composite genotypes of *CYP2B6* 15582/516/983 were assigned as extensive, intermediate and slow metabolisers as follows: 15582CC-516GG-983TT or 15582CT-516GG-983TT as extensive metaboliser genotypes; 15582TT-516GG-983TT, 15582CC-516GT-983TT, 15582CC-516GG-983CT, 15582CT-516GT-983TT or 15582CT-516GG-983CT as intermediate metaboliser genotypes; and 15582CC-

516GT-983CT, 15582CC-516GG-983CC or 15582CC-516TT-983TT as slow metaboliser genotypes (2).

Statistical analysis

The demographic characteristics of participants in the pharmacogenetic sub-study were described using medians (interquartile ranges) and proportions for continuous and categorical data, respectively. The data was analysed using R-Software (3). Boxplots, Kruskal-Wallis test, univariate and multivariate linear regression analyses were used to determine the relationships between the *CYP2B6* composite metaboliser genotypes and the percentage difference (which is the difference or change from baseline to week 48) of the following metabolic parameters: total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, fasting glucose and ALT. Participants with *CYP2B6* extensive metaboliser genotypes were used as a reference group to compare with the intermediate and slow metaboliser groups. Univariate linear regression determined the association between the *CYP2B6* metaboliser genotypes and metabolic parameters. In multivariate linear regression, we adjusted for age, sex, weight, CD4 cell count at baseline and viral load at baseline.

References:

1. Venter WDF, Moorhouse M, Sokhela S, Fairlie L, Mashabane N, Masenya M, et al. Dolutegravir plus Two Different Prodrugs of Tenofovir to Treat HIV. *N Engl J Med*. 2019;381(9):803-15.
2. Sinxadi PZ, Leger PD, McIlleron HM, Smith PJ, Dave JA, Levitt NS, et al. Pharmacogenetics of plasma efavirenz exposure in HIV-infected adults and children in South Africa. *Br J Clin Pharmacol*. 2015;80(1):146-56.
3. R: a language and environment for statistical computing [Internet]. R Foundation for Statistical Computing. 2014 [cited 18 October 2020]. Available from: <https://www.r-project.org/>.

Appendix 3
BJCP Author Guidelines

Author Guidelines for *British Journal of Clinical Pharmacology*

ORIGINAL ARTICLES

Original articles can be unsolicited, or they may be commissioned as standalone papers or as part of a Themed Issue or Series.

- **Word limit:** 4,000 words.
If your Original Article is going to exceed significantly 4,000 words, we require justification for this on submission.
- **Abstract:** 250 words maximum; structured with the following headings: Aim, Methods, Results, Conclusion.
- **Sections:** Original Articles should be structured using the headings: Introduction, Methods, Results and Discussion.
- **References:** No limit.
- **Figures/tables:** Preferably no more than 8 figures and tables.
- **Bullet point summary:** After the title page, you should include two statements, which should be succinct, accurate and specific:
 1. **What is already known about this subject:** In up to 3 short bullet point sentences (no more than 50 words), summarise the state of scientific knowledge on this subject before you did your study and say why this study needed to be done.
 2. **What this study adds:** In up to 3 short bullet point sentences (no more than 50 words), give a simple answer to the questions “What do we now know as a result of this study that we did not know before?” and “What take-home message do you want to impart to readers?”

PREPARING THE SUBMISSION

The main text should be uploaded as a word document or equivalent editable format. Tables should be included at the end of the document. All figures, supplementary information and Graphical Abstracts should be uploaded separately.

General Style Points

The following points provide general advice on formatting and style:

- **Stereoisomers.** When a drug can exist as stereoisomers or diastereomers (e.g. geometrical isomers), the form of compound studied must be designated as follows in the methods section. In the case of racemates the prefix *rac-* should precede the drug name (for example *rac*-propranolol). When possible the absolute configuration of enantiomers should be indicated (for example (*S*)-warfarin). Similarly, geometrical isomerism should be indicated by the prefixes *Z/E* or *cis/trans*. When appropriate, the interpretation of data obtained using mixtures of isomers should take account of stereochemical aspects.

- **Drug names.** Prescribed drugs should be designated by an International Non-proprietary Name (recommended, rINN, or proposed, pINN). If such a name is not available, a drug should be designated by its British Approved Name (BAN; for example hyoscyamine) or its chemical name (for example glyceryl trinitrate). When a mixture of drugs has a combination BAN (for example co-trimoxazole, co-fluampicil), that should be used.
For brevity, a company's code name may be used, but in that case the full chemical name or a figure showing the structure of the drug should be given in the introduction or a reference provided that gives this information.
Some mediators with well-established common names (*e.g.* prostacyclin) are also prescribed as licensed preparations with an rINN (*e.g.* epoprostenol). In such cases the rINN should be used in the context of therapeutic use. Sometimes English and American usage varies, as with adrenaline / epinephrine and noradrenaline / norepinephrine. “Adrenaline / noradrenaline” relate clearly to terms such as “noradrenergic”, “adrenergic” and “adrenal gland” but we will accept the term preferred by authors.
- **Symbols.** A set of standard symbols in pharmacokinetics and pharmacodynamics can be found [here](#).
 - **Abbreviations.** In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Articles should not include an abbreviations section. Each abbreviation must be defined at first use, either in the text, in a figure or table, or in their associated legends.
 - **Numbers.** Under 10 are spelled out, except for measurements with a unit (8mmol/l), age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).
- **Units.** SI units (mass or molar units) should be used. If other units are used, a conversion factor should be included in the Methods section. Solidus should be avoided as far as possible and the negative index used instead, *e.g.* mg kg⁻¹ rather than mg/kg.

Structuring your submission

- a. **Title page**, which should contain:
 - i. The title should give an informative and accurate indication of the content of the paper. It should be no longer than 150 characters (including spaces). The title should not contain abbreviations (see [Wiley's best practice SEO tips](#));
 - ii. A short running title of less than 40 characters;
 - iii. The full names of the authors;
 - iv. The author's institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
 - v. For original articles and short reports where interventions were performed with human subjects/patients and or substances administered, please add a Principal Investigator statement ‘The authors confirm that the Principal Investigator for this paper is X and that he/she [delete as appropriate] had direct clinical responsibility for patients.’ If there is no Principal Investigator, please also state this;

- vi. Keywords (these are used to identify potential referees and as indexing terms);
- vii. Word count, table count, figure count.
- b. **‘What is already known about this subject’ and ‘What this study adds’** statements (if applicable to the manuscript type).
- c. **Abstract** (un/structured, depending on manuscript type):
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