

**Pharmacogenomics and Pharmacokinetics of dolutegravir and
tenofovir in Southern Africans living with HIV**

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DECLARATION

I, Zinhle Andile Cindi, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

I, hereby declare that this thesis includes three journal manuscripts. Two (Chapters 3 and 5) of the three manuscripts included in this thesis have been published in international peer reviewed journals. The last manuscript (Chapter 4) has been accepted for publication. As the leading author, I performed genetic association analyses, wrote the first drafts, and revised the manuscripts with inputs from all co-authors for all included publications. I provided responses to journal reviewers and revised the manuscripts accordingly

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publications:

1. **Cindi Z**, Kawuma AN, Maartens G, Bradford Y, Venter F, Sokhela S, Chandiwana N, Venter F, Wasmann RE, Denti P, Wiesner L, Ritchie MD, Haas DW, Sinxadi P.
Pharmacogenetics of Dolutegravir Plasma Exposure Among Southern Africans living with HIV. *J Infect Dis.* 2022; jiac174 (**Chapter 3 of thesis**)
2. **Cindi Z**, Kawuma AN, Maartens G, Bradford Y, Sokhela S, Chandiwana N, Venter F, Wasmann RE, Denti P, Wiesner L, Ritchie MD, Haas DW, Sinxadi P.
Pharmacogenetics of tenofovir clearance among Southern Africans living with HIV.
(Accepted for publication) (**Chapter 4 of thesis**)

3. **Cindi Z**, Maartens G, Bradford Y, Venter WDF, Sokhela S, Chandiwana NC, Haas DW, Sinxadi P.

Genetic Associations with Weight Gain among South Africans who Initiated Dolutegravir-Containing and Tenofovir-Containing Regimens. *J Acquir Immune Defic Syndr*. 2021;87(3):1002–9 (**Chapter 5 of thesis**)

ABBREVIATIONS

ART	Antiretroviral therapy
AUC	Area under the concentration time curve
AUC _{VAR}	Unexplained variability in dolutegravir area under the concentration-time curve
BCRP	Breast cancer resistance protein
BLQ	Below limit of quantification
CAB	Cabotegravir
CL _{BSV}	Unexplained variability in dolutegravir/tenofovir clearance
C _{max}	Maximum concentration
C _{min}	Minimum concentration
CPIC	Clinical Pharmacogenetics Implementation Consortium
C _{trough}	Trough plasma concentration
CWRES	Conditional weighted residual
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
FFM	Fat-free mass
FTC	Emtricitabine
GWAS	Genome-wide association study
HBV	Hepatitis B virus
HOMA-IR	Homeostasis model assessment of insulin resistance
HREC	Human Research Ethics Committee
HWE	Hardy-Weinberg Equilibrium
IDB	Identity by descent
INSTI	Integrase strand transfer inhibitor
IQR	Interquartile range

LD	Linkage disequilibrium
LMICs	Low- and middle-income countries
MAF	Minor allele frequency
MDS	Multidimensional scaling
MRP	Multidrug resistance protein
MTT	Mean transit time
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitors
OAT	Organic anion transporter
OCT	Organic cation transporter
OFV	Objective function value
P-gp	P-glycoprotein
PBMCs	Peripheral blood mononuclear cells
PD	Pharmacodynamics
PEPFAR	US President Emergency Plan For AIDS Relief
PI	Protease inhibitor
PK	Pharmacokinetics
PLWHIV	People living with HIV
PrEP	Pre-exposure prophylaxis
RCT	Randomized controlled trial
SNPs	Single nucleotide polymorphisms
STR	Single-tablet regime
TAF	Tenofovir alafenamide
TDF	Tenofovir disoproxil fumarate
TEE	Tenofovir, emtricitabine, efavirenz

TLD	Tenofovir disoproxil fumarate, lamivudine and dolutegravir
TLE	Tenofovir, lamivudine and emtricitabine
T _{max}	Time to maximum concentration
TRPML1	Transient receptor potential mucolipin-1
UGT1A1	Uridine diphosphate (UDP)-glucuronosyltransferase 1A1
VANGARD	Vanderbilt Technologies for Advanced Genomics Analysis and Research Design
VANTAGE	Vanderbilt Technologies for Advanced Genomics
VPC	Visual predictive checks
WHO	World Health Organization

ABSTRACT

Background

The World Health Organization (WHO) recommends dolutegravir in combination with a nucleos(t)ide reverse transcriptase inhibitor (NRTI) backbone as the preferred first-line regimen for people living with HIV (PLWHIV) initiating antiretroviral therapy (ART). Tenofovir alafenamide (TAF) and tenofovir disoproxil fumarate (TDF) are both prodrugs of tenofovir. However, plasma tenofovir exposure is higher when given as TDF, and TAF yields lower plasma but higher intracellular tenofovir concentrations. Although generally well tolerated, excessive weight gain has been associated with dolutegravir and (TAF) in PLWHIV initiating ART or those switching from efavirenz- or TDF-containing ART. Interindividual variability in dolutegravir and tenofovir pharmacokinetics or the interindividual differences in host response may, in part, be explained by host genetics. We characterized associations between genetic polymorphisms and dolutegravir exposure, tenofovir clearance and magnitude of weight gain in Southern Africans initiating ART.

Methods

We collected clinical and laboratory data in adults randomized to initiate TAF or TDF in dolutegravir-containing arms of the ADVANCE trial (NCT03122262). We measured dolutegravir and tenofovir concentrations and developed population pharmacokinetic models for dolutegravir and tenofovir using non-linear mixed-effects modelling. Genome-wide genotyping followed by imputation was performed. Linear regression models examined associations between genetic polymorphisms and unexplained variability in dolutegravir area under the concentration-time curve (AUC_{VAR}), unexplained variability in tenofovir clearance, and percentage weight gain from baseline to week 48.

Results

Considering genetic associations with unexplained variability in dolutegravir area under the concentration-time curve (AUC_{VAR}), the lowest P-value with AUC_{VAR} was *UGT1A1* rs887829 ($P = 1.8 \times 10^{-4}$), which was also associated with $\log_{10}$ bilirubin ($P = 8.6 \times 10^{-13}$). After adjusting for rs887829, AUC_{VAR} was independently associated with rs28899168 in the *UGT1A* locus ($P = 0.02$), as were bilirubin concentrations ($P = 7.7 \times 10^{-8}$). In the population pharmacokinetic model, compared to C/C, rs887829 T/T and C/T were associated with 25.9% and 10.8% decreases in dolutegravir clearance, respectively. There were no significant genome-wide associations.

Considering genetic associations with unexplained variability in tenofovir clearance, we found no significant associations with tenofovir clearance for either TAF or TDF among 5 polymorphisms previously associated with tenofovir pharmacokinetics (lowest P-value > 0.3 for each drug). Among 11 polymorphisms selected based on both prior strong association with any drug phenotype in PharmGKB and any genome-wide association with any trait in the GWAS catalog, *IFNL4* rs12979860 T>C was significantly associated with increased tenofovir clearance (TAF: $P = 0.003$; TDF: $P = 0.003$). In genome-wide analyses, the lowest P-values for tenofovir clearance in the TAF and TDF arms were with *LINC01684* rs9305223 ($P = 3.0 \times 10^{-8}$) and intergenic rs142693425 ($P = 1.4 \times 10^{-8}$), respectively. Four additional polymorphisms were genome-wide significant.

In genome-wide multivariate linear regression analyses adjusting for baseline age, sex, concomitant NRTI, and population stratification, there were no significant associations between 59 polymorphisms relevant to dolutegravir and tenofovir disposition and the percentage weight gain. We found a genome-wide significant association between *TMEM163* rs7590091 and percentage weight gain from baseline to week 48 ($P = 3.7 \times 10^{-8}$).

Conclusion

Among Southern African people living with HIV randomized to TAF or TDF, we identified several potential genetic associations with dolutegravir exposure, tenofovir clearance and weight gain. The novel associations between dolutegravir AUC_{VAR} and rs28899168, tenofovir clearance and *IFNL4* rs12979860, and weight gain and *TMEM163* rs7590091 require independent replication. These findings enhance our understanding of dolutegravir and tenofovir pharmacogenetics among Southern Africans living with HIV.

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Chapter 1

Introduction

Context

For people living with HIV (PLWHIV), using antiretrovirals has improved morbidity and mortality.¹ The increased use of antiretroviral therapy (ART) in sub-Saharan Africa is a major achievement in response to the burden of HIV infections. As of March 2022, approximately 3.2 million PLWHIV in South Africa were on a dolutegravir-based regimen.² South Africa accounts for over 10 percent of HIV-related deaths and 15 percent of new infections.³

Unfortunately, there are challenges with ART which include increasing transmitted drug resistance,⁴ poor coverage of viral load monitoring,⁵ and low numbers of people who are verified to have failed first-line regimen ART switching to second-line regimens.⁶

The WHO had recommended as first-line regimen tenofovir, lamivudine (or emtricitabine) and efavirenz, and a second-line regimen of zidovudine, lamivudine, with a protease inhibitor in people who failed first-line ART.⁷ Currently the recommended first-line regimen is dolutegravir with tenofovir and lamivudine in people initiating ART. This regimen can be extended to people with a recent viral load measurement of less than 1000 copies per mL.⁸ People using tenofovir, lamivudine and efavirenz with virological failure are recommended to switch to dolutegravir, lamivudine and zidovudine.⁹

With dolutegravir-based regimens, there is a reduced need to switch to other antiretroviral drugs and a reduced risk of developing major drug resistance mutations compared to efavirenz-based regimens.^{10,11} Dolutegravir-based regimens are the preferred first-line treatment in sub-Saharan Africa because of the high potency of dolutegravir, good tolerability and low cost.^{9,12} The fixed-dose combination of dolutegravir, tenofovir (tenofovir disoproxil

fumarate (TDF)) and lamivudine (TLD), has been introduced in many countries in sub-Saharan Africa, including South Africa.¹³

South Africa adapted to the TLD regimen in 2019.¹⁴ Patients can switch from a first-line TEE regimen (tenofovir, emtricitabine, efavirenz) or TLE regimen (tenofovir, lamivudine, emtricitabine) or nevirapine-based regimens to TLD regardless of viral load results.¹⁵ This combination is generally well tolerated and is easier to take because of its size (one small tablet daily).³ However, if patients fail on TEE or TLE, they are not transitioned to TLD because of the feared recycling of tenofovir which may cause drug resistance.¹⁴ In such cases, patients are switched to a second-line regimen of zidovudine, lamivudine and dolutegravir.

There are potential issues with dolutegravir and tenofovir. For dolutegravir-based regimes, issues include potential for virological failure and drug resistance if dolutegravir is used with nucleoside reverse transcriptase inhibitors that HIV is resistant to,⁹ and the possible association of dolutegravir with increased risk of neural tube defects in infants.¹⁶ With tenofovir, given as tenofovir alafenamide (TAF) or TDF, potential issues such as bone and renal toxicities have been associated with TDF,^{7,17} while weight gain has been associated with TAF.¹⁸

Pharmacogenetics of antiretroviral drugs

Pharmacogenetics refers to the effect of genetic polymorphisms on drug response.

Pharmacogenetics aids in identifying individuals who are likely to experience adverse events or sub-optimal response to standard care therefore improving treatment efficacy and safety and reducing treatment costs caused by adverse drug reactions.¹⁹ It has been observed that genetics differences among PLWHIV may have variability in antiretroviral drug response and toxicity.

Pharmacogenetics of antiretroviral drugs in Africa

People of African descent have high genetic variability.²⁰ This makes pharmacogenetics research valuable for Africans. In a recent publication on whole genome sequencing among Africans, a large number of new single nucleotide variants were identified.²¹ This knowledge is important because <25% of participants in genomics research are non-Europeans, consequently missing many human genetic variants.²² This supports the need for the emerging role of pharmacogenetics studies across Africa which will identify variants that are applicable to populations of African descent.^{22–24}

Pharmacogenetics research among PLWHIV in Africa can provide novel approaches to precision medicine. This may aid policy makers to base healthcare decisions for ART on genetic makeup of populations and communities.²³ This is important because of the inter- and intra-population differences in pharmacokinetics (PK) and pharmacodynamics (PD) of a single prescribed dose that may not be appropriate for treatment across different populations.²⁵ The genetics of antiretrovirals affecting PK and PD is poorly understood in African populations. This limits treatment optimization because identified variants may be useful for screening prior to treatment among PLWHIV.^{24,26}

International cohort studies have determined the safety and efficacy of ART in HIV cohorts and controls. However, a few have investigated the role of genetics on drug disposition and treatment outcomes in African populations.²³ This is worrisome as Africa is the epicentre of HIV, and African populations have the greatest genetic diversity worldwide.^{27–29} This highlights the urgency of pharmacogenetics studies in Africa to determine genetic variants that can be used to screen patients prior to initiating treatment. Knowledge on the pharmacogenetics of dolutegravir and tenofovir is important as they are part of the preferred ART regimens in South Africa.

Project Rationale

Dolutegravir is replacing non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as efavirenz in first-line ART and HIV-1 protease inhibitors in second-line ART regimens. The standard of care recommended by WHO and in use in South Africa is (efavirenz + TDF + emtricitabine). The SPRING-2 trial showed that dolutegravir has improved safety and efficacy on a backbone of abacavir and lamivudine.³⁰ Dolutegravir is well tolerated with a greater resistance barrier than efavirenz, it requires a smaller dose than efavirenz (50 mg versus both recommended efavirenz doses (600/400 mg)), which would likely reduce manufacturing costs.

TAF, on the other hand, is replacing TDF in standard of care in some settings.³¹ TAF is reported to have an improved safety profile, as it is associated with less renal and bone toxicity than TDF. The manufacturing costs are reduced with TAF because of the low dose requirements (25 mg versus 300 mg for TDF).³² With the better tolerability of dolutegravir and TAF, and the higher barrier to resistance of dolutegravir, we may expect improved patient outcomes and more durable treatment.

Objectives of the PhD project

This project was a sub-study of the ADVANCE clinical trial. We hypothesized that the pharmacogenetics of dolutegravir and tenofovir may affect drug exposure and toxicity, e.g., weight gain among PLWHIV in Southern Africa. The key research objectives addressed in this thesis are listed below:

1. The pharmacogenetics of dolutegravir plasma exposure among Southern Africans living with HIV (**Chapter 3**). This study helps to identify genetic polymorphisms that may affect dolutegravir plasma exposure.

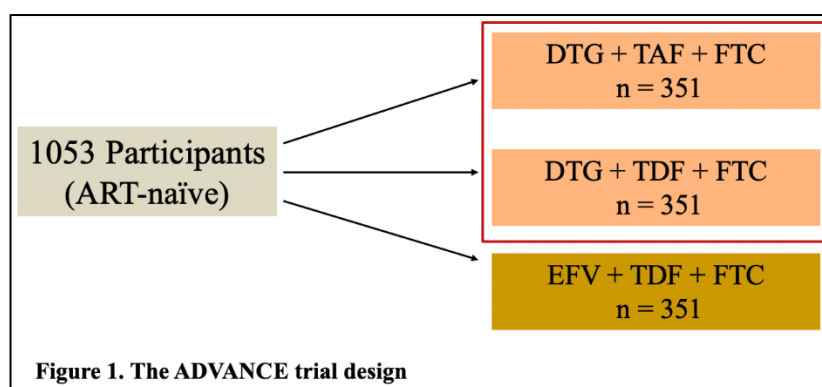
2. The pharmacogenetics of tenofovir clearance among Southern Africans living with HIV (**Chapter 4**). This study investigates genetic polymorphisms that affect tenofovir clearance.
3. Genetic associations with weight gain among South Africans who initiated dolutegravir-containing and tenofovir-containing regimens (**Chapter 5**). This study will help better define human genetics impact on the safety profile of dolutegravir and tenofovir.

Overview of study design and setting

Design of parent study and population

The ADVANCE study in South Africa is a 96-week randomized, phase 3 non-inferiority clinical trial (**Figure 1**). 1053 HIV-positive, ART-naïve individuals were randomly assigned

to one of three treatment arms, with each arm comprising a three-drug regimen. Participants received either: 1) dolutegravir, TAF, and



emtricitabine; 2) dolutegravir, TDF, and emtricitabine; or 3) efavirenz, TDF, and emtricitabine.

Eligibility

Inclusion criteria: Participants in ADVANCE were at least 12 years of age and weighed at least 40 kg. Patients had a documented laboratory diagnosis of infection with HIV-1 (positive enzyme-linked immunosorbent assay HIV-1 antibody test) and with plasma HIV-1 RNA $\geq$ 500 copies/mL at screening. The present analyses included individuals who participated in

ADVANCE, consented to the genetic sub-study from the dolutegravir containing arms (**Figure 1** highlighted in red) and provided a sample for human genetic analysis.

Exclusion criteria: Patients were excluded from ADVANCE if they had previously received more than 30 days of treatment with any form of ART or received any ART within the prior six months of screening. In addition, patients who had participated in a study with an investigational drug within 60 days of screening or who were receiving treatment with any other investigational drug or device were ineligible to participate. Patients with active tuberculosis and/or were on anti-tuberculous therapy at the time of the screening or enrolment visits were ineligible to participate.

Confidentiality

This study used deidentified data and specimens. The investigators did not have any access to any participant-identifying information. The ADVANCE protocol was approved at the University of the Witwatersrand (WRHI 060). Ethical approval for this PhD project was obtained from the University of Cape Town Human Research Ethics Committee (HREC 403/2019), and from the Vanderbilt Institutional Review Board (IRB #080478).

Genotyping

DNA extraction was performed at Sydney Brenner Institute for Molecular Bioscience at the University of the Witwatersrand, using the salting out method as described elsewhere.³³ Samples were labelled with coded identifiers. Stored DNA was genotyped using the Illumina Infinium Multi-Ethnic Global BeadChip (MEGA^{EX}) at Vanderbilt Technologies for Advanced Genomics (VANTAGE). Post-genotype quality control was performed by Vanderbilt Technologies for Advanced Genomics Analysis and Research Design (VANGARD).

All quality control steps were performed using PLINK version 1.9.³⁴ Genotyping efficiency per participant was > 95% for all samples. After quality control, data were imputed and excluded polymorphisms with imputation scores < 0.3, genotyping call rates < 99%, minor allele frequency (MAF) < 0.05, or Hardy-Weinberg Equilibrium (HWE) P values < 1.0×10^{-8} .

Pharmacokinetic sampling and analysis

Forty-one participants were enrolled for intense pharmacokinetic sampling for dolutegravir and tenofovir at steady state, with plasma samples collected pre-dose and 1, 2, 4, 6, 8 and 24-hours post-dose. Doses preceding intense sampling were observed and taken after a standard meal. For all other individuals, at least one plasma sample (sparse pharmacokinetic sampling) was collected at weeks 24, 36 or 48.

Summary of thesis structure

Chapter 2 is a literature review describing existing evidence on the pharmacokinetics, pharmacogenetics, safety, efficacy and tolerability of dolutegravir and tenofovir.

Chapter 3 presents findings on the pharmacogenetics of dolutegravir plasma exposure among Southern Africans living with HIV. The study is published in the Journal of Infectious Diseases but presented in a format consistent with the thesis document.

Cindi Z, Kawuma AN, Maartens G, Bradford Y, Venter F, Sokhela S, Chandiwana N, Wasmann RE, Denti P, Wiesner L, Ritchie MD, Haas DW, Sinxadi P. Pharmacogenetics of Dolutegravir Plasma Exposure Among Southern Africans living with HIV. *J Infect Dis.* 2022: jiac174.

Chapter 4 presents findings from the pharmacogenetics of tenofovir clearance among Southern Africans living with HIV. The study has been accepted for publication in the

Pharmacogenetics and Genomics journal but presented in a format consistent with the thesis document.

Chapter 5 presents findings on the genetic associations with weight gain among South Africans who initiated dolutegravir-containing and tenofovir-containing regimens. The study is published in the Journal of Acquired Immune Deficiency Syndromes but presented in a format consistent with the thesis document.

Cindi Z, Maartens G, Bradford Y, Venter WDF, Sokhela S, Chandiwana NC, Haas DW, Sinxadi P. Genetic Associations with Weight Gain among South Africans who Initiated Dolutegravir-Containing and Tenofovir-Containing Regimens. *J Acquir Immune Defic Syndr*. 2021;87(3):1002–9.

Chapter 6 presents a summary of all the preceding chapters as conclusions to the thesis.

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Chapter 2

Literature review

Background on dolutegravir and tenofovir

Dolutegravir

Dolutegravir, a second-generation integrase strand transfer inhibitor (INSTI), is highly potent and dissociates at a slower rate from integrase-DNA complexes than first generation INSTIs.¹

Dolutegravir has a plasma half-life of 14 hours, and is approved for once-daily dosing without the need for pharmacokinetic enhancement.^{2,3} In 2019, WHO recommended that dolutegravir should replace efavirenz, in combination with tenofovir disoproxil fumarate (TDF) and either lamivudine or emtricitabine for patients initiating or currently on ART, and for patients on subsequent-line therapy.^{4,5} A regimen comprising dolutegravir plus two nucleoside reverse transcriptase inhibitors (NRTIs) has proved to be more effective antiretroviral therapy (ART) than efavirenz plus two NRTIs, based on better tolerability, greater barrier to viral drug-resistance, and fewer drug-drug interactions.⁵

When used in first-line ART, dolutegravir has shown higher efficacy than efavirenz, atazanavir, and darunavir. It is also non-inferior to the first generation INSTI raltegravir.^{6–10}

Dolutegravir is active against some HIV-1 strains that are resistant to elvitegravir and raltegravir. This makes dolutegravir a viable option for patients with prior or current virological failure with elvitegravir- or raltegravir-based regimens.¹¹ A twice daily dolutegravir dose in INSTI experienced patients was shown to be more effective with minimal dolutegravir resistance, this indicates its potential use in salvage therapy.^{11–13} Dual therapy of dolutegravir and lamivudine was also shown to be an effective maintenance therapy for patients with viral suppression.¹⁴ In addition, when used as dual therapy in combination with lamivudine in first-line regimen, the combination was non-inferior to triple-

drug ART in patients with at most 500 000 copies per mL of plasma viral load and without pre-existing HIV drug resistance.¹⁵

Previously, because of the high cost, dolutegravir has been scarce in low- and middle-income countries (LMICs). However, an affordable new low-cost generic fixed-dose drug combination (300 mg tenofovir disoproxil fumarate, 300 mg lamivudine and 50 mg dolutegravir [TLD]) is now available.¹³ The Medicine Patent Pool has licensed 92 LMICs since September 2017 to obtain TLD at a median price of US\$75 per person per year. This price is similar to the current non-nucleoside reverse transcriptase inhibitors (NNRTIs)-based ART or even lower.¹⁶ There has been accelerated access of TLD, initiated by the US President Emergency Plan For AIDS Relief (PEPFAR), in countries supported by PEPFAR. This is to maximize the benefits of TLD and reduce the logistics for multiple drugs therapy.¹³

Tenofovir

Tenofovir, a nucleotide analogue, is not a substrate, inducer or inhibitor of cytochrome P450 and therefore has fewer drug interactions.^{17–19} Tenofovir, given as tenofovir disoproxil fumarate or tenofovir alafenamide, is part of the most preferred combination antiretroviral regimens because of its potency and ease of use.²⁰ It is given as once daily therapy with or without food. In clinical trial participants, it has been found that tenofovir in combination with emtricitabine or lamivudine and a third agent (NNRTI, or boosted protease inhibitor (PI), or an INSTI) results in potent antiretroviral effect leading to durable viral suppression.²¹ In addition, tenofovir with emtricitabine has been approved for pre-exposure prophylaxis (PrEP) in individuals at a high risk of HIV acquisition.²² A summary of the differences between TDF and TAF are shown on **Table 1**.

Table 1. Comparisons between TDF and TAF

TDF	TAF
Approved in 2001	Approved in 2015
Higher plasma tenofovir exposure	Lower plasma tenofovir exposure
Lower intracellular tenofovir diphosphate	Higher intracellular tenofovir diphosphate
Associated with bone and kidney toxicities	Associated with weight gain
The prescribed dose is 300 mg	The prescribed dose is 25 mg, but also available at 10 mg with a pharmacokinetic booster

Tenofovir disoproxil fumarate (TDF)

The WHO recommended phasing out stavudine due to toxicities such as irreversible mitochondrial toxicity, including lipodystrophy, peripheral neuropathy, and lactic acidosis.²³ Stavudine was rapidly replaced by TDF.²⁴ Studies in India and South Africa have shown that TDF has cost effectiveness benefits when measured against conventionally dosed stavudine,^{25–27} however, these findings may not be true when compared with lower doses of stavudine.²⁸ A trial in the United States including mostly Caucasian males compared 40 mg stavudine with TDF and found no differences in virological suppression or incidence of resistance after three years. However, lipid profiles were better with TDF, and lipodystrophy was strongly associated with stavudine 19% of patients compared to 3% in the TDF arm. TDF has well-described renal and bone effects.^{29,30}

TDF is the most widely used antiretroviral agent because of its long plasma, and intercellular $t_{1/2}$ (14-17 and >60 hours, respectively), and overall tolerability. The combination of TDF and emtricitabine is well tolerated and commonly used for PrEP, hepatitis B virus (HBV) and as the NRTI backbone in HIV-1 ART.³¹ When compared to TAF, TDF has more favourable metabolic effects with lipid suppressive effects demonstrated in previous studies.^{32,33}

Tenofovir Alafenamide (TAF)

Tenofovir alafenamide was developed to promote antiviral potency and clinical safety. TAF is recommended than TDF in some high-income countries because it is less toxic and has a smaller tablet size.³⁴ There is improved kidney safety because of the lower circulating plasma concentrations of tenofovir¹ which may result in lower concentrations in proximal tubule cells and less nephrotoxicity.³⁵ This suggests that, when compared to TDF, when a correct dose of TAF is administered, there could be improved clinical efficacy and long-term safety. For example, there are substantially reduced tenofovir exposures with improved pharmacodynamics of TAF dosing at 25 mg compared to 300 mg TDF.³⁶ This supports previous reports that TAF may confer similar clinical efficacy to TDF, whilst offering a favourable safety profile.^{37,38}

A monotherapy study in HIV treatment-naïve patients tested the hypothesis that TAF could be a safer and more effective prodrug for the delivery of tenofovir in HIV-infected cells. In this study, doses of 40 and 120 mg of TAF versus 300 mg of TDF were compared following 14 days of monotherapy.³⁹ In the TAF arms, there was greater intracellular tenofovir exposure relative to TDF suggesting that TAF could be a more suitable tenofovir prodrug.

With TDF-related nephrotoxicity risk factors, lower doses of TAF were studied with the aim of reducing overall systemic exposure of tenofovir while maintaining efficacy at least equivalent to TDF. A monotherapy study in HIV treatment-naïve patients was designed to compare 8, 25 and 40 mg TAF to 300 mg of TDF during a 10-day period.³⁶ The results were surprising. The 8 mg of TAF and the 300 mg TDF resulted in similar viral load reduction, while the 25 mg TAF resulted in a more significant drop than TDF (-1.46 versus -0.97 log₁₀ copies/ml). Interestingly, with 25 mg of TAF the systemic exposure of tenofovir is approximately 1/10 that of 300 mg TDF. This proves that when comparing the tenofovir

prodrugs, circulating tenofovir concentrations are unrelated to the antiviral effect.³⁶ In addition, the intracellular concentrations of tenofovir in peripheral blood mononuclear cells (PBMCs) were 7-fold higher in 25 mg TAF patients compared to 300 mg TDF. The high intracellular concentration but low plasma concentration of tenofovir in TAF^{20,40,41} are thought to be a pharmacokinetic attribute that may protect against adverse effects. This is because of the reduced serum concentration which results in lower exposure of kidneys and bone.³⁶

Multiple studies have confirmed the efficacy of TAF combination regimens.^{42–44} These studies included treatment-naïve adults and adolescents, treatment-experienced adults after regimen switches, and renal impairment studies. In summary, these studies found no significant differences in virological response compared to TDF in any of these patient populations. However, there were significantly smaller increases in serum creatinine, smaller decreases in bone mineral density, and less proteinuria and albuminuria, and all were consistent with lower levels of circulating tenofovir.

Currently, TAF is approved in five fixed-dose combination products for the treatment of HIV

(Table 2).⁴⁵ With the lower mass of TAF (25 mg) versus TDF (300 mg), a smaller single-tablet regime (STR) can be produced. For

Table 2. TAF approved fixed-dose combination drugs

Approved drug	Combination products
Symtuza	TAF/emtricitabine/darunavir/cobicistat
Genvoya	TAF/emtricitabine/elvitegravir/cobicistat
Descovy	TAF/emtricitabine
Odefsey	TAF/emtricitabine/rilpivirine
Biktarvy	TAF/emtricitabine/bictegravir

example, for Symtuza this was previously not possible.⁴⁵ The dose of TAF can be reduced to 10 mg when TAF is combined with a pharmacokinetic booster like cobicistat or ritonavir, which results in the same plasma TAF exposure as the un-boosted 25 mg dose.⁴⁶

The FDA approved the fixed-dose combination of Descovy for PrEP.⁴⁷ This was based on the DISCOVER PrEP trial where they compared the safety and efficacy of Descovy (n=2694) to Truvada (TDF/emtricitabine) (n=2693) in adult cisgender men who have sex with men and transgender women who have sex with men.⁴⁸ Descovy was non-inferior to Truvada and demonstrated statistically significant advantages with regards to renal and bone effects.

Dolutegravir and tenofovir pharmacokinetics

Dolutegravir pharmacokinetics

Dolutegravir is metabolized in the liver primarily by uridine diphosphate (UDP)-glucuronosyltransferase 1A1 (UGT1A1) with a small amount metabolized by cytochrome P450 (CYP) 3A, and its urinary excretion is <1%.^{49–51} Dolutegravir, administered orally, undergoes rapid absorption, with time to maximum concentration (T_{max}) ~2hr following a 50 mg dose,⁴⁹ and undergoes extensive metabolism.⁵² Though clinically not significant, higher fat content of a meal increases dolutegravir exposure.⁵³

Dolutegravir is a P-glycoprotein (P-gp) substrate and breast cancer resistance protein (BCRP). *In vitro* dolutegravir does not exhibit induction or inhibition of the following transporters: P-gp, BCRP, organic anion transporter (OAT)P1B1, OATP1B3, multidrug resistance protein (MRP) 2 or organic cation transporter (OCT)1. At clinically relevant concentrations, it may inhibit the renal OCT2 transporter.⁵² Dolutegravir has few drug-drug interactions⁵⁴ and is minimally excreted unchanged in the urine.⁴⁹ **Table 3** summarises dolutegravir pharmacokinetics parameters reported in healthy and HIV-infected individuals. These studies were not conducted in Africa. Participants were randomized to receive dolutegravir 2, 5, 10, 25, 50 and 100 mg or placebo.^{2,49} Dolutegravir demonstrated low interindividual pharmacokinetic variability, potent antiviral activity, and a predictable pharmacokinetics and pharmacodynamics interaction, supporting once-daily dosing without a

pharmacokinetic booster.² In addition, with dolutegravir's ease of dosing, and low drug interaction profile, it has become part of the most preferred combination of ART therapy.

Table 3. Dolutegravir pharmacokinetics parameters reported in uninfected and HIV infected individuals

Dolutegravir daily dose (mg)	Number of participants	C _{max} (µg/ml)	T _{max} (hour)	AUC _t (µg•h/ml)	C _{min} (µg/ml)	C _{trough} (µg/ml)
2 ^a	9	0.25 (25)	1.0 (0.42-3.00)	2.56 (29)	NA	0.04 (50)
10	8 ^b	1.47 (24)	0.50 (0.25-2.00)	16.7 (15)	0.27 (25)	0.35 (20)
	7 ^a	0.80 (23)	1.48 (0.50-3.00)	10.1 (20)	NA	0.19 (25)
25 ^b	10	3.09	1.00 (0.50-2.00)	38.4 (23)	0.66 (32)	0.84 (33)
50	8 ^b	6.16 (15)	1.00 (0.50-2.00)	76.8 (19)	1.48 (25)	1.64 (25)
	10 ^a	3.34 (16)	2.00 (0.97-4.00)	43.4 (20)	NA	0.83 (26)

All pharmacokinetic parameters except T_{max} are reported as mean (% coefficient of variation); T_{max}, is reported as median (range).

C_{max}, maximum concentration; T_{max}, time to maximum concentration; AUC_t, area under the concentration time curve during a dosage interval; C_{min}, minimum concentration; C_{trough}, trough plasma concentration.

^a Steady state concentrations obtained from HIV infected adults after 10 doses of monotherapy with dolutegravir tablets.⁴⁹

^b Steady state concentrations obtained from healthy adults after 10 doses of dolutegravir suspension.²

*Table adapted from⁵⁴

Tenofovir pharmacokinetics

Tenofovir is not a substrate, inducer or inhibitor of CYP450 enzymes and has limited drug interactions.¹⁹ When orally administered, it is rapidly absorbed and primarily eliminated unchanged in urine by both glomerular filtration and active proximal tubular secretion.^{18,21}

TDF is rapidly converted to tenofovir in plasma and metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFV-DP), which incorporates into proviral DNA and impairs its transcription to terminate the growing DNA chain.¹⁸ In addition, TDF does not modify the metabolism of other drugs.¹⁸

There is high variability in TAF metabolism within populations and between different populations. In a Chinese study including 64 participants, the C_{max} of TAF varied up to tenfold, while in fasted participants, the AUC_{0-t} varied up to 15-fold.⁵⁵ The association between race or ethnicity and the pharmacokinetics of TAF have previously been reported. In

a pharmacokinetics study of 20 Japanese and non-Japanese participants, TAF and tenofovir AUCs were approximately 30% higher in Japanese participants. In addition, the $C_{\max}$ values were 32% for TAF and 45% higher for tenofovir in the Japanese participants compared to non-Japanese.⁵⁶ These differences can be explained in part by genetic polymorphisms.

Dolutegravir and tenofovir pharmacogenetics

Pharmacogenetics is the effect of genes on drug response. Described below is an overview of the pharmacogenetics of dolutegravir and tenofovir.

Dolutegravir pharmacogenetics

Dolutegravir has fewer drug-drug interactions than many other antiretroviral drugs, in part because it does not induce or inhibit CYP isoforms or UDP-glucuronosyltransferases (UGTs).⁵⁷ However, drugs that have been reported to interact with dolutegravir include NNRTIs (efavirenz, darunavir, atazanavir, etc.) by altering *UGT1A1*.⁵⁸ There are several polymorphisms in the *UGT1A1* gene. In the promoter region, there are normally six TA repeats in the TATA box, in comparison to seven repeats observed in the *UGT1A1**28 (rs8175347) polymorphism, which decreases mRNA and protein expression levels.⁵⁹ Dolutegravir has been identified as a substrate for ATP-binding cassette transporters B1 (*ABCB1*) and G2 (*ABCG2*).⁶⁰

With dolutegravir primarily metabolized by UGT1A1, functional polymorphisms in the *UGT1A1* gene may affect dolutegravir exposure. In an analysis of pooled data from 89 individuals who had participated in nine Phase I and II clinical studies, frequent *UGT1A1* polymorphisms that are associated with Gilbert's syndrome (a condition that can lead to accumulation of unconjugated bilirubin) were associated with increased plasma dolutegravir exposure.⁶¹ Bilirubin is conjugated by UGT1A1.⁶² A population pharmacokinetic analysis found a negative correlation between bilirubin and the apparent clearance of dolutegravir,

which was attributed to competition of dolutegravir and bilirubin for UGT1A1. This suggests that the effect of plasma dolutegravir concentrations may be affected by changes in enzymatic activity and protein level of UGT1A1.⁶³

The frequency of *UGT1A1* differs according to race. *UGT1A1**28 (rs8175347) is relatively common, approximately 30-40% in Caucasian and African American populations, compared to *UGT1A1**6 (rs4148323) which is rarely found in Caucasians and African American populations.⁶⁴ However, in Asian populations *UGT1A1**6 has a higher frequency of approximately 16%.⁶⁵ Slow metabolizer *UGT1A1* genotypes (*UGT1A1* *28/*28, *28/*37, and *37/*37, which are in tight linkage disequilibrium with rs887829) have been associated with approximately 46% increased plasma AUC, 32% increased maximum concentration, and 32% decreased oral clearance compared to *UGT1A1* normal metabolizer genotypes (*1/*1 and *1/*36).⁶¹

A study reported 1.7 times higher median dolutegravir trough concentration in patients carrying the *UGT1A1**6 homozygous allele than patients carrying both normal alleles. This suggests that the effect of *UGT1A1**6 on dolutegravir trough concentrations is minimal, but this interpretation was limited by the small number of *6 homozygous patients.⁶⁶ In the same study, age over 40 years was independently associated with high dolutegravir trough concentrations. However, in another study, increased age was associated with decreased dolutegravir plasma trough concentrations.⁶³ This study also reported that the *UGT1A1* gene, gender, body weight, smoking and food consumption affect the pharmacokinetics of dolutegravir.

Tenofovir pharmacogenetics

Tenofovir entry into the kidney tubular cells through the basolateral membrane involves primarily OAT1 and to a less extent OAT3.^{35,67} The secretion of tenofovir is an active process

that depends on efflux by the transporter proteins MRP2 and MRP4, encoded by *ABCC2* and *ABCC4*, respectively.^{68–72} The two efflux transporters have also been reported to play a role in the elimination of tenofovir.^{35,69,70} *In vitro* studies have suggested that tenofovir is not a substrate of MRP2 and proposed that there may be an indirect mechanism explaining the observed association with *ABCC2* polymorphisms.⁷³ Higher tenofovir concentrations have been associated with polymorphisms in *ABCC2*, *ABCC4* and *SCL22A6* in candidate gene studies.^{74–76} The *ABCC4* rs374206 SNP has been associated with increased intracellular concentrations of tenofovir triphosphate (35% higher when using peripheral blood mononuclear cells).⁷⁷

In vitro studies have demonstrated that tenofovir is a substrate of MRP7 and polymorphisms in *ABCC10* may affect tenofovir disposition.⁷⁸ Two *ABCC10* polymorphisms (rs9349256 and rs2125739) have been associated with kidney toxicity.⁷³ These findings have not been replicated. Should these studies be replicated, pre-treatment screening of the *ABCC10* polymorphisms may aid in the exclusion of patients for the PI/TDF combination.⁷⁹ In addition, *ABCC10* rs9349256 and protease inhibitor co-administration were independently associated with the urinary to plasma tenofovir ratio.⁷⁹

A mice study found significantly high tenofovir associated tissue damage in *ABCB1* and *SLC22A6* knock-out mice.⁸⁰ eGFR, PI co-administration and *SLC28A2* CT/TT genotypes have been independently associated with plasma tenofovir exposure.⁷⁹ However, it has not been confirmed that tenofovir is a concentrating nucleoside transporter-2 substrate.¹⁹ In addition to factors such as age, weight, estimated creatinine clearance and anti-hepatitis C virus positivity that were found to be associated with tenofovir plasma concentrations, renal filtration rate, PI co-administration and variants in *CNT2* gene were independent predictors.⁷⁹

Table 4 shows single nucleotide polymorphisms (SNPs) that have previously been associated with dolutegravir and tenofovir disposition.

Table 4. SNPs affecting dolutegravir and tenofovir disposition

Dolutegravir	
Gene	Polymorphisms
<i>ABCB1</i>	rs1922242, rs10276036, rs1045642, rs3842, rs2235015, rs3213619, rs2235067, rs10267099, rs1128503, rs4728709, rs4148740, rs10280101
<i>ABCG2</i>	rs13137622, rs2725252, rs2231137, rs10011796, rs12505410, rs7699188, rs3114020
<i>CYP3A4</i>	rs2246709, rs2740574, rs35599367, rs4646440, rs2242480, rs4646437
<i>CYP3A5</i>	rs776746
<i>NR1I2</i>	rs2472677, rs1523130
<i>UGT1A1</i>	rs1042640, rs10929302, rs1976391, rs887829, rs4124874, rs8330, rs4148323, rs8175347
Tenofovir	
<i>ABCC2</i>	rs8187707, rs3740066, rs17222723, rs7080681, rs2273697, rs8187710
<i>ABCC4</i>	rs3742106, rs11568655, rs1751034, rs2274405, rs1557070, rs1059751, rs11568695, rs2274407, rs2274406, rs899494
<i>ABCC10</i>	rs2487663
<i>SLC22A2</i>	rs3127573
<i>SLC22A11</i>	rs11231809
<i>SLC28A2</i>	rs11854484
<i>SLOC1B3</i>	rs7311358

Dolutegravir and tenofovir toxicities

The limitations with HIV therapy have not necessarily been efficacy but safety and tolerability.⁴⁵ With the rollout of dolutegravir-based regimens in LMICs such as Brazil, Kenya, Botswana and Uganda, results from an observational study conducted in Botswana suggested an increased risk of neural tube defects in infants born from HIV-positive women who were pre-exposed to dolutegravir at conception.⁸¹ The most reported adverse effects in clinical trials for dolutegravir are headache (2%) and insomnia (3%).²¹ Another adverse effect of dolutegravir has been neuropsychiatric events which have led to treatment discontinuation in some phase 3 clinical trials.^{6,82–84}

Weight changes

It has been observed that plasma dolutegravir concentrations may be higher in individuals with lower body weights.⁶³ Safety signals may be missed during drug development and early clinical usage and not become apparent until years after the drug has been approved.

Continued pharmacovigilance of dolutegravir is therefore important.⁸⁵ This requires assessing the safety profile of dolutegravir beyond standard Phase 3 studies, and in a broader range of patients.⁸⁵

Integrase inhibitor-based ART has been associated with excessive weight gain, especially with second-generation integrase inhibitors and TAF.^{86–91} Studies have shown that switching from TDF to TAF is associated with weight and lipid changes.^{92–96} While in Black women who initiated TAF, there is increased weight to a greater extent than TDF especially in combination with integrase inhibitors.^{97,98} PrEP studies have reported weight loss associated with TDF (**Table 5**). This is thought to be caused by the effect of TDF on lipid suppression as observed in the DISCOVER and iPrEx studies.^{48,99}

Table 5. Summary of studies reporting weight loss associated with TDF

Study	Arms	Location
VOICE ¹⁰⁰	TDF or TDF/FTC (n=2010)	South Africa, Uganda, Zimbabwe
	Placebo (n=1009)	
iPrEx ¹⁰¹	TDF/FTC (n=1251)	Peru, Brazil, USA, South Africa, Thailand, Ecuador
	Placebo (n=1248)	
PARTNERS ¹⁰²	TDF or TDF/FTC (n=3163)	Kenya, Uganda
	Placebo (1584)	
TDF-2 ¹⁰³	TDF/FTC (n=611)	Botswana
	Placebo (n=608)	
FEM-PrEP ¹⁰⁴	TDF/FTC (n=1025)	Kenya, South Africa, Tanzania, Thailand
	Placebo (n=1033)	
BKK ¹⁰⁵	TDF (n=1204)	Thailand

	Placebo (n=1209)	
HPTN 084 ¹⁰⁶	TDF/FTC (n=1610)	Argentina, Brazil, Peru, USA, South Africa, Thailand, Vietnam
	CAB (n=1614)	

FTC=Emtricitabine, CAB=Cabotegravir

Renal toxicity

In the kidney, the primary transporters of organic anions are members of the organic anion transporters (OATs) family 22a (SLC22A), primarily OAT1 and OAT3.¹⁹ They are involved in the renal secretion of drugs and their metabolites, such as nucleoside or nucleotide analogs.¹⁹ The organic cation transporter 2 (OCT2) is responsible for creatinine uptake at the basolateral membrane of the proximal tubular kidney cells.¹⁰⁷ There have not been reports of dolutegravir effects on the CYP system or drug transporters.¹⁰⁷ However, with the inhibition of *OCT2* by dolutegravir, there have been indications of dolutegravir causing an increase in serum creatinine by up to 0.4 mg/dl (44 µmol/L) with predictable decrease in eGFR by 10-15 ml/min per 1.73 m² without altering true GFR.¹⁰⁸ This change may be manageable under steady state in patients with normal kidney function but may be challenging in patients with chronic kidney disease (CKD). Kidney toxicities have not been described with dolutegravir. Given that dolutegravir is metabolized in the liver, no dose adjustments are required for those with kidney disease.¹ However, patients with severe kidney impairment may have increased dolutegravir exposure.¹⁰⁹

The human renal OAT1 (hOAT1) encoded by *SLC22A6*, plays an important role in the elimination of tenofovir, cidofovir and adefovir.¹¹⁰ In non-mammalian cells, the over expression of hOAT1 induced cytotoxicity after exposure to cidofovir and adefovir and demonstrated significant effects on the growth of the renal proximal epithelial cells.¹⁹ In contrast, tenofovir does not show toxicity towards non-mammalian cells expressing hOAT1

and shows minimal effect on the growth of the renal proximal epithelial cells.¹⁹ Studies have found no relationship with abnormal tenofovir clearance nor with toxicity with *SLC22A6* 453G>A (rs4149170) and 728G>A (rs11568626) polymorphisms.^{78,111}

ART regimens containing TDF and PIs are associated with higher tenofovir plasma exposure and a higher risk of developing renal toxicity. These higher concentrations (above 160 ng ml⁻¹ in two reports) may be associated with a higher incidence of proximal tubular dysfunction.^{112–115} This may be caused by the PIs inhibitory effect on P-gp and MRP efflux pumps.^{116–119} The loss of function variants in MRP2 and MRP7 have been associated with a rise in several tubular toxicity markers.^{73,78,120} Ritonavir and cobicistat act as pharmacokinetic boosters when co-administered with TDF as they increase the maximum concentration (C_{max}) and absorption of tenofovir.¹²¹ When these boosters are used, TAF doses are reduced from 25 mg to 10 mg daily,³⁷ whereas 300 mg TDF dose is not adjusted.^{41,122,123} This pharmacokinetic enhancement may result in lesser tolerability of TDF compared with TAF regimens overall.¹²¹

Studies have reported associations between polymorphisms of the efflux transporters and tenofovir-associated tubulopathy. With tenofovir-associated tubulopathy, a high degree of interindividual variability in disease characteristics and severity has been observed,¹⁹ and genetic variants of transporters have been investigated.^{78,124} Variants in both *ABCC2*¹²⁴ and *ABCC4*^{78,124} have shown association, but variants in *SLC22A6* and *ABCB1* have found no associations.⁷⁸ In one study, they reported an association between the CATC haplotype and a greater risk of proximal tubulopathy in patients receiving tenofovir. The authors suggested that this haplotype might impair tenofovir secretion by tubular cells. In addition, there was complete linkage between *ABCC2* 1249G>A (rs2273697) and the CATC haplotype.¹²⁴ In another study designed to look for associations between genetic polymorphisms and

tenofovir-associated tubulopathy, an association was found between the *ABCC2* -24C (rs717620) allele and tubular damage while using tenofovir. However, the association between the CATC haplotype and tenofovir-associated tubulopathy was not confirmed.⁷⁸ These differences may be due to the study population or the criteria used to define renal toxicity.¹⁹ *ABCC4* plays an important role in the excretion of tenofovir.¹⁹ The *ABCC4* 669C>T (rs899494) polymorphism was found at a higher proportion of patients receiving tenofovir who were developing renal tubular damage.¹²⁴ This finding was however not confirmed by others.¹²⁵ Additional SNPs from *ABCC4* (559G>T [rs11568658], 912G>T [rs2274407], 951G>A [rs2274406], 969G>A [rs2274405], 1497C>T [rs1557070], 3310T>C [rs11568655] and 3348A>G [rs1751034]) have been examined but none have proven to be associated with a greater risk of tenofovir associated kidney damage.^{78,124}

The pathogenesis of tenofovir-associated nephrotoxicity is potentially as a result of effects on the proximal tubule epithelial cell mitochondria and altered mitochondrial cytochrome c oxidase activity^{126,127} as well as decreased expression of megalin and cubilin, variety of ion transporters and down regulatory effects on endothelial nitric oxidesynthase.^{127,128} There is an association between tenofovir use and reduced bone mineral density and increases in biomarkers associated with bone metabolism, which may increase fracture risk.^{129,130} In patients with proximal renal tubulopathy, osteomalacia and hypophosphatemia with symptomatic bone and muscular pain have been reported.^{131,132} Evidence of proximal tubular injury can be recognized by using light microscopy.¹ However, with tenofovir nephrotoxicity, it is the proximal tubular eosinophilic inclusions representing giant mitochondria that are considered distinctive features of tenofovir nephrotoxicity.¹²⁶

The use of TDF with PIs (such as atazanavir or ritonavir) increases TDF drug concentrations and enhances its potential for nephrotoxicity and incident chronic kidney disease.¹³³ Other

risk factors for TDF-induced proximal tubular injury include prolonged exposure, concomitant use of didanosine or PIs (such as ritonavir), polymorphisms of transporters involved in drug secretion by the kidney, pre-existing kidney disease, diabetes mellitus, immunodeficiency and aging.^{45,134–136} From the TDF Expanded Access Program, post-marketing analyses showed that renal failure, tubular dysfunction and elevated serum creatinine were rare but significant (0.3%, 0.05%, and 0.1%, respectively).¹³⁷

For HIV-positive patients on maintenance haemodialysis, it is recommended that TDF be dosed once weekly.¹ However, a pharmacokinetics study determined that once weekly dosing with haemodialysis resulted in steady-state plasma and increased intracellular PBMC concentrations than those found in patients with normal kidney function who are taking TDF daily.¹³⁸ This suggests that for patients on dialysis, less frequent dosing of TDF may be appropriate.¹

Summary of literature review

Dolutegravir and tenofovir form part of the preferred first- or second-line regimens for treating HIV. Dolutegravir is replacing efavirenz as it has shown to have higher efficacy. The affordable fixed-dose drug combination (300 mg tenofovir disoproxil fumarate, 300 mg lamivudine and 50 mg dolutegravir [TLD]) is now available in low-and middle-income countries. Dolutegravir is primarily metabolized in the liver by UGT1A1 and minimally by CYP3A. It is minimally excreted unchanged in the urine. Polymorphisms in UGT1A1 have shown to affect dolutegravir activity. For example, *UGT1A1**28 (rs8175347) which is in strong linkage disequilibrium with *UGT1A1**80 (rs887829) decreases mRNA and protein expression levels. Slow metabolizer UGT1A1 genotypes (*UGT1A1* *28/*28, *28/*37, and *37/*37) have been associated with increased plasma AUC, increased maximum concentration and decreased oral clearance compared to normal metabolizer genotypes (*1/*1

and **1/*36*). *UGT1A1* **6* homozygous allele has been associated with higher median dolutegravir trough concentration. Dolutegravir is a substrate of *ABCB1* and *ABCG2*. Dolutegravir has previously been associated with increased risk of neural tube defects and the most common reported adverse effects for dolutegravir are headache and insomnia.

TDF, first generation tenofovir prodrug, has higher plasma tenofovir exposure but lower intracellular TFV-DP, can cause bone and kidney toxicities. TAF has lower plasma tenofovir exposure but higher intracellular TFV-DP has been associated with weight gain. Tenofovir is not a substrate, inducer or inhibitor of CYP450 enzymes. In candidate gene studies, higher tenofovir concentrations have been associated with polymorphisms in *ABCC2*, *ABCC4* and *SCL22A6*. While two *ABCC10* polymorphisms (rs9349256 and rs2125739) have been associated with kidney toxicity.

Knowledge gaps identified

- There are currently no studies that have investigated the pharmacogenetics of dolutegravir and tenofovir exposure among Africans.
- There is limited knowledge of weight gain associated with dolutegravir and tenofovir.
- With dolutegravir and tenofovir the most favoured ART regimens in Africa, the studies reported in this thesis will enhance the understanding of the pharmacogenetics and pharmacokinetics of these drugs among Southern Africans.

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Chapter 3

Pharmacogenetics of dolutegravir plasma exposure among Southern Africans living with HIV

Abstract

Background: Dolutegravir is a component of preferred antiretroviral therapy (ART) regimens. We characterized the pharmacogenetics of dolutegravir exposure following ART initiation in the ADVANCE trial in South Africa.

Methods: Genome-wide genotyping followed by imputation was performed. We developed a population pharmacokinetic model for dolutegravir using non-linear mixed-effects modelling. Linear regression models examined associations with unexplained variability in dolutegravir area under the concentration-time curve (AUC_{VAR}).

Results: Genetic associations were evaluable in 284 individuals. Of nine polymorphisms previously associated with dolutegravir pharmacokinetics, the lowest P-value with AUC_{VAR} was *UGT1A1* rs887829 ($P = 1.8 \times 10^{-4}$), which was also associated with $\log_{10}$ bilirubin ($P = 8.6 \times 10^{-13}$). After adjusting for rs887829, AUC_{VAR} was independently associated with rs28899168 in the *UGT1A* locus ($P = 0.02$), as were bilirubin concentrations ($P = 7.7 \times 10^{-8}$). In the population pharmacokinetic model, rs887829 T/T and C/T were associated with 25.9% and 10.8% decreases in dolutegravir clearance, respectively, compared to C/C. The lowest P-value for AUC_{VAR} genome-wide was *CAMKMT* rs343942 ($P = 2.4 \times 10^{-7}$).

Conclusions: In South Africa, rs887829 and rs28899168 in the *UGT1A* locus were independently associated with dolutegravir AUC_{VAR} . The novel rs28899168 association warrants replication. This study enhances understanding of dolutegravir pharmacogenetics in Africa.

Keywords: Antiretroviral therapy, dolutegravir, HIV, pharmacogenetics, pharmacokinetics

Introduction

Sub-Saharan Africa has the highest prevalence of HIV-1 infection worldwide.¹ Dolutegravir is an HIV-1 integrase strand transfer inhibitor with a favorable safety profile and high barrier to viral resistance.² The World Health Organization recommends dolutegravir with tenofovir and lamivudine as a preferred regimen for ART-naïve patients, and dolutegravir-based ART as a switch option when failing ART or transitioning for programmatic reasons.³ In South Africa, preferred initial regimens include dolutegravir, together with either tenofovir and emtricitabine, or abacavir with lamivudine.⁴

Dolutegravir undergoes glucuronidation by hepatic uridine glucuronosyltransferase 1A1 (UGT1A1), with minimal contributions of cytochrome P450 (CYP) 3A4, UGT1A3 and UGT1A9,⁵ and is a substrate of transporter proteins P-glycoprotein (encoded by *ABCB1*) and breast cancer resistance protein (encoded by *ABCG2*).⁶

Studies of ART pharmacogenetics may guide ART optimization in different populations. Africa has the world's greatest genetic diversity,⁷ but data are limited regarding dolutegravir pharmacogenetics in Africa. We characterized genetic associations with dolutegravir exposure among ART-naïve African participants in the ADVANCE study.⁸

Methods

Study population

The ADVANCE study in South Africa was a phase 3 clinical trial (ClinicalTrials.gov Identifier NCT03122262) in which 1,053 HIV-positive, ART-naïve participants were randomly assigned to one of 3 treatment arms: 1) dolutegravir, tenofovir alafenamide (TAF) and emtricitabine; 2) dolutegravir, tenofovir disoproxil fumarate (TDF) and emtricitabine; or 3) efavirenz, TDF and emtricitabine.⁸ The present study included dolutegravir arm participants who consented to genetic testing.

Pharmacokinetic sampling and analysis

Intense pharmacokinetic sampling at steady state was performed in a subset of participants receiving dolutegravir, equally divided between TDF- and TAF-containing arms. Samples were taken pre-dose and at 1, 2, 4, 6, 8, and 24 hours post-dose. Doses preceding intense sampling were observed following standard meal. For all other individuals, sparse pharmacokinetic sampling (at least one sample) was performed at either week 48 or 96.

Dolutegravir was quantified with a validated assay developed at the Division of Clinical Pharmacology, University of Cape Town. Samples were processed with a liquid-liquid extraction method using dolutegravir-d4 as an internal standard followed by high-performance liquid chromatography with tandem mass spectrometry detection using an AB SCIEX API 4000 instrument. Analyte and internal standard were monitored at mass transitions of the protonated precursor ions m/z 420.1 and m/z 424.2 to the productions m/z 277.2 and m/z 279.1, respectively. The calibration curve fitted a quadratic regression over a range of 0.030 to 10.0 $\mu\text{g/mL}$. Combined accuracy and precision statistics of quality control samples during validation were between 103.5% and 106.0%, and 4.6% and 6.1%, respectively. The laboratory participated in the Clinical Pharmacology Quality Assurance external quality control program under a contract with the Division of AIDS of the National Institute of Allergy and Infectious Diseases, through which this assay was approved.

Genetic polymorphisms

Whole blood was collected from consenting participants, and deoxyribonucleic acid (DNA) was extracted as described elsewhere.⁹ Samples were labelled with coded identifiers. Stored DNA was genotyped using the Illumina Infinium Multi-Ethnic Global BeadChip (MEGA^{EX}) at Vanderbilt Technologies for Advanced Genomics (VANTAGE). Post-genotype quality control included sex checks, call rates by marker and sample, identity by descent plots, assessment for batch effects, concordance between duplicate samples, and HapMap controls.

Quality control steps were performed using PLINK version 1.9.¹⁰ Genotyping efficiency per participant was >95% for all samples. After quality control, data were imputed using the TOPMed reference panel after transforming to genome build 38 using liftOver and stratification by chromosome to parallelize the imputation process.¹¹ We excluded imputed polymorphisms with imputation scores < 0.3, genotyping call rates < 95%, minor allele frequency (MAF) < 0.05, or Hardy-Weinberg Equilibrium P-values < 1.0×10^{-8} . Linkage disequilibrium (LD) r^2 values were determined using PLINK.

Population pharmacokinetic modelling (without genetics)

Concentration-time data were analysed by nonlinear mixed-effects modelling with NONMEM (v7.5.0; ICON Development Solutions, Ellicott City, MD). First-order conditional estimation with eta-epsilon interaction were used to fit models to the observations. Pearl-speaks-NONMEM (PsN) v4.7.0, Pirana v2.9.7, and R v3.6.1 were used for model automation and tracking and to create and visualize model diagnostics. We tested various structural models to describe the pharmacokinetics of dolutegravir including 1- and 2-compartment disposition models, first-order elimination and absorption, with or without absorption lag time, and transit compartments.¹² We included between-individual and between-occasion random effects on the model parameters with an assumption of a log-normal distribution. A combined (additive and proportional) error model was used to describe the residual unexplained variability, with the additive component of the error constrained to be at least 20% of the lower limit of quantification.

Throughout the pharmacokinetic analysis, model development was guided by inspection of diagnostic plots, including visual predictive checks (VPC), and decreases in the NONMEM objective function value (OFV), which were assumed to follow a χ^2 distribution. To discriminate between nested models, a decrease in OFV of -3.84 was equivalent to model

improvement at $P < 0.05$. To adjust for the effect of body size on disposition parameters, we added allometric scaling to the model. We tested total body weight and fat-free mass (FFM) with the allometric exponents for clearance and volume parameters fixed to 0.75 and 1, respectively.¹³ After the inclusion of allometric scaling, we investigated effects of the following covariates: sex, age, and TAF- versus TDF-containing ART. Covariates were assessed by stepwise inclusion followed by backward elimination and were retained at a significance level of 0.01. To evaluate the robustness of parameter estimates in the final model, we ran a sampling-importance resampling exercise and generated 95% confidence interval.

Unexplained variability to be regressed against genetics

The final model was used to generate individual steady-state estimates of area under the concentration-time curve (AUC_{0-24h}) and unexplained variability in AUC_{0-24h} (AUC_{VAR}) for individuals with at least 1 pharmacokinetic sample. Individual estimates of all model parameters were obtained from the final model by a post hoc Bayes estimation method, considering an individual's pharmacokinetic data and characteristics (i.e., FFM). Individual estimates of AUC_{0-24h} were obtained using the formula $AUC_{0-24h} = F_i \times Dose_i / CL_i$, where $Dose_i$ represents the actual dose given to each individual and CL_i and F_i represent individual estimates of clearance and bioavailability, respectively.

Genetic association analyses

The outcome of primary interest was AUC_{VAR} , and secondarily unexplained variability in dolutegravir clearance (CL_{BSV}). Multivariable linear regression models characterized associations with genetic polymorphisms. To adjust for genetic ancestry, we estimated continuous axes of ancestry incorporating the intersection of common autosomal genotypes using EIGENSTRAT.¹⁴ Principal components scree plots were used to assess whether

components included in analyses were informative; based on this, two principal components were sufficient. Other than principal components, additional covariates were not included in association analyses because they were accounted for in the population pharmacokinetic model. We report regression coefficients (β) for additive associations with polymorphisms, where positive β values indicate an association with increased AUC_{VAR}. The Bonferroni method was used to determine significance thresholds, with 0.05 divided by the number of polymorphisms tested in targeted polymorphism and gene analyses, and $P < 5.0 \times 10^{-8}$ for genome-wide analyses.

We *a priori* selected 12 polymorphisms previously reported to be associated with dolutegravir exposure (*ABCB1* rs1128503, rs2032582, rs1045642 and rs3842; *ABCG2* rs2231142 and rs2231137; *CYP3A4* rs35599367; *CYP3A5* rs776746 (*3 allele); *NR1I2* rs2472677 and rs1523130; and *UGT1A1* rs4148323 (*6 allele) and rs8175347 (*28 allele)). We also selected rs887829 which is in strong linkage with rs8175347. Beyond these polymorphisms, we then used a stepwise approach to prioritize sets of polymorphisms to interrogate, in order to decrease the burden of multiple testing. We reasoned that polymorphisms previously and strongly associated with at least 1 drug-related phenotype, or that have been significantly genome-wide associated with any trait, are most likely to be true associations. We used the Pharmacogenomics Knowledge Base ([PharmGKB] accessed 13 July 2021)¹⁵ and NHGRI-EBI GWAS Catalog (accessed 19 January 2021) as references.¹⁶ In PharmGKB, 173 polymorphisms were previously associated with at least 1 drug-related phenotype (pharmacokinetics, efficacy, or toxicity) with levels of evidence of 1 (preponderance of evidence shows an association, replicated in multiple cohorts, and preferably with strong effect size) or 2 (moderate evidence of association, replicated but some studies may not show statistical significance, or with small effect size). In the GWAS Catalog, 89 716 polymorphisms were previously associated with any trait at $P < 5.0 \times 10^{-8}$ in at least 1 study.

We prioritized polymorphisms common to both PharmGKB and the GWAS Catalog, considering these to have the most robust evidence for true associations. We secondarily explored all polymorphisms from PharmGKB and the GWAS Catalog (based on criteria described above) and all polymorphisms in our imputed genome-wide genotype data. As a positive control, we tested for associations between *UGT1A* locus polymorphisms and screening bilirubin concentrations. Bilirubin is conjugated by UGT1A1, and there are strong associations between *UGT1A1* polymorphisms (e.g., rs887829) and bilirubin concentrations.¹⁷

Population pharmacokinetic modelling with genetic information

Based on results of our genetic association analyses, we assessed effects of selected polymorphisms on dolutegravir clearance, on all available pharmacokinetic data (intensive and sparse samples). In addition to participants with genotype data, both intensive and sparse pharmacokinetic data were available from other ADVANCE participants. For such individuals, we assigned a phenotype using mixture modelling as described elsewhere.¹⁸

Results

Study population

Among 340 ADVANCE participants who consented for genetic analyses, 284 (84%) were successfully genotyped and had pharmacokinetic data. Participant disposition is presented in **Figure 1**. All participants were Black Africans and 62% were females. Study participant characteristics are shown in **Table 1**.

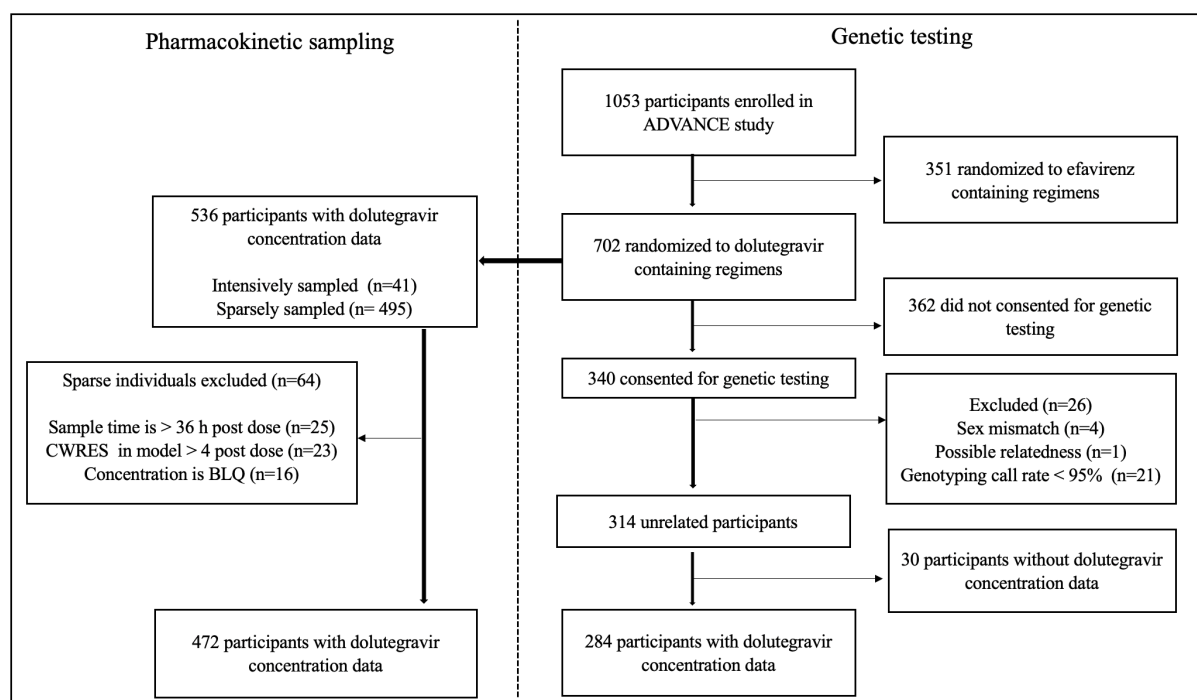


Figure 1. Of 1,053 participants enrolled in the ADVANCE study, 284 who were randomized to dolutegravir-containing regimens and with available pharmacokinetic sampling data were evaluable for genetic associations. CWRES, conditional weighted residual; BLQ, below limit of quantification.

Table 1: Baseline characteristics of ADVANCE participants included in genetic association analyses

Characteristic	Dolutegravir recipients (n = 284)
Age in years, median (IQR) ^a	33 (27, 38)
Sex	
Male, n (%)	108 (38)
Female, n (%)	176 (62)
BMI, kg/m ² , median (IQR)	23.2 (20.4, 26.9)
CD4 T-cell count in cells/mm ³ , median (IQR)	292 (163, 459)
Plasma HIV-1 RNA in copies/mL, median (IQR)	26 003 (6 044, 74 037)

^a Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; RNA, ribonucleic acid.

Dolutegravir population pharmacokinetics without genetics

For this model, data were available from 41 intensively sampled individuals who provided 276 dolutegravir concentrations (40 complete profiles, and 1 pre-dose sample from an individual for whom intravenous access difficulty precluded additional samples). Median (interquartile range) weight and age were 73.1 (67.2 – 85.2) kg and 31 (29 – 36) years, respectively. A two-compartment disposition model ($\Delta\text{OFV} = -47$, $P < 0.001$ compared to one-compartment) with first-order elimination and transit compartments absorption ($\Delta\text{OFV} = -4.8$, $P = 0.028$ compared to absorption lag) best described pharmacokinetics. Allometric scaling with FFM best described the effect of body size on disposition parameters and was applied to all clearance and volume parameters. We estimated clearance of 0.732 L/h, central volume of 12.2 L, and peripheral volume of 5.87 L for a typical individual with a 47 kg FFM and included between-subject variability on clearance and between-occasion variability on mean transit time (MTT), absorption rate constant, and bioavailability. Final parameter estimates, precision and the VPC shows that the model described the data adequately (**Table S1**).

Genetic associations with dolutegravir pharmacokinetics

Seven of 12 polymorphisms previously associated with dolutegravir pharmacokinetics were present in our genetic data. Of the 5 not included, 3 (rs4148323, rs8175347, and rs35599367) are very infrequent in African populations (MAF approximately 1% or less), and 1 was not genotyped (*UGT1A1**28 rs8175347) but is in strong LD with rs887829.¹⁹ Of the remaining 7 polymorphisms, the lowest P value for association with AUC_{VAR} was *UGT1A1* rs887829 ($\beta = 0.14$, $P = 1.8 \times 10^{-4}$) with a MAF of 0.41 (**Table 2**). This withstood correction for multiple testing (cut-off $P < 5.6 \times 10^{-3}$) and was also most strongly associated with CL_{BSV} ($\beta = -0.09$, $P = 8.4 \times 10^{-6}$) (**Table S2**). Association of the other 6 polymorphisms with CL_{BSV} were consistent with those for AUC_{VAR} (data not shown). This was expected, since AUC is derived

from CL ($AUC_{0-24h} = F_i \times Dose_i / CL_i$) and these parameters were highly correlated (Spearman $r^2 = -0.96$).

Table 2: Genetics associations with AUC_{VAR} and log₁₀ bilirubin concentrations

Association analyses	Polymorphism	Gene	MAF	Beta	P-value ^b
Associations with SNPs selected <i>a priori</i> with AUC _{VAR}	rs887829 ^c	<i>UGT1A</i>	0.41	0.14	1.8 x 10 ⁻⁴
	rs2472677	<i>NR1I2</i>	0.36	0.05	0.2
	rs776746	<i>CYP3A5</i>	0.19	0.04	0.44
	rs3842	<i>ABCB1</i>	0.28	-0.02	0.59
	rs1045642	<i>ABCB1</i>	0.12	-0.03	0.66
	rs1128503	<i>ABCB1</i>	0.08	0.02	0.8
	rs1523130	<i>NR1I2</i>	0.06	-0.01	0.9
	rs2231137	<i>ABCG2</i>	0.06	-0.01	0.94
Genome-wide associations	AUC _{VAR}				
	rs343942	<i>CAMKMT</i>	0.12	0.29	2.4 x 10 ⁻⁷
	rs9980715	<i>MIR99AHG</i>	0.29	0.19	6.6 x 10 ⁻⁷
	rs75466245	<i>CAMKMT</i>	0.05	0.38	7.2 x 10 ⁻⁷
	rs1038692137	Intergenic	0.19	0.23	9.8 x 10 ⁻⁷
	rs343960	<i>CAMKMT</i>	0.05	0.38	1.4 x 10 ⁻⁶
	rs76142931	Intergenic	0.06	-0.37	2.0 x 10 ⁻⁶
	rs140435425 ^c	Intergenic	0.07	-0.34	2.2 x 10 ⁻⁶
	rs112238172	Intergenic	0.11	0.27	2.7 x 10 ⁻⁶
	rs111066265	<i>MAD1L1</i>	0.21	-0.21	2.7 x 10 ⁻⁶
	rs343950	<i>CAMKMT</i>	0.06	0.34	2.7 x 10 ⁻⁶
	Log ₁₀ bilirubin				
	rs6742078	<i>UGT1A</i>	0.4	0.11	7.0 x 10 ⁻¹³
	rs887829 ^c	<i>UGT1A</i>	0.41	0.12	8.6 x 10 ⁻¹³
	rs4148325	<i>UGT1A</i>	0.41	0.12	9.4 x 10 ⁻¹³
	rs4148324	<i>UGT1A</i>	0.41	0.11	1.3 x 10 ⁻¹²
	rs11888459 ^c	<i>UGT1A</i>	0.38	0.1	2.1 x 10 ⁻¹⁰
	rs28899168	<i>UGT1A</i>	0.15	-0.14	2.5 x 10 ⁻¹⁰
	rs34352510	<i>UGT1A</i>	0.37	0.1	2.7 x 10 ⁻¹⁰
	rs7604115	<i>UGT1A</i>	0.37	0.1	3.7 x 10 ⁻¹⁰
	rs11673726 ^c	<i>UGT1A</i>	0.37	0.1	3.8 x 10 ⁻¹⁰
	rs7564935	<i>UGT1A</i>	0.36	0.1	6.8 x 10 ⁻¹⁰

^a Abbreviations: AUC_{VAR}, unexplained variability in population estimates of AUC values; MAF, Minor allele frequency, SNP, single-nucleotide polymorphism.

^b Significance threshold was 5.6 x 10⁻³ for the subset of nine polymorphisms selected *a priori*. Genome-wide significance threshold was 5.0 x 10⁻⁸. For each pharmacokinetic parameter, the 10 lowest P-values are shown.

^c These polymorphisms were in linkage with other polymorphisms in our imputed data, which gave identical association results. These included: rs140435425 with rs139714988, rs144113286 and rs150029007; rs887829 with rs1976391 and rs1368812138; rs11888459 with rs10178992; and rs11673726 with rs3771341.

To interrogate *UGT1A1* more thoroughly, we considered 853 polymorphisms within the *UGT1A* locus $\pm 50\text{kb}$ in each direction. The *UGT1A* locus includes *UGT1A1*, 3, 4, 5, 6, 7, 8, 9 and 10 which create transcripts by differential splicing. A LocusZoom plot representing *UGT1A* locus associations is presented in **Figure 2**. For AUC_{VAR} , the lowest P-value was

rs6736508 ($\beta = 0.18$, $P = 1.2 \times 10^{-5}$) (**Figure 2A**), which withstood correction for multiple testing (cut-off $P < 5.9 \times 10^{-5}$). This polymorphism was not in strong LD with rs887829 ($r^2 = 0.32$). By comparison, for $\log_{10}$ bilirubin

concentrations the lowest P-value was rs6742078 ($\beta = 0.11$, $P = 7.0 \times 10^{-13}$), which was in strong

linkage disequilibrium with rs887829 (**Figure 2B**). Results for CL_{BSV} were consistent with results for AUC_{VAR} , although for CL_{BSV} no polymorphism had substantially lower P-values than rs887829. For CL_{BSV} , the lowest P-value was rs201393786 ($\beta = 0.1$, $P = 2.6 \times 10^{-7}$) (**Table S3**).

To determine whether rs6736508 or other *UGT1A* locus polymorphism were associated with AUC_{VAR} independent of rs887829, we repeated the above analyses after adjusting for rs887829, which should strengthen any true independent association. In this analysis, rs6736508 became 2-log less significant ($\beta = 0.13$, $P = 4.2 \times 10^{-3}$), and no longer withstand

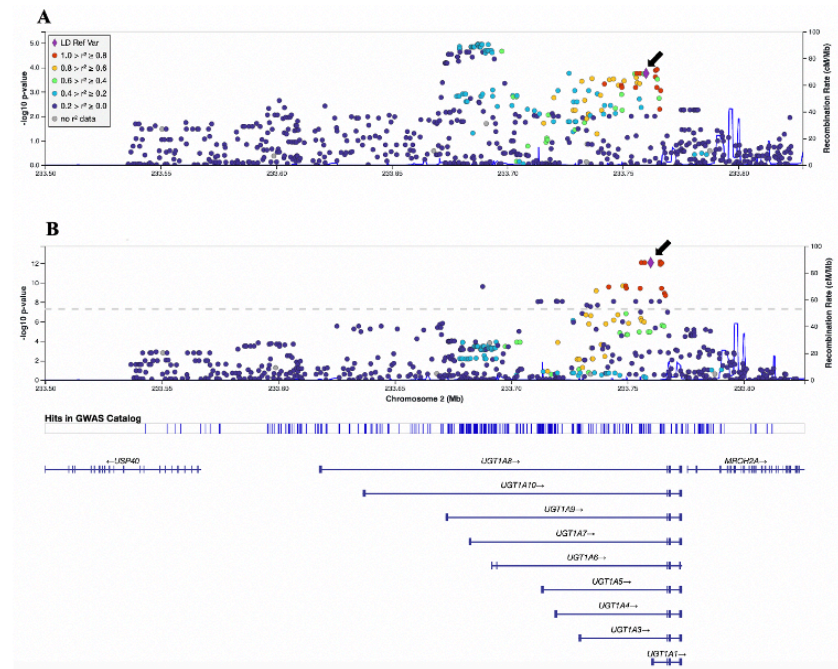


Figure 2: LocusZoom plots of *UGT1A* locus associations with plasma dolutegravir exposure and $\log_{10}$ bilirubin concentrations. The figure shows $-\log_{10}$ P-values for associations of 853 polymorphisms in the *UGT1A* locus ($\pm 50\text{ kb}$ in either direction) among 284 individuals evaluable for genetic associations. The purple diamond identifies *UGT1A1* rs887829 which we selected to be the reference polymorphism for linkage disequilibrium (LD) values because it has been most consistently associated with bilirubin concentrations in prior genome-wide association studies. Note the different Y-axis scales. *Panel A*: Associations with unexplained variability in dolutegravir AUC_{VAR} . *Panel B*: Associations with baseline $\log_{10}$ bilirubin concentrations. Marker colors indicate LD r^2 values in relation to rs887829, based on selecting the ALL populations option in LocusZoom.

correction for multiple testing, indicating that its association was largely if not entirely dependent on rs887829. Results for CL_{BSV} were consistent with results for AUC_{VAR} (data not shown). To guide next steps, we used $\log_{10}$ bilirubin as a biomarker to identify additional variants that may affect UGT1A1 activity independent of rs887829. After adjusting for rs887829, a *UGT1A* locus polymorphism rs28899168 was independently associated with lower $\log_{10}$ bilirubin concentrations ($\beta = -0.11$, $P = 7.7 \times 10^{-8}$). This polymorphism was also independently associated with lower AUC_{VAR} ($\beta = -0.12$, $P = 0.02$). P-values for *UGT1A* locus polymorphisms with AUC_{VAR} and CL_{BSV} after adjusting for rs887829 are presented in **Table S4**.

In analyses that explored genome-wide associations with AUC_{VAR} , none withstood correction for multiple testing. The lowest P-value was *CAMKMT* rs343942 ($\beta = 0.29$, $P = 2.4 \times 10^{-7}$), (**Figure 3A**). By comparison, the lowest P-value with $\log_{10}$ bilirubin concentrations was

rs6742078 in the *UGT1A*

locus ($\beta = 0.11$, $P = 7.0 \times$

10^{-13}) (**Figure 3B**). The

10 lowest P-values for

AUC and for $\log_{10}$

bilirubin concentrations

are presented in **Table 2**.

Results for CL_{BSV} were

consistent with results

for AUC_{VAR} (data not

shown). For CL_{BSV} , the

lowest P-value was *LOC105377607* rs201393786 on chromosome 4 ($\beta = 0.10$, $P = 2.6 \times 10^{-7}$)

(**Figure S1**). As we did for *UGT1A* locus polymorphisms, we characterized associations

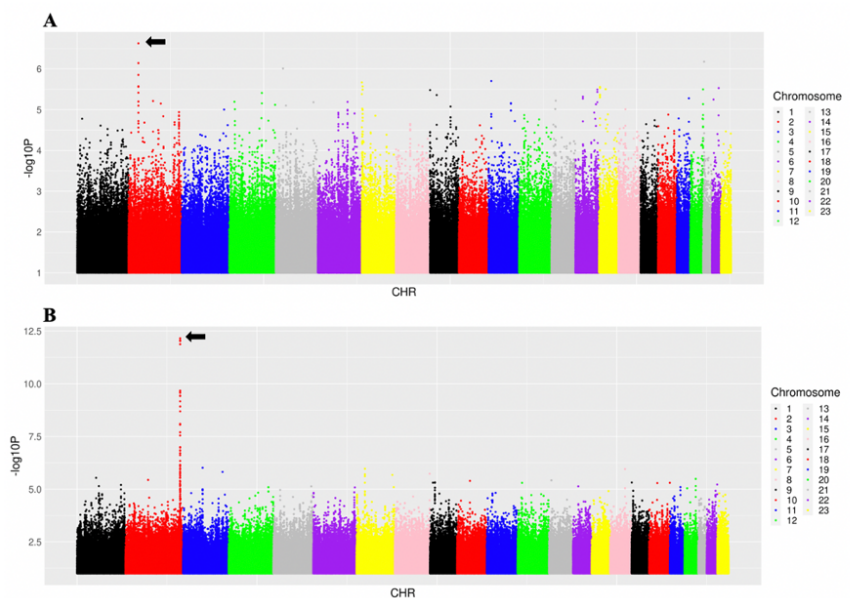


Figure 3: Manhattan plots of genome-wide associations with dolutegravir pharmacokinetic parameter and $\log_{10}$ bilirubin concentrations. The figure shows $-\log_{10}$ P-values for association among 284 individuals who were evaluable for genetic associations. The black arrows indicate the lowest P-value in each figure. Note the different Y-axis scales. *Panel A:* Associations with dolutegravir AUC_{VAR} . The lowest P-value was *CAMKMT* rs343942 ($P = 2.4 \times 10^{-7}$). *Panel B:* Associations with $\log_{10}$ bilirubin concentrations. The lowest P-value was rs6742078 in the *UGT1A* locus ($P = 7.0 \times 10^{-13}$).

genome-wide after adjusting for rs887829. In these analyses, the lowest P-value for dolutegravir AUC was *ADGRE4P* rs7256367 ($\beta = 0.20$, $P = 2.6 \times 10^{-7}$), which was also the lowest P-value for CL_{BSV} ($\beta = -0.11$, $P = 2.3 \times 10^{-7}$), and did not withstand correction for multiple testing. The 10 lowest P-values for AUC after adjusting for rs887829 are in **Table S4**.

We next considered 89,716 polymorphisms previously associated with any trait in the GWAS Catalog. In these analyses, the lowest P-value for AUC was *CAMKMT* rs343968 ($\beta = 0.33$, $P = 2.8 \times 10^{-6}$). The 10 lowest P-values for association with AUC_{VAR} are presented in **Table 3**.

Table 3: Associations between unexplained variability in population estimates of AUC values (AUC_{VAR}) and polymorphisms previously associated with any trait in the GWAS Catalog

Polymorphism	Gene	Chromosome	MAF ^a	Beta	P-value ^b	GWAS Catalog trait
rs343968	<i>CAMKMT</i>	2	0.07	0.33	2.8×10^{-6}	Height
rs4418728	Intergenic	10	0.20	-0.19	8.4×10^{-5}	Triglyceride levels
rs4148325	<i>UGT1A5</i>	2	0.41	0.15	1.2×10^{-4}	Bilirubin concentrations
rs4148324	<i>UGT1A10</i>	2	0.41	0.14	1.3×10^{-4}	Bilirubin concentrations
rs10473629	Intergenic	5	0.13	0.22	1.3×10^{-4}	Self-reported math ability
rs35207189	Intergenic	13	0.37	-0.15	1.6×10^{-4}	Household income (MTAG)
rs887829 ^c	<i>UGT1A1</i>	2	0.41	0.14	1.8×10^{-4}	Bilirubin concentrations

^a Abbreviations: AUC_{VAR} , unexplained variability in population estimates of area under the concentration-time curve values; MAF, minor allele frequency; MTAG, multi-trait analysis of GWAS.

^b The 7 lowest P values for associations with GWAS Catalog traits are shown.

^c Polymorphism rs887829 was in complete linkage with rs1976391 in our imputed genotype data, so gave identical results.

PharmGKB and GWAS Catalog polymorphisms included in our analyses are provided in **Table S5** and **Table S6**. Results for CL_{BSV} were consistent with results for AUC_{VAR} (data not shown), with the lowest the lowest P-value being rs4148325 in the *UGT1A* locus ($\beta = -0.09$, $P = 5.0 \times 10^{-6}$), which is in strong LD with rs887829 ($r^2 = 0.99$).

Including genotype in the population pharmacokinetic model

Among the 41 intensively sampled individuals, 26 (8 with C/C, 10 with C/T, 8 with C/T) had *UGT1A1* rs887829 genotype information and when this was tested on dolutegravir clearance,

there was a graded trend towards reduced clearance for C/T and T/T individuals of borderline significance. However, when we fitted a mixture model to all available pharmacokinetic data (intensive and sparse samples) there was a significant and graded effect of *UGT1A1* rs887829 genotype on dolutegravir clearance ($\Delta\text{OFV} = -24.7$, $P < 0.00001$ compared to no genotype). We estimated a clearance of 0.78 L/h for C/C, with a 10.8% and 25.9% decrease in clearance for C/T, and T/T, respectively. This included 472 individuals (188 not genotyped) who provided 742 concentrations. Simulations performed using the final model and highlighted in **Figure 4** show that dolutegravir trough concentrations are highest with T/T genotypes.

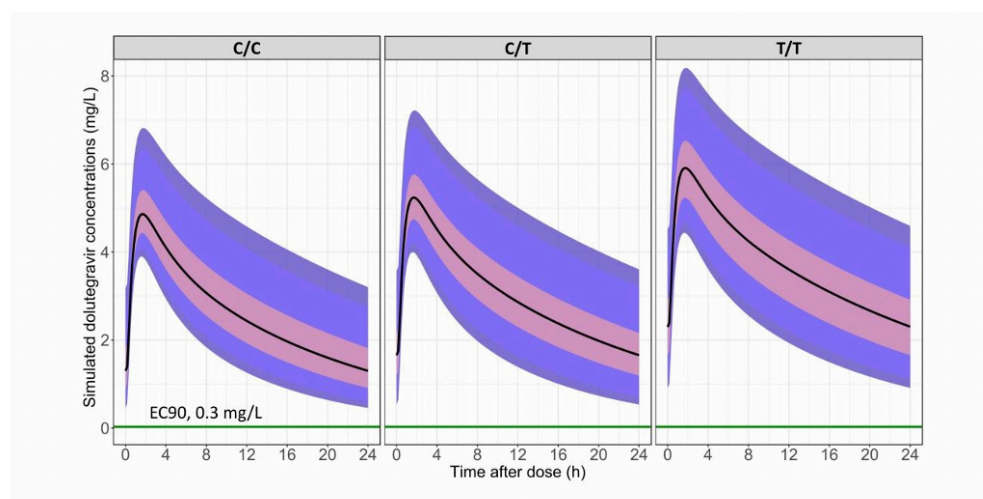


Figure 4. Simulated dolutegravir concentration-time profiles. Simulations are made with the final dolutegravir model and based on 1200 typical individuals they were categorized either as C/C, C/T, or T/T for rs887829 (dolutegravir is administered at 50 mg once daily). For each panel, the solid black line in the middle represents the median simulated concentration, the pink shaded area represents the 75th percentile, the blue shaded area is the 95th percentile, and the darkest blue at the extremes is the 97.5th percentile of the simulated concentrations. The green horizontal line is dolutegravir's effective concentration 90 (0.3 mg/L).

A VPC and a schematic of the model are in **Figure S2**. Testing the effect of rs6736508 on dolutegravir clearance within a model that already included rs887829 only improved the model by -5.73 points ($P = 0.057$) and therefore, rs6736508 was not retained in the model. On the other hand, testing the effect of rs28899168 within a model that already included rs887829 improved the model by -7.42 points ($P = 0.0244$). However, this was not significant

at the backward elimination step where we used a threshold of $P < 0.01$ and therefore, rs28899168 was not retained in the final model.

Discussion

We characterized genetic associations with between-individual variability in dolutegravir exposure among participants in the ADVANCE study in South Africa. Among polymorphisms previously associated with plasma dolutegravir exposure,^{20–23} the *UGT1A1* rs887829 T allele was associated with greater AUC_{VAR} ($P = 1.8 \times 10^{-4}$). This allele is known to be in strong linkage with the Gilbert trait decreased expression allele, *UGT1A1**28, a promoter TA_n dinucleotide.¹⁹ Our finding supports previous reports associating *UGT1A1**28 with dolutegravir concentrations.^{20,21,23} In prior genome-wide association studies of bilirubin concentrations, rs887829 was most strongly and consistently associated.^{17,24} When included in the population pharmacokinetic model, rs887829 C/T and T/T genotypes were associated with 10.8% and 25.9% decreases in dolutegravir clearance, respectively, and thus higher exposures compared to C/C individuals.

To identify novel *UGT1A* locus associations with dolutegravir exposure we used screening bilirubin concentration as a biomarker for *UGT1A1* activity. After controlling for rs887829, a second *UGT1A* locus polymorphism, rs28899168 (intronic in *UGT1A8*, *UGT1A9*, and *UGT1A10*) was independently associated with $\log_{10}$ bilirubin concentrations ($P = 7.7 \times 10^{-8}$, below the Bonferroni P-value cut-off of 5.9×10^{-5}), suggesting that rs28899168 was associated with increased *UGT1A1* expression or activity independent of rs887829 (albeit with a P-value 5-log greater than that for rs887829). After adjusting for rs887829, rs28899168 was also independently associated with lower dolutegravir AUC_{VAR} ($P = 0.02$). Although the P-value for rs28899168 did not withstand correction for multiple comparisons,

it is likely true given its independent association with bilirubin. This polymorphism has not been previously associated with dolutegravir pharmacokinetics or bilirubin.

In genome-wide analyses for associations with AUC_{VAR} , the lowest P-value was *CAMKMT* rs343942 ($P = 2.4 \times 10^{-7}$), which has not been previously associated with dolutegravir pharmacokinetics. *CAMKMT* catalyses Lys-116 trimethylation in calmodulin,²⁵ and other *CAMKMT* polymorphisms have been associated with anxiety risk.^{25,26} The second lowest P-value was *MIR99AHG* rs9980715 ($P = 6.6 \times 10^{-7}$). *MIR99AHG* is a potential noncoding tumor suppressor gene in lung adenocarcinoma.²⁷ These seem unlikely to represent true associations with dolutegravir pharmacokinetics.

Previous reports have associated *UGT1A1**28 rs8175347 with dolutegravir pharmacokinetics. Using data from nine Phase I and II studies involving 89 participants of European-American or African-American descent, *UGT1A1**28 was associated with a 32% decrease in dolutegravir oral clearance, a 46% increase in AUC, and a 32% increase in C_{max} .²⁰ Among 107 Japanese patients, *UGT1A1**28 and *UGT1A1**6 (rs4148323) were associated with increased dolutegravir concentrations.²¹ *UGT1A1**6 is rare among Africans and Europeans but frequent among Asians. Using data from three Phase I and one Phase III clinical trials involving 93 Caucasian and Black African or Caribbean participants, *UGT1A1**28 was associated with 28% increased dolutegravir plasma AUC_{0-24h} .²³ The *UGT1A1* rs887829 association in our study supports previous reports. Beyond *UGT1A1*, *ABCG2* rs2231142 and *NR1I2* rs2472677 in combination were suggested to be associated with increased dolutegravir C_{max} , while no further associations were found in *ABCG2* rs2231137, *CYP3A4**22 rs35599367, *CYP3A5**3 rs776746, and *NR1I2* rs1523130.²³ A Japanese study of 42 patients also found higher dolutegravir concentrations associated with *ABCG2* rs2231142 and no associations with polymorphisms with *ABCB1* rs1128503, rs1045642, rs2032582, or

rs3842.²² We found no associations with these *ABCB1*, *ABCG2*, *CYP3A4*, *CYP3A5* and *NR1I2* polymorphisms.

During HIV-1 treatment, plasma dolutegravir concentrations considerably exceed what is required to inhibit wild-type virus replication. Therefore, loss-of-function polymorphisms in *UGT1A1* are unlikely to increase antiviral efficacy. However, lower plasma dolutegravir exposure with the *UGT1A1* rs887829 C allele may be important in some situations, such as in patients receiving concomitant medications that increase dolutegravir clearance or decrease absorption, or in patients harbouring HIV-1 with reduced susceptibility to dolutegravir. Conversely, higher plasma dolutegravir concentrations associated with *UGT1A1* rs887829 T alleles may increase the risk of intolerance, supported by a Japanese study of 107 patients which reported a greater incidence of selected grade 1 or 2 neuropsychiatric adverse events among individuals carrying *UGT1A1**6 or *UGT1A1**28 ($P = 0.05$).

Our study had limitations. Although sample size was modest, this was the largest pharmacogenetic study of dolutegravir to date. A larger sample size may have identified novel genome-wide significant associations. We could not replicate some polymorphisms that were very infrequent in Africans. We did not directly genotype *UGT1A1**28. However, we selected *UGT1A1* rs887829 which is in strong linkage disequilibrium with *UGT1A1**28.

Conclusions

In summary, 2 *UGT1A* locus polymorphisms were independently associated with dolutegravir AUC_{VAR} in a Black African population, 1 of which was expected (rs887829) and 1 of which was novel (rs28899168). The latter association should be replicated in other large cohorts. This study extends our understanding of dolutegravir pharmacogenetics in Africa, which is important given the widespread prescribing of dolutegravir in Africa.

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Supplementary material

Table S1: Final population parameter estimates for dolutegravir

Parameter description	Typical Value (95% CI) ^a	
	Intensive samples only	Intensive and sparse
	<i>n</i> =41 participants	<i>n</i> =472 participants* (With genetic information included)
Clearance (CL) (L/h) ^c	0.732 (0.666–0.801)	0.786 (0.705–0.879)
Effect of Homozygous for <i>UGT1A1</i> rs887829 on CL (%)		-25.9% (-37.2– -11.7)
Effect of Heterozygous for <i>UGT1A1</i> rs887829 on CL (%)		-10.8% (-21.7–3.00)
Central volume (L) ^c	12.2 (1.12–13.4)	10.5 (8.69–12.4)
Inter-compartmental clearance (L/h) ^c	0.509 (0.252–3.86)	1.29 (0.521–2.15)
Peripheral volume (L) ^c	5.87 (1.74–41.6)	3.77 (2.36–5.59)
Relative bioavailability (<i>F</i>)	1 Fixed	1 Fixed
Absorption mean transit time (MTT) (h)	0.166 (0.00625–0.396)	0.180 (0.099–0.288)
Number of Transit compartments (<i>n</i>)	7.85 (2.64–22.3)	5 Fixed
Absorption rate constant (<i>k_a</i>) (/h)	2.41 (0.242–4.28)	1.67 (1.11–2.25)
Parameter Variability (% CV)^b		
Between-subject variability in CL	22.3% (14.1–27.8)	27.9% (23.6–32.9)
Between-occasion variability in <i>F</i>	31.6 % (21.4–41.7)	38.7% (30.3–47.5)
Between-occasion variability in MTT	130 % (63.5–445)	184% (134–234)
Between-occasion variability in <i>k_a</i>	69.9 % (30.9–97.1)	60.7% (43.4–75.4)
Residual unexplained variability		
Proportional error (%)	7.41 (4.51–10.2)	7.52 (5.25–10.1)
Additive error (mg/L)	0.191 (0.0602–0.256)	0.180 (0.119–0.255)

^a 95% confidence intervals were obtained by sampling importance resampling FFM, fat-free mass (calculated according to Janmahasatian et al)²⁸ %CV, coefficient of variation.

^b Calculated by %CV = $\sqrt{\omega}$. 100%.

^c Clearance and volume of distribution parameters were scaled with fat-free mass (FFM) using the exponents 0.75 and 1, respectively. The typical values reported here refer to an individual with an FFM of 47 kg.

*Of these, 384 were genotyped and those with missing genotype were assigned one via mixture modeling as described here¹⁸

Table S2: Associations between unexplained variability (between-individual variability) in the population parameter estimates of dolutegravir clearance (CL_{BSV}) and selected polymorphisms of potential relevance to dolutegravir

Polymorphism	Gene	MAF	Beta	P value^b
rs887829 ^c	<i>UGT1A</i>	0.41	-0.09	8.4 x 10 ⁻⁶
rs2472677	<i>NR1I2</i>	0.36	-0.02	0.28
rs3842	<i>ABCB1</i>	0.28	0.01	0.64
rs776746	<i>CYP3A5</i>	0.19	-0.01	0.67
rs1128503	<i>ABCB1</i>	0.08	-0.01	0.75
rs1523130	<i>NR1I2</i>	0.06	4.2 x 10 ⁻³	0.92
rs2231137	<i>ABCG2</i>	0.06	3.4 x 10 ⁻³	0.94
rs1045642	<i>ABCB1</i>	0.12	1.6 x 10 ⁻³	0.96

^a Abbreviations: MAF, Minor allele frequency.

^b Significance threshold was 5.6 x 10⁻³ for the subset of nine polymorphisms.

^c Polymorphism rs887829 was in complete linkage with rs1976391 in our imputed genotype data, so gave identical results.

Table S3: Genome-wide associations with unexplained variability (between-individual variability) in the population parameter estimates of dolutegravir clearance (CL_{BSV})

Polymorphism	Gene	MAF ^a	Beta	P value ^b
rs201393786	<i>LOC105377607</i>	0.49	0.1	2.6 x 10 ⁻⁷
rs7298064	<i>AEBP2</i>	0.21	0.12	3.6 x 10 ⁻⁷
rs10841238 ^c	<i>AEBP2</i>	0.21	0.12	5.0 x 10 ⁻⁷
rs10770496	<i>AEBP2</i>	0.19	0.12	7.2 x 10 ⁻⁷
rs7958449	<i>AEBP2</i>	0.16	0.12	1.4 x 10 ⁻⁶
rs4900552	Intergenic	0.11	0.16	1.6 x 10 ⁻⁶
rs10841224	<i>AEBP2</i>	0.19	0.12	1.8 x 10 ⁻⁶
rs7971277	<i>AEBP2</i>	0.18	0.12	1.9 x 10 ⁻⁶
rs4906258	Intergenic	0.1	0.17	1.9 x 10 ⁻⁶

^a Abbreviations: MAF, Minor allele frequency.

^b Genome-wide significance threshold was 5.0 x 10⁻⁸. The 10 lowest P-values are shown.

^c These polymorphisms were in linkage with other polymorphisms in our imputed data, which gave identical association results, rs10841238 with rs7298006.

Table S4: GWAS with dolutegravir pharmacokinetic parameters adjusted for *UGT1A1* rs887829

Polymorphism	Gene	MAF	Beta	P value
AUC_{VAR}				
rs7256367	<i>ADGRE4P</i>	0.29	0.20	2.6 x 10 ⁻⁷
rs583310	Intergenic	0.14	-0.25	4.9 x 10 ⁻⁷
rs1352224	<i>LINC02462</i>	0.29	0.19	5.8 x 10 ⁻⁷
rs1486986	<i>LINC02462</i>	0.29	0.19	5.8 x 10 ⁻⁷
rs1578706247	Intergenic	0.29	0.19	5.8 x 10 ⁻⁷
CL_{BSV}				
rs7256367	<i>ADGRE4P</i>	0.29	-0.11	2.3 x 10 ⁻⁷
rs201393786	<i>LOC105377607</i>	0.49	0.09	6.2 x 10 ⁻⁷
rs1352223	<i>LINC02462</i>	0.29	-0.10	9.3 x 10 ⁻⁷
rs1256609206	<i>RAB28</i>	0.29	-0.10	9.3 x 10 ⁻⁷
rs1486986	<i>LINC02462</i>	0.29	-0.10	9.3 x 10 ⁻⁷
Log₁₀ bilirubin				
rs28899168	<i>UGT1A</i>	0.15	-0.11	7.7 x 10 ⁻⁸
rs374147899	<i>COL11A1</i>	0.31	0.08	6.3 x 10 ⁻⁷
rs114707025	<i>GPAT3</i>	0.06	-0.16	6.5 x 10 ⁻⁷
rs877736	<i>PPP2R2B</i>	0.08	0.14	8.7 x 10 ⁻⁷
rs9853252	Intergenic	0.05	0.16	9.2 x 10 ⁻⁷

^a Abbreviations: AUC_{VAR}, unexplained variability in population estimates of AUC values; CL_{BSV}, unexplained variability (between-individual variability) in the population parameter estimates of dolutegravir clearance; MAF, Minor allele frequency.

^b Genome-wide significance threshold was $P < 5.0 \times 10^{-8}$.

For each pharmacokinetic parameter, the top five lowest P-values are shown

Table S5: Polymorphisms included in our analyses from PharmGKB

Polymorphism	Gene	Chromosome	MAF	Beta	P value
AUC_{VAR}					
rs887829	<i>UGT1A6</i>	2	0.410	0.143	1.8 x 10 ⁻⁴
rs6295	Intergenic	5	0.421	-0.089	0.020
rs20455	<i>KIF6</i>	6	0.178	0.097	0.054
rs4917639	<i>CYP2C9</i>	10	0.174	0.070	0.130
rs4673993	<i>ATIC</i>	2	0.067	-0.111	0.138
rs7270101	<i>ITPA</i>	20	0.092	-0.084	0.181
rs4713916	<i>FKBP5</i>	6	0.093	0.083	0.185
rs16960228	<i>PRKCA</i>	17	0.380	-0.048	0.219
rs4961	<i>ADD1</i>	4	0.073	-0.091	0.223
rs1695	<i>GSTP1</i>	11	0.486	-0.045	0.225
rs1801133	<i>MTHFR</i>	1	0.090	0.084	0.236
rs1051730	<i>CHRNA3</i>	15	0.053	-0.091	0.267
rs924607	<i>LOC100996325</i>	5	0.072	0.080	0.300
rs3812718	<i>SCN1A</i>	2	0.287	-0.040	0.336
rs489693	Intergenic	18	0.438	0.036	0.345
rs678849	<i>OPRD1</i>	1	0.237	0.041	0.353
rs3212986	<i>ERCC1</i>	19	0.221	-0.040	0.359
rs1954787	<i>GRIK4</i>	11	0.120	0.050	0.397
rs11881222	<i>IFNL3</i>	19	0.382	0.032	0.433
rs4444903	<i>EGF</i>	4	0.227	-0.033	0.440
rs776746	<i>CYP3A5</i>	7	0.185	0.039	0.440
rs12777823	Intergenic	10	0.293	-0.034	0.447
rs12979860	<i>IFNL4</i>	19	0.414	0.028	0.460
rs8050894	<i>VKORC1</i>	16	0.243	-0.030	0.482
rs8099917	Intergenic	19	0.053	0.059	0.500
rs1800629	Intergenic	6	0.154	-0.032	0.544
rs510769	<i>OPRM1</i>	6	0.196	0.028	0.555
rs1051266	<i>SLC19A1</i>	21	0.276	-0.025	0.560
rs7294	<i>VKORC1</i>	16	0.408	0.021	0.573
rs3745274	<i>CYP2B6</i>	19	0.380	-0.021	0.588
rs6065	<i>GP1BA</i>	17	0.167	0.026	0.605
rs7297610	Intergenic	12	0.363	0.019	0.628
rs11615	<i>ERCC1</i>	19	0.056	-0.040	0.629
rs1045642	<i>ABCB1</i>	7	0.120	-0.025	0.656
AUC_{VAR}					
rs2236857	<i>OPRD1</i>	1	0.220	-0.017	0.692
rs578776	<i>CHRNA3</i>	15	0.405	0.013	0.747
rs25487	<i>XRCC1</i>	19	0.103	-0.018	0.772
rs3766951	<i>OPRD1</i>	1	0.312	0.010	0.792

rs1799930	<i>NAT2</i>	8	0.235	-0.010	0.824
rs4680	<i>COMT</i>	22	0.332	-0.006	0.876
rs10306114	<i>PTGS1</i>	9	0.132	0.008	0.879
rs2740574	<i>LOC110366354</i>	7	0.252	0.006	0.886
rs2298383	<i>ADORA2A-AS1</i>	22	0.290	0.005	0.904
rs2359612	<i>VKORC1</i>	16	0.260	0.004	0.911
rs1800566	<i>NQO1</i>	16	0.176	-0.005	0.916
rs1042713	<i>ADRB2</i>	5	0.472	0.003	0.940
rs1051740	<i>EPHX1</i>	1	0.218	0.002	0.956
rs17782313	Intergenic	18	0.252	-0.002	0.957
rs11045879	<i>SLCO1B1</i>	12	0.065	-0.003	0.965
rs7412	<i>APOE</i>	19	0.168	0.002	0.974
rs2011425	<i>UGT1A8</i>	2	0.083	0.001	0.989
CL_{BSV}					
rs887829	<i>UGT1A3</i>	2	0.410	-0.089	8.6 x 10 ⁻⁶
rs6295	Intergenic	5	0.421	0.049	0.015
rs20455	<i>LOC107986594</i>	6	0.178	-0.046	0.079
rs7270101	<i>ITPA</i>	20	0.092	0.054	0.097
rs4917639	<i>CYP2C9</i>	10	0.174	-0.037	0.124
rs8050894	<i>VKORC1</i>	16	0.243	0.031	0.168
rs1051730	<i>CHRNA3</i>	15	0.053	0.057	0.185
rs4713916	<i>FKBP5</i>	6	0.093	-0.043	0.195
rs4673993	<i>ATIC</i>	2	0.067	0.051	0.197
rs924607	<i>LOC105374608</i>	5	0.072	-0.052	0.201
rs3812718	<i>SCN1A</i>	2	0.287	0.028	0.202
rs16960228	<i>PRKCA</i>	17	0.380	0.025	0.212
rs4961	<i>ADD1</i>	4	0.073	0.044	0.260
rs1695	<i>GSTP1</i>	11	0.486	0.021	0.272
rs7294	<i>VKORC1</i>	16	0.408	-0.022	0.277
rs4444903	<i>EGF</i>	4	0.227	0.022	0.327
rs3766951	<i>OPRD1</i>	1	0.312	-0.020	0.348
rs489693	Intergenic	18	0.438	-0.018	0.359
CL_{BSV}					
rs678849	<i>OPRD1</i>	1	0.237	-0.021	0.371
rs11881222	<i>IFNL3</i>	19	0.382	-0.019	0.380
rs7297610	Intergenic	12	0.363	-0.017	0.396
rs1801133	<i>MTHFR</i>	1	0.090	-0.030	0.413
rs1954787	<i>GRIK4</i>	11	0.120	-0.021	0.505
rs8099917	Intergenic	19	0.053	-0.029	0.528
rs12777823	Intergenic	10	0.293	0.013	0.587
rs11615	<i>ERCC1</i>	19	0.056	0.022	0.608
rs1042713	<i>ADRB2</i>	5	0.472	0.009	0.625
rs25487	<i>XRCC1</i>	19	0.103	0.016	0.626

rs3212986	<i>CD3EAP</i>	19	0.221	0.011	0.628
rs776746	<i>ZSCAN25</i>	7	0.185	-0.011	0.666
rs4680	<i>COMT</i>	22	0.332	0.008	0.682
rs3745274	<i>CYP2B6</i>	19	0.380	0.007	0.726
rs2011425	<i>UGT1A9</i>	2	0.083	0.012	0.743
rs1800629	Intergenic	6	0.154	0.009	0.749
rs2359612	<i>VKORC1</i>	16	0.260	-0.007	0.752
rs1799930	<i>NAT2</i>	8	0.235	0.007	0.753
rs7412	<i>APOE</i>	19	0.168	0.007	0.782
rs17782313	Intergenic	18	0.252	0.006	0.783
rs6065	<i>GP1BA</i>	17	0.167	-0.007	0.784
rs12979860	<i>IFNL4</i>	19	0.414	-0.005	0.789
rs2298383	<i>ADORA2A-AS1</i>	22	0.290	-0.006	0.795
rs1800566	<i>NQO1</i>	16	0.176	-0.006	0.826
rs10306114	<i>PTGS1</i>	9	0.132	-0.004	0.885
rs1051266	<i>SLC19A1</i>	21	0.276	0.003	0.890
rs2740574	<i>LOC110366354</i>	7	0.252	0.003	0.892
rs578776	<i>CHRNA3</i>	15	0.405	-0.002	0.923
rs2236857	<i>OPRD1</i>	1	0.220	-0.002	0.932
rs510769	<i>OPRM1</i>	6	0.196	0.002	0.942
rs1051740	<i>EPHX1</i>	1	0.218	-0.001	0.956
rs1045642	<i>ABCB1</i>	7	0.120	0.002	0.957
rs11045879	<i>SLCO1B1</i>	12	0.065	0.001	0.976

^a Abbreviations: AUC_{VAR}, unexplained variability in population estimates of AUC values; CL_{BSV}, unexplained variability (between-individual variability) in the population parameter estimates of dolutegravir clearance; MAF, Minor allele frequency.

Table S6: Polymorphisms previously associated with any trait from the GWAS Catalog included in our analyses

Polymorphism	Gene	Chromosome	MAF	Beta	P value	Disease trait
AUC_{VAR}						
rs343968	<i>CAMKMT</i>	2	0.07	0.33	2.8 x 10 ⁻⁶	Height
rs4418728	Intergenic	10	0.20	-0.19	8.4 x 10 ⁻⁵	Triglyceride levels
rs4148325	<i>UGT1A5</i>	2	0.41	0.15	1.2 x 10 ⁻⁴	Bilirubin concentrations
rs4148324	<i>UGT1A10</i>	2	0.41	0.14	1.3 x 10 ⁻⁴	Bilirubin concentrations
rs10473629	Intergenic	5	0.13	0.22	1.3 x 10 ⁻⁴	Self-reported math ability
rs35207189	Intergenic	13	0.37	-0.15	1.6 x 10 ⁻⁴	Household income (MTAG)
rs887829	<i>UGT1A1</i>	2	0.41	0.14	1.8 x 10 ⁻⁴	Bilirubin concentrations
rs111741722	<i>UGT1A5</i>	2	0.41	0.14	1.8 x 10 ⁻⁴	Blood protein levels
rs2069568	<i>SLA</i>	8	0.19	-0.17	1.8 x 10 ⁻⁴	Eosinophil counts
rs10929301	<i>UGT1A5</i>	2	0.30	-0.16	1.8 x 10 ⁻⁴	Bilirubin concentrations
rs3755319	<i>UGT1A8</i>	2	0.30	-0.16	1.8 x 10 ⁻⁴	Bilirubin concentrations
rs2516599	<i>NID2</i>	14	0.48	0.13	1.8 x 10 ⁻⁴	Lung function (FEV1/FVC)
rs9376091	Intergenic	6	0.20	0.18	2.2 x 10 ⁻⁴	Neutrophil count
rs6742078	<i>UGT1A8</i>	2	0.40	0.14	2.5 x 10 ⁻⁴	Bilirubin concentrations
rs9387925	Intergenic	6	0.35	-0.14	2.5 x 10 ⁻⁴	Intelligence (MTAG)
rs7821092	<i>RP1L1</i>	8	0.44	-0.14	2.5 x 10 ⁻⁴	LDL cholesterol
rs6747843	<i>UGT1A10</i>	2	0.27	0.15	2.6 x 10 ⁻⁴	Bilirubin concentrations
rs10804990	<i>TBC1D14</i>	4	0.29	0.15	2.7 x 10 ⁻⁴	Mean corpuscular volume
rs1122794	<i>FAM234A</i>	16	0.20	0.16	2.8 x 10 ⁻⁴	Mean corpuscular haemoglobin
rs1006548	<i>FANCA</i>	16	0.28	-0.15	2.8 x 10 ⁻⁴	Low tan response
rs7564935	<i>UGT1A6</i>	2	0.36	0.14	2.9 x 10 ⁻⁴	Bilirubin concentrations
rs11695484	<i>UGT1A8</i>	2	0.31	0.14	3.0 x 10 ⁻⁴	Bilirubin concentrations
rs4332347	<i>DISC1</i>	1	0.20	-0.16	3.0 x 10 ⁻⁴	Adolescent idiopathic scoliosis
rs17864701	<i>UGT1A10</i>	2	0.30	0.14	3.2 x 10 ⁻⁴	Bilirubin concentrations
rs7102705	Intergenic	11	0.33	0.15	3.3 x 10 ⁻⁴	Breast size
rs10866054	<i>CADPS</i>	3	0.41	-0.14	3.5 x 10 ⁻⁴	Risk-taking tendency (4-domain principal component model)
rs2885296	<i>UGT1A7</i>	2	0.30	0.14	3.6 x 10 ⁻⁴	Bilirubin concentrations
rs5510	<i>SERPINA4</i>	14	0.42	-0.13	3.8 x 10 ⁻⁴	Protein levels in obesity
rs6714634	<i>UGT1A10</i>	2	0.30	0.14	4.2 x 10 ⁻⁴	Bilirubin concentrations
rs10859277	<i>LINC01619</i>	12	0.24	0.16	4.3 x 10 ⁻⁴	Eosinophil counts
rs2268796	<i>SRD5A2</i>	2	0.29	0.14	4.3 x 10 ⁻⁴	Blood protein levels
AUC_{VAR}						
rs12988520	<i>UGT1A8</i>	2	0.26	0.15	4.5 x 10 ⁻⁴	Bilirubin concentrations
rs10929302	<i>UGT1A8</i>	2	0.29	0.14	4.6 x 10 ⁻⁴	Bilirubin concentrations
rs2068888	Intergenic	10	0.21	-0.16	4.6 x 10 ⁻⁴	Sex hormone-binding globulin levels adjusted for BMI

rs11597947	<i>ZMIZ1</i>	10	0.08	-0.23	4.7×10^{-4}	Monocyte count
rs34352510	<i>UGT1A10</i>	2	0.37	0.13	4.7×10^{-4}	Bilirubin concentrations
rs11080055	<i>TMEM97</i>	17	0.49	-0.13	4.8×10^{-4}	Blood protein levels
rs10819335	Intergenic	9	0.47	0.13	4.9×10^{-4}	Monocyte count
rs168643	Intergenic	5	0.15	0.19	4.9×10^{-4}	Diastolic blood pressure
rs12611922	<i>BAZ2B</i>	2	0.39	0.14	5.0×10^{-4}	Neutrophil percentage of white cells
rs11638908	<i>MEGF11</i>	15	0.05	0.26	5.1×10^{-4}	Mean corpuscular haemoglobin
rs3008892	<i>WDR78</i>	1	0.08	0.25	5.2×10^{-4}	Height
rs1595217	Intergenic	13	0.32	-0.14	5.3×10^{-4}	Reaction time
rs10178992	<i>UGT1A5</i>	2	0.38	0.13	5.4×10^{-4}	Bilirubin concentrations
rs11888459	<i>UGT1A7</i>	2	0.38	0.13	5.4×10^{-4}	Bilirubin concentrations
rs10178992	<i>UGT1A10</i>	2	0.38	0.13	5.4×10^{-4}	Bilirubin concentrations
rs11888459	<i>UGT1A2P</i>	2	0.38	0.13	5.4×10^{-4}	Bilirubin concentrations
rs10178992	<i>UGT1A8</i>	2	0.38	0.13	5.4×10^{-4}	Bilirubin concentrations
rs11888459	<i>UGT1A5</i>	2	0.38	0.13	5.4×10^{-4}	Bilirubin concentrations
rs10937763	<i>TBC1D14</i>	4	0.30	0.14	5.7×10^{-4}	Mean platelet volume
CL_{BSV}						
rs4148325	<i>UGT1A3</i>	2	0.41	-0.09	5.0×10^{-6}	Bilirubin concentrations
rs4148324	<i>UGT1A3</i>	2	0.41	-0.09	5.9×10^{-6}	Bilirubin concentrations
rs887829	<i>UGT1A3</i>	2	0.41	-0.09	8.4×10^{-6}	Bilirubin concentrations
rs111741722	<i>UGT1A3</i>	2	0.41	-0.09	8.4×10^{-6}	Blood protein levels
rs6742078	<i>UGT1A3</i>	2	0.40	-0.09	9.4×10^{-6}	Bilirubin concentrations
rs343968	<i>CAMKMT</i>	2	0.07	-0.16	1.1×10^{-5}	Height
rs6747843	<i>UGT1A3</i>	2	0.27	-0.09	1.3×10^{-5}	Bilirubin concentrations
rs7564935	<i>UGT1A3</i>	2	0.36	-0.09	1.5×10^{-5}	Bilirubin concentrations
rs34352510	<i>UGT1A3</i>	2	0.37	-0.08	2.6×10^{-5}	Bilirubin concentrations
rs11673726	<i>UGT1A3</i>	2	0.37	-0.08	3.0×10^{-5}	Bilirubin concentrations
rs3771341	<i>UGT1A3</i>	2	0.37	-0.08	3.0×10^{-5}	Bilirubin concentrations
rs7604115	<i>UGT1A3</i>	2	0.37	-0.08	3.0×10^{-5}	Bilirubin concentrations
rs11888459	<i>UGT1A3</i>	2	0.38	-0.08	3.0×10^{-5}	Bilirubin concentrations
rs10178992	<i>UGT1A3</i>	2	0.38	-0.08	3.0×10^{-5}	Bilirubin concentrations
CL_{BSV}						
rs2885296	<i>UGT1A3</i>	2	0.30	-0.08	3.5×10^{-5}	Bilirubin concentrations
rs3755319	<i>UGT1A3</i>	2	0.30	0.09	3.7×10^{-5}	Bilirubin concentrations
rs10929301	<i>UGT1A3</i>	2	0.30	0.09	3.7×10^{-5}	Bilirubin concentrations
rs11695484	<i>UGT1A3</i>	2	0.31	-0.08	3.9×10^{-5}	Bilirubin concentrations
rs17864701	<i>UGT1A3</i>	2	0.30	-0.08	3.9×10^{-5}	Bilirubin concentrations
rs6714634	<i>UGT1A3</i>	2	0.30	-0.08	4.2×10^{-5}	Bilirubin concentrations
rs61912333	Intergenic	12	0.20	0.09	5.1×10^{-5}	Diastolic blood pressure
rs10929302	<i>UGT1A3</i>	2	0.29	-0.08	5.3×10^{-5}	Bilirubin concentrations

rs17863787	<i>UGT1A9</i>	2	0.19	-0.10	5.5×10^{-5}	Bilirubin concentrations
rs12988520	<i>UGT1A9</i>	2	0.26	-0.09	7.3×10^{-5}	Bilirubin concentrations
rs73339979	Intergenic	17	0.20	0.09	1.0×10^{-4}	Total cholesterol levels
rs5510	<i>SERPINA4</i>	14	0.42	0.07	1.4×10^{-4}	Protein levels in obesity
rs2516599	<i>NID2</i>	14	0.48	-0.07	1.4×10^{-4}	Lung function (FEV1/FVC)
rs146543016	<i>CRTC1</i>	19	0.14	0.11	1.5×10^{-4}	Self-reported math ability
rs674514	<i>LOC105377392</i>	4	0.38	-0.08	1.5×10^{-4}	Neuroticism
rs708100	<i>TMEM199</i>	17	0.49	-0.07	1.7×10^{-4}	Blood protein levels
rs11638908	<i>MEGF11</i>	15	0.05	-0.15	2.1×10^{-4}	Mean corpuscular hemoglobin
rs9376091	Intergenic	6	0.20	-0.09	2.1×10^{-4}	Neutrophil count
rs10804990	<i>TBC1D14</i>	4	0.29	-0.08	2.2×10^{-4}	Mean corpuscular volume
rs704	<i>VTN</i>	17	0.50	-0.07	2.2×10^{-4}	Blood protein levels
rs10866054	<i>CADPS</i>	3	0.41	0.08	2.2×10^{-4}	Risk-taking tendency (4-domain principal component model)
rs4418728	Intergenic	10	0.20	0.09	2.3×10^{-4}	Triglyceride levels
rs203425	<i>EEF1DP3</i>	13	0.07	0.13	2.5×10^{-4}	DNA methylation variation (age effect)
rs13401281	<i>UGT1A4</i>	2	0.43	0.07	2.5×10^{-4}	Total bilirubin concentrations
rs1006548	<i>FANCA</i>	16	0.28	0.08	2.5×10^{-4}	Low tan response
rs9892407	Intergenic	17	0.21	0.09	2.6×10^{-4}	Blood protein levels
rs2069568	<i>TG</i>	8	0.19	0.09	2.9×10^{-4}	Eosinophil counts
rs241775	Intergenic	17	0.46	-0.07	2.9×10^{-4}	Blood protein levels
rs2599469	Intergenic	19	0.44	-0.07	3.0×10^{-4}	Adolescent idiopathic scoliosis
rs11080055	<i>TMEM97</i>	17	0.49	0.07	3.4×10^{-4}	Blood protein levels
rs9387925	Intergenic	6	0.35	0.07	3.7×10^{-4}	Intelligence (MTAG)
rs2070959	<i>UGT1A9</i>	2	0.16	-0.10	3.8×10^{-4}	Bilirubin concentrations
rs10173355	<i>UGT1A9</i>	2	0.16	-0.10	3.8×10^{-4}	Bilirubin concentrations
CL_{BSV}						
rs10819335	Intergenic	9	0.47	-0.07	3.9×10^{-4}	Monocyte count
rs10179091	<i>UGT1A3</i>	2	0.48	0.07	3.9×10^{-4}	Bilirubin concentrations
rs35207189	Intergenic	13	0.37	0.07	4.0×10^{-4}	Household income (MTAG)

^a Abbreviations: AUC_{VAR}, unexplained variability in population estimates of AUC values; CL_{BSV}, unexplained variability (between-individual variability) in the population parameter estimates of dolutegravir clearance; MAF, Minor allele frequency.

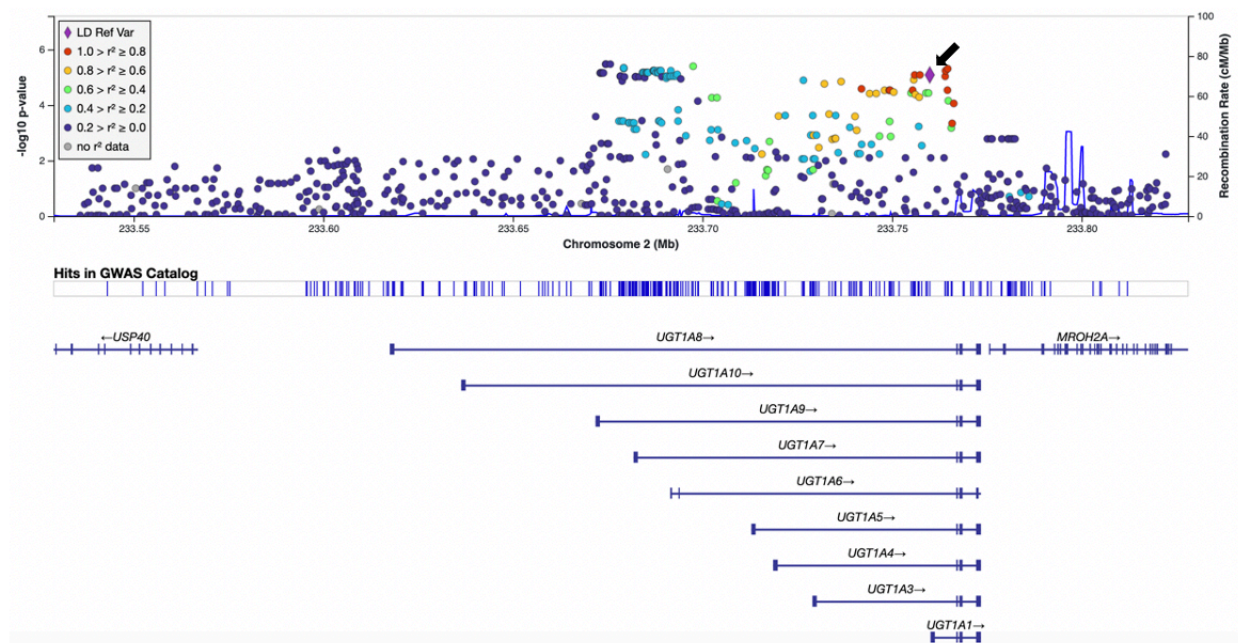


Figure S1: LocusZoom plot of *UGT1A* locus associations with between-individual variability in dolutegravir clearance. The figure shows $-\log_{10}$ P-values for associations of 853 polymorphisms in the *UGT1A* locus (± 50 kb in either direction) among 284 individuals who were evaluable for genetic associations. The purple diamond identifies *UGT1A1* rs887829 which we selected to be the reference polymorphism for linkage disequilibrium (LD) values because it has been most consistently associated with bilirubin concentrations in prior genome-wide association studies. The lowest P-value was rs201393786 in *LOC105377607* ($P = 2.6 \times 10^{-7}$).

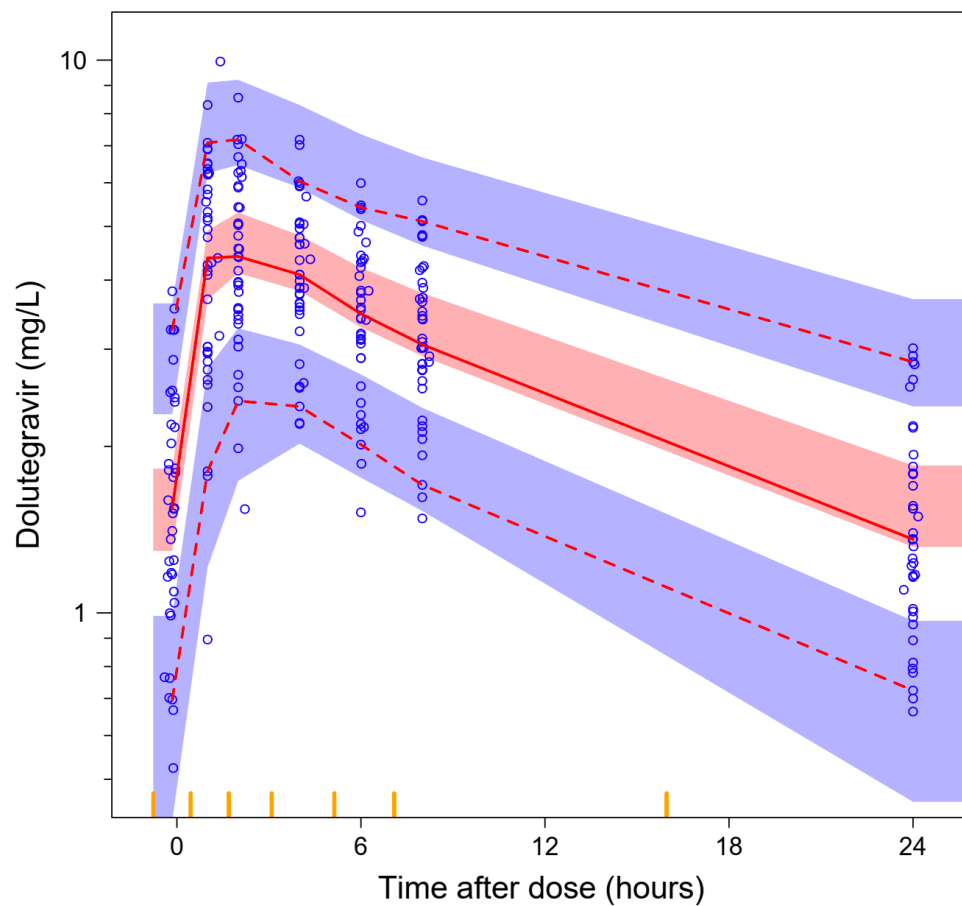


Figure S2: Visual predictive check of the final model fitted to intense samples only (without genetic information). Blue 12 circles represent observed plasma concentrations. The solid line in the middle represents the median observed 13 concentration, the dashed lines below and above represent the 5th and 95th percentiles of the observed concentrations, 14 respectively. The shaded areas around each line represent the 95% confidence interval for the same percentiles based 15 on simulations with the model.

Chapter 4

Pharmacogenetics of tenofovir clearance among Southern Africans living with HIV

Abstract

Background: Tenofovir is a component of preferred combination antiretroviral therapy (ART) regimens in Africa. Few pharmacogenetic studies have been conducted of tenofovir exposure in Africa, where genetic diversity is greatest.

Objective: We characterized the pharmacogenetics of plasma tenofovir clearance in Southern Africans receiving tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF).

Methods: Adults randomized to TAF or TDF in dolutegravir-containing arms of the ADVANCE trial (NCT03122262) were studied. Linear regression models stratified by study arm examined associations with unexplained variability in tenofovir clearance. We investigated genetic associations with polymorphisms selected *a priori* followed by genome-wide associations.

Results: A total of 268 participants (138 and 130 in the TAF and TDF arm, respectively) were evaluable for associations. Among polymorphisms previously associated with any drug-related phenotype, *IFNL4* rs12979860 was associated with more rapid tenofovir clearance in both arms (TAF: $P = 0.003$; TDF: $P = 0.003$). Genome-wide, the lowest P -values for tenofovir clearance in TAF and TDF arms were *LINC01684* rs9305223 ($P = 3.0 \times 10^{-8}$) and intergenic rs142693425 ($P = 1.4 \times 10^{-8}$), respectively.

Conclusion: Among Southern Africans randomized to TAF or TDF in ADVANCE, unexplained variability in tenofovir clearance was associated with a polymorphism in *IFNL4*, an immune response gene. It is unclear how this gene would affect tenofovir disposition.

Keywords: antiretroviral therapy, HIV, tenofovir, tenofovir disoproxil fumarate, tenofovir alafenamide, pharmacokinetics, pharmacogenetics

Introduction

South Africa is home to an estimated 8.2 million people living with HIV (PLWH),¹ including 5.2 million on antiretroviral therapy (ART).² The World Health Organization recommends initiating ART using dolutegravir in combination with nucleoside reverse-transcriptase inhibitors (NRTI).³ The preferred first-line regimen in South Africa is tenofovir disoproxil fumarate (TDF), lamivudine and dolutegravir,³ which is available as a fixed-dose formulation.

Tenofovir disoproxil fumarate, a prodrug of tenofovir, is a substrate for the efflux transporter P-glycoprotein (P-gp).⁴ After oral administration, TDF undergoes rapid metabolism in plasma to tenofovir, which is activated intracellularly.^{5,6} Tenofovir is excreted into urine via multidrug resistance protein 4 (MRP4).⁷ TDF is generally well tolerated but can cause nephrotoxicity and decreases in bone mineral density.^{8–10}

Tenofovir alafenamide (TAF), a newer oral prodrug of tenofovir, has improved renal and bone safety outcomes. Unlike TDF, TAF is predominantly metabolized intracellularly to tenofovir by cathepsin A and is more stable in plasma.^{5,11,12} This results in higher intracellular concentrations of the active metabolite, tenofovir diphosphate, and approximately tenfold lower plasma tenofovir concentrations than TDF,^{5,11,13} which is thought to explain the reduced risk of bone and renal toxicity.¹⁴ Transport of TDF through the basolateral membrane of renal cells involves organic anion transporter 1 (OAT1) and, to a lesser extent, OAT3,¹⁵ while TAF is not a substrate of either OAT1 or OAT3 but does undergo minor metabolism by CYP3A4.¹⁶ Better understanding of the pharmacogenetics of tenofovir clearance may inform optimize prescribing in different populations and reduce tenofovir-associated toxicities.

In candidate gene studies, higher tenofovir concentrations have been associated with polymorphisms in *SCL22A6*, *ABCC2* and *ABCC4*.^{17–19} In a Chinese cohort, *SLCO1B3*

rs7311358 was independently associated with TAF AUC_{0-t}, while *ABCC2* rs3740066 was associated with tenofovir half-life. Higher TAF AUC and shorter tenofovir half-life with the *ABCB1* rs2032582 T allele and *ABCC4* rs3742106 CC genotype were noted though neither were statistically significant.²⁰ Carriers of the *ABCC4* rs1751034 variant had slower tenofovir renal clearance.²¹ *CYP3A4* rs35599367 was associated with higher plasma TAF AUC_{0-24h}.²² Despite Africa having the world's greatest genetic diversity, there are limited data on the pharmacogenetics of plasma tenofovir exposure in Africans. We hypothesized that polymorphisms in *ABCB1*, *ABCC2*, *ABCC4*, *ABCG2*, *CYP3A4*, *SLC28A2* and *SLCO1B3* may be associated with interindividual differences in plasma tenofovir clearance among Southern African PLWH receiving TAF or TDF. We also hypothesized that we would identify novel associations genome-wide.

Methods

Ethics

The present study was conducted in accordance with the Declaration of Helsinki and the ADVANCE protocol WRHI 060 (NCT03122262) received ethics and regulatory approvals from the Wits Human Research Ethics Committee (REF 160606B) and the South African Health Products Regulatory Authority (REF 20160620), respectively. Ethics approval for the pharmacogenetics sub-study was also granted by the University of Cape Town Health Sciences Human Research Ethics (REF 403/2019). Written informed consent for genetic research was obtained from study participants.

Study population

The ADVANCE study in South Africa was a phase 3 non-inferiority clinical trial in which 1053 HIV-positive, ART-naïve participants were randomly assigned to one of three treatment arms: 1) dolutegravir, TAF and emtricitabine; 2) dolutegravir, TDF and emtricitabine; or 3)

efavirenz, TDF and emtricitabine.^{23,24} The present analyses included arm 1 and arm 2 participants who consented to genetic analysis.

Tenofovir assay

Plasma tenofovir concentrations were determined with a validated liquid chromatography tandem mass spectrometry assay developed at the Division of Clinical Pharmacology, University of Cape Town. The method utilised plasma protein precipitation followed by high performance liquid chromatography with tandem mass spectrometry detection.

Chromatographic separation was achieved on a Waters Atlantis T3 column. The AB Sciex 5500 Qtrap mass spectrometer was used to monitor the transition of the protonated precursor ions 288.1 and 294.1 to the product ions 176.1 and 182.1 for tenofovir and tenofovir-d6 (internal standard), respectively. Electrospray ionisation was used for ion production. The calibration curve fitted a quadratic (weighted by 1/concentration) regression based on peak area ratios over the range 0.5 to 300 ng/mL. The combined accuracy (%Nom) of the limit of quantification, low, medium, and high-quality controls (3 validation batches, N=18) were between 93.8% and 103.8%, with precision (%CV) less than 13%.

Genetic polymorphisms

Whole blood was collected from consenting participants, and DNA extracted using the salting out method as described elsewhere.²⁵ Samples were labelled with coded identifiers. Genotyping utilized the Illumina Infinium Multi-Ethnic Global BeadChip (MEGA^{EX}) at Vanderbilt Technologies for Advanced Genomics (VANTAGE). Post-genotype quality control was performed by Vanderbilt Technologies for Advanced Genomics Analysis and Research Design (VANGARD), and included sex checks, call rates by marker and sample, identity by descent (IBD) plots to identify and remove related individuals, assessment for batch effects, concordance between duplicate samples, and HapMap controls.

All quality control steps were performed using PLINK version 1.9.²⁶ Genotyping efficiency per participant was > 95% for all samples. After quality control, data were imputed using the TOPMed reference panel after transforming to genome build 38 using liftOver and stratification by chromosome to parallelize the imputation process.^{27,28} We excluded polymorphisms with imputation scores < 0.3, genotyping call rates < 99%, minor allele frequency (MAF) < 0.05, or Hardy-Weinberg Equilibrium (HWE) P-values < 1.0x10⁻⁸. Linkage disequilibrium (LD) r^2 values were determined using PLINK.

Pharmacokinetic sampling and analysis

In a subset of participants (equally divided between the TDF- and TAF-containing arms), intense pharmacokinetic sampling was done at steady state, with plasma samples collected pre-dose and 1, 2, 4, 6, 8 and 24 hours post dose. Doses preceding intense sampling were observed and taken after a standard meal. For all other individuals, at least one plasma sample (sparse pharmacokinetic sampling) was collected at week 48 or 96 at random times post dose.

Deriving individual parameters and unexplained variability from a population pharmacokinetic model

We used a previously published pharmacokinetic model of tenofovir to estimate individual values of clearance and variability in clearance. Details about the population pharmacokinetic model have been presented elsewhere.²⁹ Briefly, the 2-compartment model describing tenofovir disposition was developed with data from the 41 intensively sampled individuals in a 1:1 ratio (21 on TDF, 20 on TAF) from the ADVANCE study. By employing a post-hoc Bayes estimation method and considering an individual's pharmacokinetic data and characteristics (i.e., weight), the tenofovir model was used to generate individual estimates of

clearance and unexplained variability in clearance for all individuals including those who underwent sparse sampling only.

Genetic association analyses

The outcome of interest was unexplained variability in plasma tenofovir clearance (CL_{BSV}) among individuals receiving TAF or TDF. To adjust for genetic ancestry, we estimated continuous axes of ancestry incorporating the intersection of common autosomal genotypes using EIGENSTRAT.³⁰ We projected our individuals into a principal components analysis on the 1000Genomes. Principal component scree plots were visually inspected to determine how many components to include in analyses. Based on this, just the first two principal components were included. Other than principal components, no additional covariates were included in association analyses because they were already accounted for in the population pharmacokinetic model. We report regression coefficients (β) for additive associations with polymorphisms, where positive β values indicate an association with increased clearance. The Bonferroni method was used to correct for multiple testing, with significance thresholds of 0.05 divided by the number of polymorphisms tested in targeted polymorphism and gene analyses, and $P < 5.0 \times 10^{-8}$ for genome-wide analyses. We present nominal P-values uncorrected for multiple testing and indicate thresholds that would be significant with multiple testing.

We *a priori* selected for analysis eight polymorphisms previously reported to affect tenofovir pharmacokinetics (*ABCB1* rs2032582; *ABCC2* rs3740066; *ABCC4* rs3742106 and rs1751034; *ABCG2* rs2231142; *CYP3A4* rs35599367; *SLC28A2* rs11854484; and *SLCO1B3* rs7311358). Beyond these polymorphisms, and to decrease the burden of multiple testing, we then used a stepwise approach to prioritize which sets of polymorphisms to interrogate, based on the rationale that polymorphisms that have previously been strongly associated

with at least one drug-related phenotype, or that have been genome-wide significantly associated with any trait, are most likely to be true associations.

We used as references the Pharmacogenomics Knowledge Base (PharmGKB, accessed 5 April 2022)³¹ and the NHGRI-EBI GWAS Catalog (accessed 5 April 2022).³² In PharmGKB, 141 polymorphisms were previously associated with at least one drug-related phenotype (pharmacokinetics, efficacy, or toxicity) with levels of evidence of 1 (the preponderance of evidence shows an association, which has been replicated in multiple cohorts, and preferably with strong effect size) or 2 (moderate evidence of association, which has been replicated but some studies may not show statistical significance, or the effect size is small). In the GWAS Catalog, 132 547 polymorphisms were previously associated with any trait at $P < 5.0 \times 10^{-8}$ in at least one published study. A subset of 22 polymorphisms were common to both PharmGKB and the GWAS Catalog. We prioritized polymorphisms common to both PharmGKB and the GWAS Catalog, considering these to have the most robust evidence for true drug-related associations. We secondarily explored all polymorphisms from PharmGKB and from the GWAS Catalog (based on criteria described above), and all polymorphisms in our imputed genome-wide genotype data.

Results

Study population

Among 340 ADVANCE participants who consented for genetic analyses, 268 were successfully genotyped and had pharmacokinetic data. Participant disposition is presented in **Figure 1**. All participants were Black Africans and 62% were females. Study participant characteristics are shown in **Table 1**.

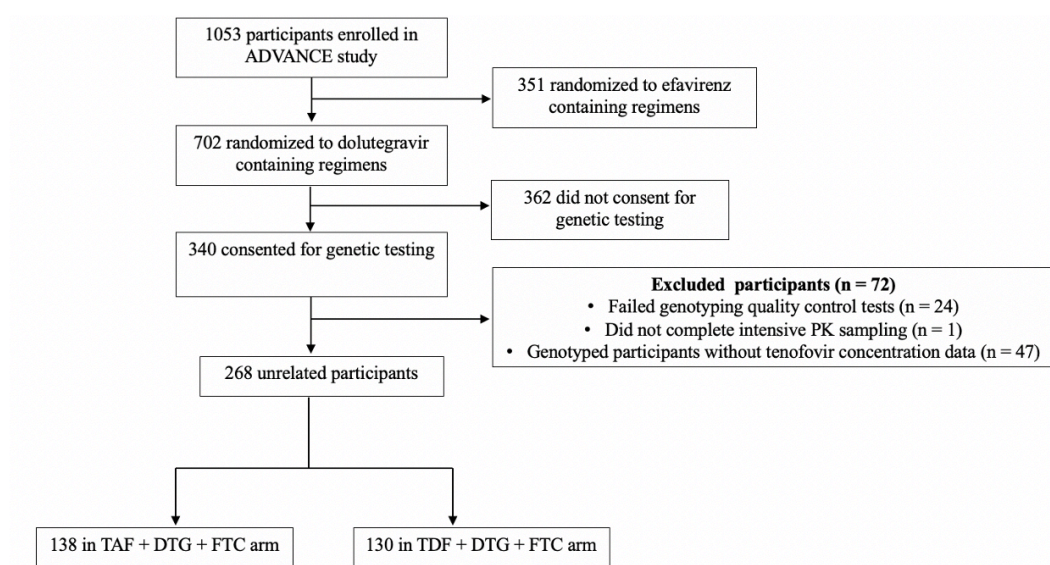


Figure 1. Disposition of study participants. Of 1053 participants enrolled in the ADVANCE study, 268 randomized to TAF- and TDF-containing regimens had available pharmacokinetic sampling data and were evaluable for genetic associations.

Table 1: Baseline characteristics for TAF and TDF recipients included in genetic analyses

Characteristic	TAF ^a , DTG and FTC (n=138)	TDF, DTG and FTC (n=130)
Age in years (IQR)	32 (27 to 38)	32 (27 to 37)
Sex		
Female, n (%)	86 (62)	84 (65)
Male, n (%)	52 (38)	46 (35)
BMI in kg/m ² , median (IQR)	23.4 (20.5 to 26.5)	22.9 (20.0 to 27.5)
CD4 T-cell count in cells/mm ³ , median (IQR)	299 (164 to 491)	283 (159 to 424)
CrCl in ml/min, median (IQR)	125 (111 to 141)	120 (104 to 148)

^a TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; DTG, dolutegravir; FTC, emtricitabine; IQR = interquartile range; BMI = body mass index; CrCl = creatinine clearance.

Population pharmacokinetic modelling

Details of the final tenofovir population pharmacokinetic model have been presented

elsewhere.²⁹ Briefly, tenofovir pharmacokinetics were best described by a two-compartment

model with clearance, central volume, and peripheral volume estimated at 44.7 L/h, 378 L, and peripheral volume of 356 L, respectively for a typical 70 kg individual.

Genetic associations with tenofovir clearance

Three of eight polymorphisms previously associated with tenofovir pharmacokinetics were not evaluable in our genetic data: *CYP3A4* rs35599367 did not meet the imputation quality threshold, while *ABCB1* rs2032582 and *ABCG2* rs2231142 are very infrequent in Africans (MAF less than approximately 5%). The lowest P-value for association with tenofovir CL_{BSV} in the TAF arm was *ABCC2* rs3740066 ($\beta = -0.1$, $P = 0.4$). The lowest P-value for association with tenofovir CL_{BSV} in the TDF arm was *ABCC2* rs3740066 ($\beta = -0.1$, $P = 0.3$) (Table 2).

Table 2: Associations with polymorphisms selected *a priori*

Tenofovir prodrug	Polymorphism	Gene	MAF ^a	Beta	P-value ^b
TAF	rs3740066	<i>ABCC2</i>	0.2	-0.1	0.4
	rs3742106	<i>ABCC4</i>	0.4	0.03	0.5
	rs11854484	<i>SLC28A2</i>	0.2	-0.03	0.6
	rs7311358	<i>SLOC1B3</i>	0.4	0.02	0.7
	rs1751034	<i>ABCC4</i>	0.4	-0.001	1.0
TDF	rs3740066	<i>ABCC2</i>	0.2	-0.1	0.3
	rs1751034	<i>ABCC4</i>	0.4	-0.03	0.4
	rs11854484	<i>SLC28A2</i>	0.2	0.01	0.8
	rs3742106	<i>ABCC4</i>	0.4	-0.002	1.0

^a TAF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; MAF, minor allele frequency.

^b Bonferroni significance threshold was 0.01 for the subset of five polymorphisms selected *a priori*.

Among polymorphisms from PharmGKB and the GWAS catalog, we report the five lowest P-value associations with TAF and TDF CL_{BSV} (Tables 3 and 4). We were able to test for

associations with 11 of 22 polymorphisms common to PharmGKB and the GWAS Catalog. Polymorphisms not included in our analyses did not meet imputation score, MAF, or HWE cut-offs. In the TAF arm the lowest P-value for association among these 11 polymorphisms was *IFNL4* rs12979860 ($\beta = 0.1$, $P = 0.003$) (**Table 3**), which withstood correction for multiple testing (cut-off $P < 0.0045$), and with the C allele being associated with increased tenofovir CL_{BSV} (**Online supplemental material Figure S1**).

Table 3: Lowest P-values for genetic association with unexplained variability in tenofovir clearance in the TAF arm

	Polymorphism	Gene	MAF	Beta	P-value
PharmGKB and GWAS catalog ^a (n = 11 polymorphisms)	rs12979860	<i>IFNL4</i>	0.4	0.1	0.003
	rs8099917	Intergenic	0.05	-0.2	0.06
	rs1801133	<i>MTHFR</i>	0.09	-0.1	0.07
	rs7412	<i>APOE</i>	0.2	-0.05	0.27
	rs396991	<i>FCGR3A</i>	0.3	0.04	0.4
PharmGKB but not in GWAS catalog ^b (n = 15 polymorphisms)	rs1051266	<i>SLC19A1</i>	0.3	0.1	0.1
	rs4673993	<i>ATIC</i>	0.07	-0.1	0.1
	rs1042713	<i>ADRB2</i>	0.5	0.1	0.1
	rs7294	<i>VKORC1</i>	0.4	0.04	0.3
	rs20455	<i>KIF6</i>	0.2	-0.1	0.3
GWAS catalog ^c (n = 82 787 polymorphisms)	rs3850736	<i>LINC01414</i>	0.2	0.2	3.5x10 ⁻⁵
	rs2356369	<i>LINC01414</i>	0.2	0.2	4.6x10 ⁻⁵
	rs7570090	<i>RPE</i>	0.4	-0.2	8.2x10 ⁻⁵
	rs7841320	<i>LINC01414</i>	0.3	0.2	9.0x10 ⁻⁵
	rs10032941	<i>CIQTNF7</i>	0.3	0.2	1.0x10 ⁻⁴
Genome-wide genotype data ^d (n = 8 903 713 polymorphisms)	rs9305223 ^e	<i>LINC01684</i>	0.3	0.2	3.0x10 ⁻⁸
	rs4816969	<i>LINC01684</i>	0.3	0.2	3.7x10 ⁻⁸
	rs2829163 ^f	Intergenic	0.4	0.2	4.5x10 ⁻⁸
	rs7282679	Intergenic	0.2	0.2	5.9x10 ⁻⁸
	rs2226443	<i>LINC01684</i>	0.3	0.2	6.6x10 ⁻⁸

^a Significance threshold was 4.5x10⁻³ for a subset of 11 polymorphisms.

^b Significance threshold was 3.3x10⁻³ for a subset of 15 polymorphisms.

^c Significance threshold was 6.0x10⁻⁷ for a subset of 82 787 polymorphisms.

^d Significance threshold was 5.0x10⁻⁹ for genome-wide analysis.

^e The rs9305223 polymorphism was in strong LD with rs4816969 and rs2226443, r² > 0.9.

^f The rs2829163 polymorphism was in LD with rs7282679, r² > 0.5.

Considering PharmGKB polymorphisms that were not in the GWAS Catalog, we were able to test for associations with 15 (11%) of 141 polymorphisms. The lowest P-value for association among these 15 polymorphisms was *SLC19A1* rs1051266 ($\beta = 0.1$, $P = 0.1$).

Considering polymorphisms previously associated with any GWAS Catalog trait, we were able to test for associations with 82 785 (62%) of 132 547. The lowest P-value for association among these was *LINC01414* rs3850736 ($\beta = 0.2$, $P = 3.5 \times 10^{-5}$), which did not withstand correction for multiple testing.

In the TDF arm, among polymorphisms common to PharmGKB and the GWAS catalog, the lowest P-values was again *IFNL4* rs12979860 ($\beta = 0.1$, $P = 0.003$) (**Table 4**); which withstood correction for multiple testing (cut-off $P < 0.0045$), with the C allele associated with increased tenofovir CL_{BSV} (**Online supplemental material Figure S2**). Considering PharmGKB polymorphisms that were not in the GWAS Catalog, the lowest P-value was *DPYD* rs2297595 ($\beta = 0.1$, $P = 0.03$). Considering polymorphisms previously associated with any GWAS Catalog trait, the lowest P-value for association among these was rs144511092 ($\beta = 0.3$, $P = 1.9 \times 10^{-5}$), which did not withstand correction for multiple testing. In genome-wide associations combining both TAF and TDF arms ($n = 268$), *IFNL4* rs12979860 had a P-value of $P = 2.1 \times 10^{-5}$ ($\beta = 0.12$), which did not achieve genome-wide significance (**Online supplemental material Figure S3**). In both analyses that considered polymorphisms in PharmGKB only, and polymorphisms from the GWAS catalog, none of the polymorphisms with the lowest P-value withstood correction for multiple testing.

Table 4: Lowest P-values for genetic association with unexplained variability in tenofovir clearance in the TDF arm

	Polymorphism	Gene	MAF	Beta	P-value
PharmGKB and GWAS catalog ^a (n = 11 polymorphisms)	rs12979860	<i>IFNL4</i>	0.4	0.1	0.003
	rs12777823	Intergenic	0.3	-0.05	0.2
	rs1800629	<i>TNF</i>	0.2	-0.06	0.2
	rs7412	<i>APOE</i>	0.2	-0.05	0.25
	rs10929302	<i>UGT1A</i>	0.3	0.02	0.6
PharmGKB but not in GWAS catalog ^b (n = 15 polymorphisms)	rs2297595	<i>DPYD</i>	0.1	0.1	0.03
	rs3745274	<i>CYP2B6</i>	0.4	0.1	0.05
	rs1042713	<i>ADRB2</i>	0.5	0.04	0.2
	rs28399499	<i>CYP2B6</i>	0.1	-0.1	0.3
	rs1801159	<i>DPYD</i>	0.2	0.05	0.3
GWAS catalog ^c (n = 82 787 polymorphisms)	rs144511092	Intergenic	0.1	0.3	1.9x10 ⁻⁵
	rs7902657	Intergenic	0.5	0.1	2.5x10 ⁻⁵
	rs4682844	<i>CCDC12</i>	0.2	0.2	2.7x10 ⁻⁵
	rs34940374	<i>GIMAP6</i>	0.1	0.2	3.4x10 ⁻⁵
	rs7970054	<i>LRIG3</i>	0.05	0.3	5.0x10 ⁻⁵
Genome-wide genotype data ^d (n = 8 903 713 polymorphisms)	rs142693425	Intergenic	0.1	0.3	1.4x10 ⁻⁸
	rs112914324	Intergenic	0.3	0.2	1.6x10 ⁻⁸
	rs11995962	Intergenic	0.1	0.3	2.3x10 ⁻⁸
	rs73151902	Intergenic	0.05	0.4	1.1x10 ⁻⁷
	rs866325353	Intergenic	0.05	0.4	2.1x10 ⁻⁷

^a Significance threshold was 4.5x10⁻³ for a subset of 11 polymorphisms.

^b Significance threshold was 3.3x10⁻³ for a subset of 15 polymorphisms.

^c Significance threshold was 6.0x10⁻⁷ for a subset of 82 787 polymorphisms.

^d Significance threshold was 5.0x10⁻⁹ for genome-wide analysis.

In analyses that explored genome-wide associations with tenofovir CL_{BSV} in the TAF arm, the lowest P-value was *LINC01684* rs9305223 ($\beta = 0.2$, $P = 3.0 \times 10^{-8}$), which was genome-wide significant. This polymorphism is in strong linkage disequilibrium ($r^2 > 0.9$) with *LINC01684* rs4816969 and rs2226443 on chromosome 21 (**Figure 2**). Intergenic rs2829163 on chromosome 21 also reached genome-wide significance ($\beta = 0.2$, $P = 4.5 \times 10^{-8}$). The five lowest P-values for tenofovir CL_{BSV} in the TAF arm are presented in **Table 3**.

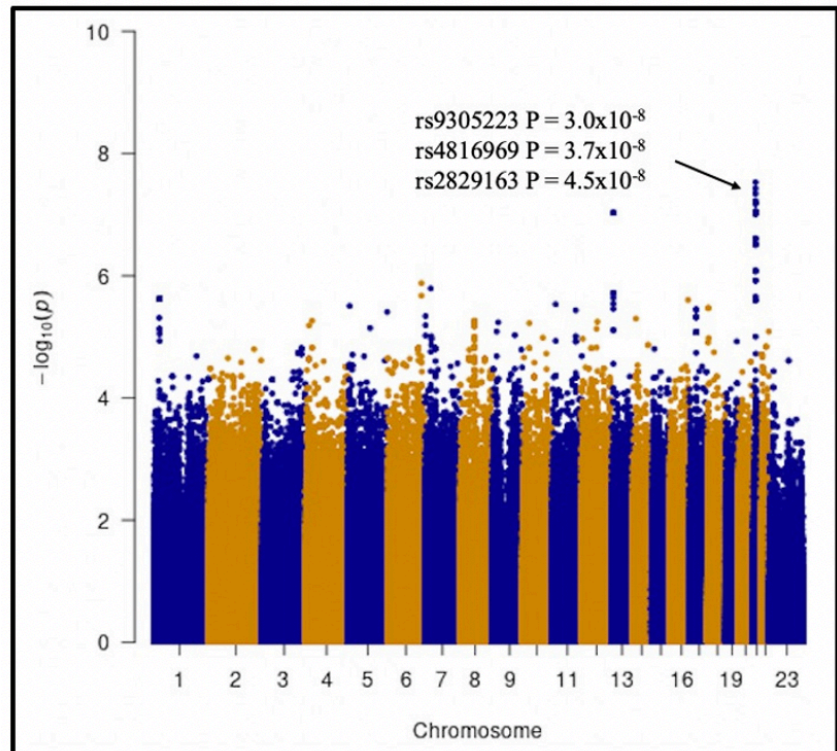


Figure 2. Manhattan plot of associations with unexplained variability in tenofovir clearance in the TAF arm. The figure shows $-\log_{10}$ P-values for association among 138 TAF recipients who were evaluable for genetic associations. The black arrow indicates polymorphisms that reached genome-wide significance at $P < 5.0 \times 10^{-8}$.

In the TDF arm, the lowest P-value was for intergenic rs142693425 on chromosome 15 ($\beta = 0.3$, $P = 1.4 \times 10^{-8}$), and rs112914324 ($\beta = 0.2$, $P = 1.6 \times 10^{-8}$) on chromosome 11 and rs11995962 ($\beta = 0.3$, $P = 2.3 \times 10^{-8}$) on chromosome 8, which were each genome-wide significant (**Figure 3**) and associated with increased tenofovir CL_{BSV} . The five lowest P-values for tenofovir CL_{BSV} in the TDF arm are presented in **Table 4**.

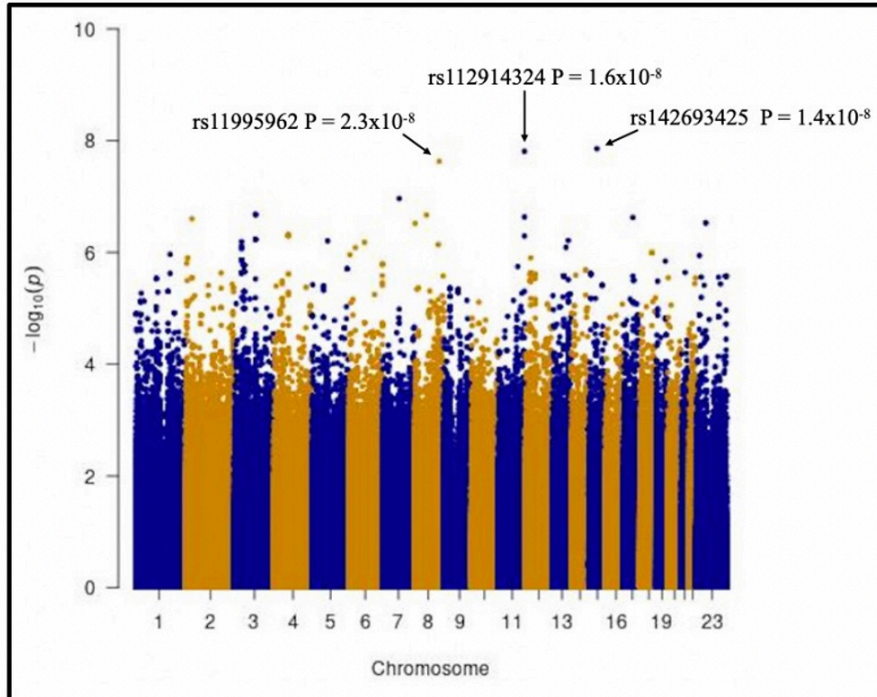


Figure 3. Manhattan plot of associations with unexplained variability in tenofovir clearance in the TDF arm. The figure shows $-\log_{10}$ P-values for association among 130 TDF recipients who were evaluable for genetic associations. The black arrows indicate polymorphisms that reached genome-wide significance at $P < 5.0 \times 10^{-8}$.

Discussion

We characterized genetic associations with unexplained variability in tenofovir clearance (CL_{BSV}) in ART-naïve participants randomized to TAF or TDF in the ADVANCE study in South Africa. We prioritized evaluation of polymorphisms from prior genetic association studies represented in PharmGKB and the GWAS Catalog, as present in our imputed genome-wide data. Among polymorphisms common to both PharmGKB and the GWAS catalog, the lowest P-value in both TDF and TAF arms was *IFNL4* rs12979860 (TAF $P = 0.003$, TDF $P = 0.003$), each of which withstood correction for multiple testing. This is a pseudogene which, in some humans, encodes the interferon lambda 4 (IFNL4) protein which is involved in immune response to viral infections. In prior reports, rs12979860 CC genotype was associated with lower hepatitis B virus plasma titers in Chinese and Moroccan patients treated with interferon-based therapy.^{33,34} In other studies, *IFNL4* rs12979860 CC genotype was independently associated with spontaneous HIV control in Caucasian Spanish individuals,³⁵ but not in African Americans.^{36,37} No association has been previously reported between *IFNL4* rs12979860 and tenofovir pharmacokinetics. While it is remarkable that *IFNL4* rs12979860 was associated with tenofovir clearance in both the TAF and TDF arms, it is unclear how this immune-response protein would mechanistically affect the pharmacokinetics of tenofovir, and there are no apparent drug metabolism or transport genes nearby on chromosome 19.

In genome-wide analyses for associations in the TAF arm, the lowest P-value was *LINC01684* rs9305223 ($P = 3.0 \times 10^{-8}$) on chromosome 21, which was in strong linkage disequilibrium with rs4816969 and rs2226443. Intergenic rs2829163 on chromosome 21 also reached genome-wide significance. In genome-wide analyses for associations in the TDF arm, the lowest P-value was intergenic rs142693425 ($P = 1.4 \times 10^{-8}$) which reached genome-wide significance, as did two additional intergenic polymorphisms, rs112914324 and

rs11995962. It is unclear how these intergenic polymorphisms could affect tenofovir disposition. These associations warrant replication in independent cohorts.

We found no significant associations between polymorphisms selected *a priori* (*ABCC2* rs3740066; *ABCC4* rs3742106 and rs1751034; *SLC28A2* rs11854484 and *SLOC1B3* rs7311358) and CL_{BSV}. In a Chinese population, *ABCC2* rs3740066, *ABCC4* rs3742106 and *SLCO1B3* rs7311358 were associated with TAF pharmacokinetics.²⁰ Carriers of the *ABCC4* rs1751034 variant had slower tenofovir clearance.²¹ In a study from Italy, *SLC28A2* rs11854484 CT/TT genotypes were associated with plasma tenofovir exposure.³⁸

Our study had limitations. Although our sample size was modest to detect genome-wide associations, this was the largest pharmacogenetic study of tenofovir clearance to date in Africa. In addition, the prioritized approach used in our analyses reduced the burden of multiple testing. A larger sample size may identify novel genome-wide significant associations with small effect sizes, or with infrequent polymorphisms. Several polymorphisms previously associated with tenofovir phenotypes could not be evaluated in our dataset.

In summary, unexplained variability in tenofovir clearance was associated with an *IFNL4* polymorphism that has previously been associated with response to hepatitis B virus treatment, and with immune control of HIV. In both TAF and TDF arms of ADVANCE study, we also identified genome-wide significant associations with intergenic polymorphisms. Further studies are needed to replicate these associations, as it is unclear mechanistically how these polymorphisms would affect tenofovir clearance.

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Supplementary material

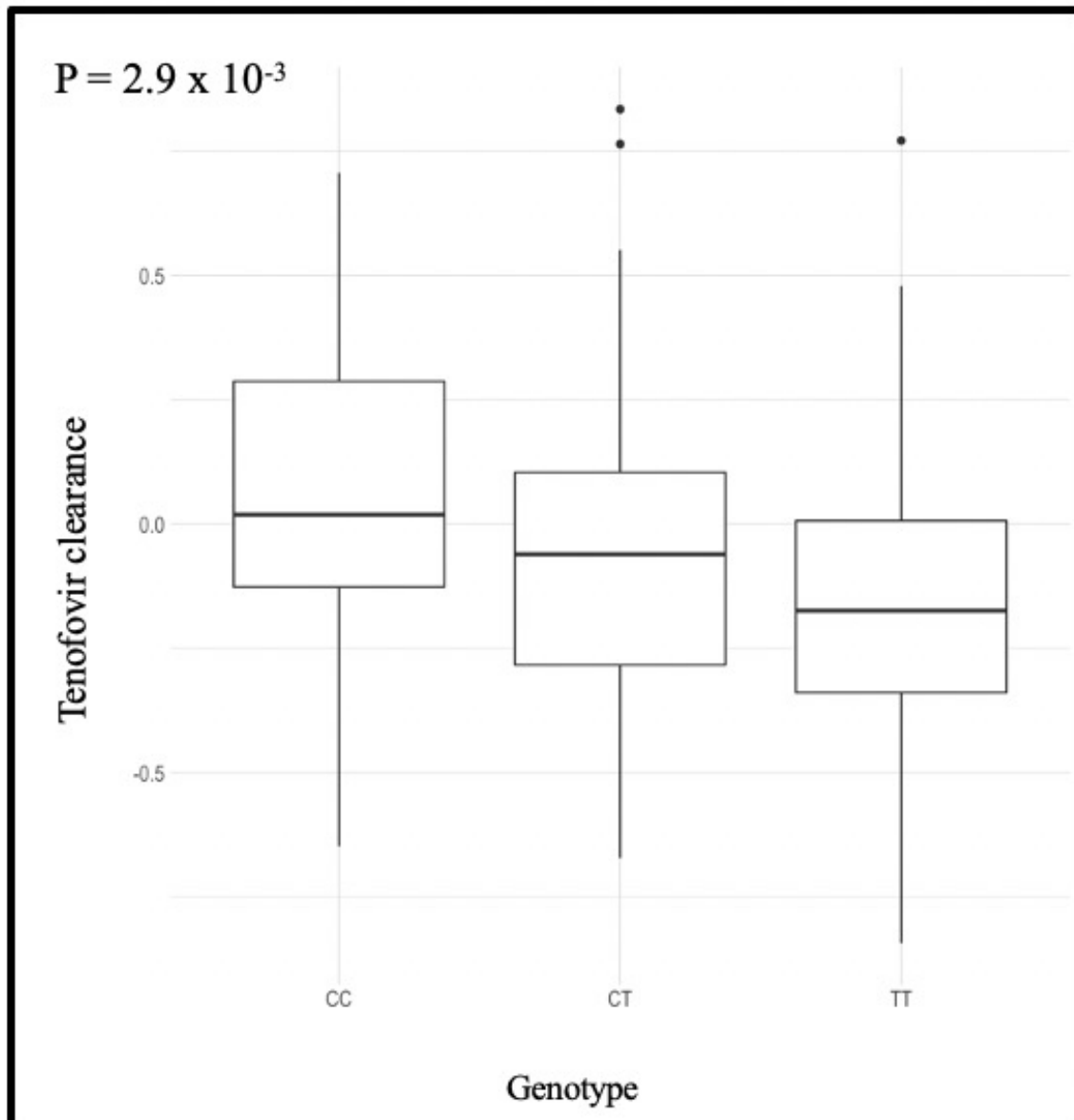


Figure S1. Box and whisker plots for *IFNL4* rs12979860 genotypes and unexplained variability in tenofovir clearance in the TAF/DTG/FTC arm of ADVANCE. The figure shows tenofovir clearance on the y-axis and *IFNL4* rs12979860 genotypes on the x-axis. The C allele was associated with increased tenofovir clearance. Analyses included 138 individuals who were evaluable for genetic associations (CC in 21, CT in 69, TT in 48).

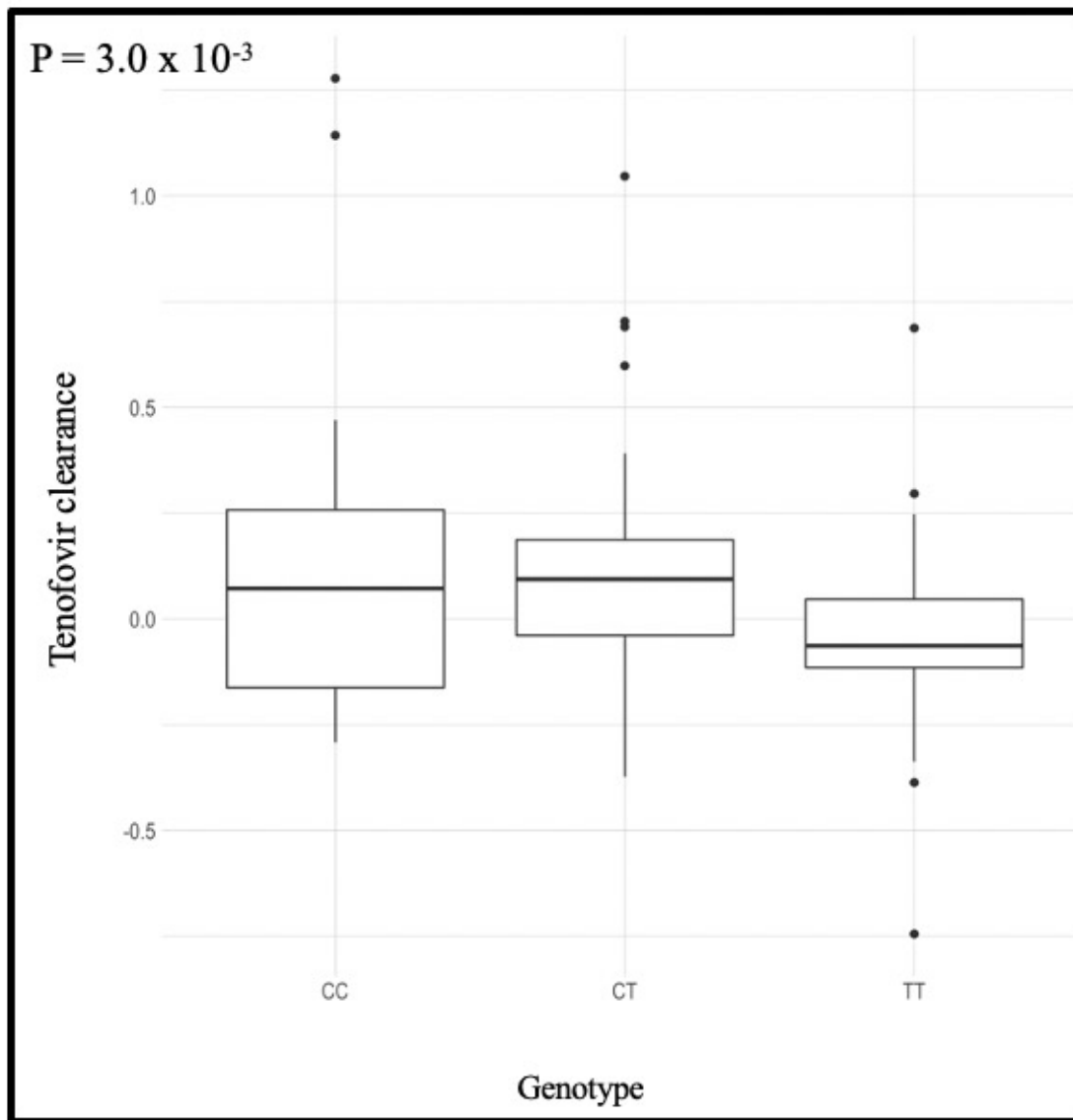


Figure S2. Box and whisker plots for *IFNL4* rs12979860 genotypes and unexplained variability in tenofovir clearance in the TDF/DTG/FTC arm of ADVANCE. The figure shows tenofovir clearance on the y-axis and *IFNL4* rs12979860 genotypes on the x-axis. The C allele was associated with increased tenofovir clearance. Analyses included 130 individuals who were evaluable for genetic associations (CC in 22, CT in 67, TT in 41).

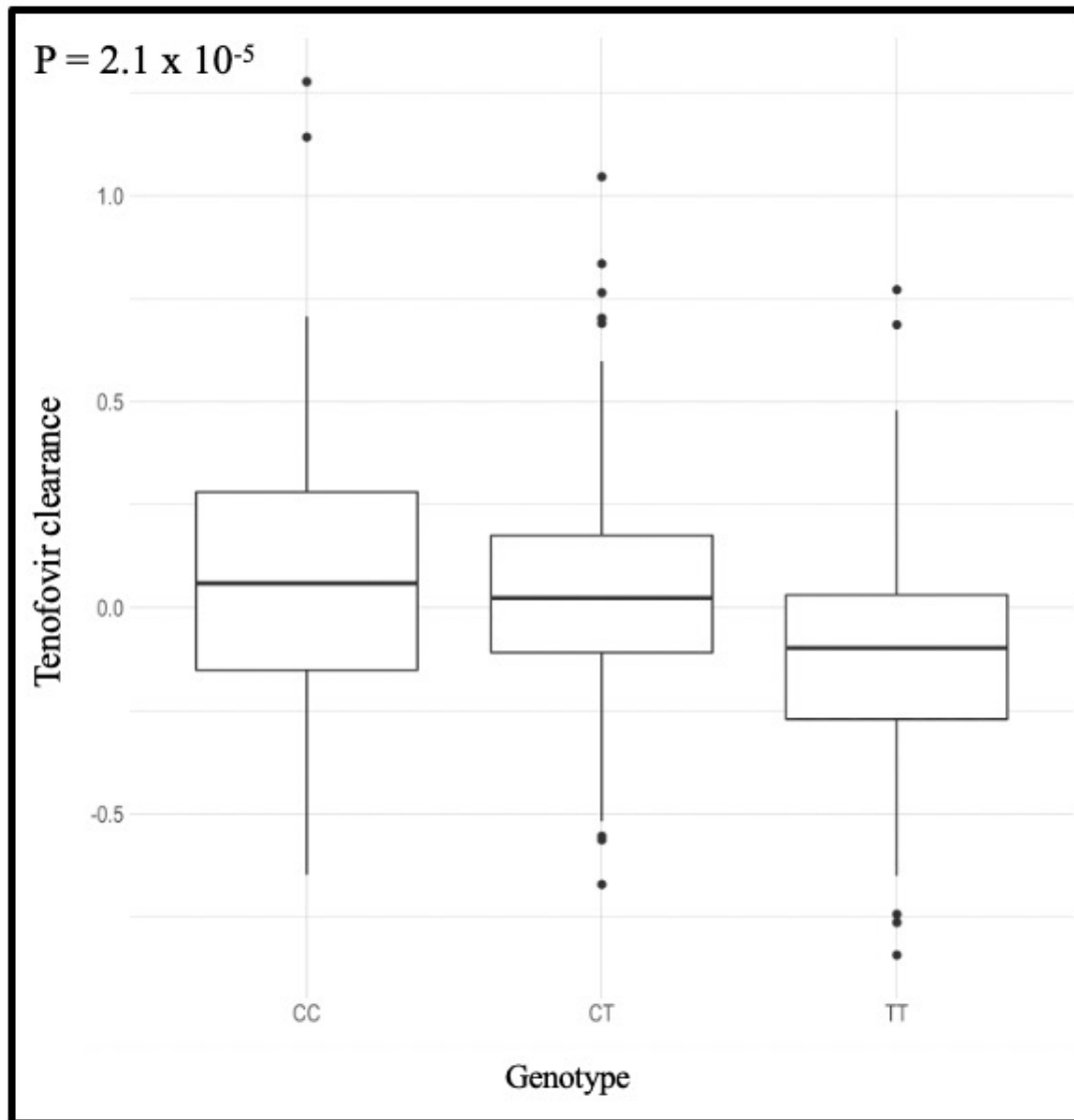


Figure S3. Box and whisker plots for *IFNL4* rs12979860 genotypes with unexplained variability in tenofovir clearance in the combined TAF/DTG/FTC and TDF/DTG/FTC arms of ADVANCE. The figure shows tenofovir clearance on the y-axis and *IFNL4* rs12979860 genotypes on the x-axis. The C allele was associated with increased tenofovir clearance. Analyses included 268 individuals who were evaluable for genetic associations (CC in 43, CT in 136, TT in 89).

Chapter 5

Genetic associations with weight gain among South Africans who initiated dolutegravir-containing and tenofovir-containing regimens

Abstract

Background: Excessive weight gain affects some HIV-positive individuals prescribed dolutegravir-containing regimens. Mechanisms underlying such weight gain are unknown.

Setting: Data and DNA from antiretroviral therapy-naïve participants who were randomized to initiate dolutegravir with emtricitabine plus either tenofovir alafenamide (TAF) or tenofovir disoproxil fumarate (TDF) in the ADVANCE study (NCT03122262) were used to characterize associations between human genetic polymorphisms and magnitude of weight gain.

Methods: Associations with percent weight gain from baseline to week 48 were assessed using multivariable linear regression models. Primary analyses a priori considered 59 polymorphisms and 10 genes of potential relevance to dolutegravir, TAF, or TDF pharmacokinetics. We also explored genome-wide associations.

Results: Among the 314 (92%) of 340 dolutegravir recipients who were successfully genotyped, 160 (47%) and 154 (45%) were randomized to TAF/emtricitabine and TDF/emtricitabine, respectively. In target gene analyses, the lowest P-values for the dolutegravir and tenofovir groups were *ABCG2* rs4148149 ($P = 7.0 \times 10^{-4}$) and *ABCC10* rs67861980 ($P = 1.0 \times 10^{-2}$), respectively, which were not significant after correction for multiple testing. In genome-wide analyses, the lowest P-values were rs7590091 in *TMEM163* ($P = 3.7 \times 10^{-8}$) for dolutegravir, rs17137701 in *LOC105379130* ($P = 6.4 \times 10^{-8}$) for TAF, and rs76771105 in *LOC105371716* ($P = 9.7 \times 10^{-8}$) for TDF.

Conclusions: Among South African participants in a randomized clinical trial of dolutegravir plus either TAF/emtricitabine or TDF/emtricitabine, we identified several potential genetic associations with weight gain. Only *TMEM163* rs7590091 withstood correction for multiple testing. These associations warrant replication in other cohorts.

Key Words: weight gain, dolutegravir, tenofovir, pharmacogenetics

Introduction

The HIV-1 integrase strand transfer inhibitor (INSTI) dolutegravir effectively controls viral replication, is generally well tolerated, and has out-performed several other drugs.¹⁻⁶ Dolutegravir and other INSTIs are preferred components of first-line antiretroviral therapy (ART) in the United States, Europe, by WHO, and increasingly worldwide.^{7,8} In Africa, dolutegravir is replacing non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as efavirenz in first-line ART and HIV-1 protease inhibitors in second-line ART regimens.^{9,10} However, recent reports have associated INSTIs, especially the second generation INSTIs dolutegravir and bictegravir, with greater weight gain than other agents either after ART initiation or switch to INSTI-containing ART.¹¹⁻¹⁷ An early report was an observational cohort study of 495 patients with sustained virologic suppression, 136 of whom switched from efavirenz-containing regimens to INSTI-containing regimens. At 18 months, patients who switched to INSTI-containing regimens gained significantly more weight, especially those who switched to dolutegravir, than those who remained on efavirenz.¹¹ Similar findings of greater weight gain with INSTI-containing regimens as compared with NNRTI or protease inhibitor-containing regimens were reported in a pooled analysis of 8 randomized controlled trials among treatment-naïve individuals initiating ART,¹⁶ and in cohort studies of patients with viral suppression on protease inhibitor-containing or NNRTI-containing ART who then switched to INSTIs.^{18,19}

Specific concomitant nucleos(t)ide reverse transcriptase inhibitors (NRTIs) have also been associated with magnitude of weight gain. In randomized controlled trials among HIV-positive patients, the tenofovir prodrug, tenofovir alafenamide (TAF), has been associated with greater weight gain than tenofovir disoproxil fumarate (TDF),¹⁶ and in a study of pre-exposure prophylaxis in HIV-negative individuals.²⁰ TAF has become the most widely prescribed NRTI in high-income countries because it causes less kidney and bone toxicity

than TDF. There is considerable interest in using TAF rather than TDF in low-income and middle-income countries based on its more favorable safety profile, and likely lower cost for generic manufacture given its much lower dose.¹⁰

Additional factors that have been associated with greater weight gain in individuals who initiate ART, and in people with viral suppression on ART who switch to INSTIs, have included Black race and female sex.^{16,18} Two randomized controlled trials of dolutegravir in Africa have reported 48-week findings including changes in weight. In the NAMSAL study, which randomized ART-naïve participants in Cameroon to dolutegravir or efavirenz, both with TDF and lamivudine, there was greater median weight gain and more treatment-emergent obesity at 48 weeks in the dolutegravir arm.¹⁵ In the ADVANCE study, a 3-arm (1) dolutegravir, TAF, and emtricitabine; 2) dolutegravir, TDF, and emtricitabine; or 3) efavirenz, TDF, and emtricitabine randomized controlled trial of ART-naïve participants in South Africa; there was greater mean weight gain and treatment-emergent obesity at 48 weeks in the dolutegravir arms, with the greatest weight increases in the dolutegravir and TAF arm.¹⁷ This may be explained, in part, by lesser weight gain among participants receiving efavirenz with TDF, particularly with *CYP2B6* slow metabolizer genotypes.^{21,22}

Genomic studies could help elucidate mechanisms underlying greater weight gain with dolutegravir and TAF, which are currently unknown. We conducted analyses to characterize associations between human genetic polymorphisms and magnitude of weight gain among ART-naïve individuals who were randomized to initiate dolutegravir-containing regimens in the ADVANCE study.

Methods

Study Population

The ADVANCE study in South Africa is a phase 3 noninferiority clinical trial in which 1053 HIV-positive, ART-naïve participants were randomly assigned to 1 of 3 treatment arms 17: (1) dolutegravir, TAF, and emtricitabine; (2) dolutegravir, TDF, and emtricitabine; or (3) efavirenz, TDF, and emtricitabine. DNA samples were collected from 340 (48%) of 702 treatment arm 1 and 2 participants who consented to genetic testing. Although some individuals opted to not participate in the genetic study, many individuals were not offered the opportunity to participate. Ethics approval was granted by the University of Cape Town and Wits University Human Research Ethics Committees.

Genetic Polymorphisms

Whole blood was collected from consenting participants, and DNA extracted using the salting out method as described elsewhere.²³ Samples were labeled with coded identifiers. Stored DNA was genotyped using the Illumina Infinium Multi-Ethnic Global BeadChip (MEGA^{EX}) at Vanderbilt Technologies for Advanced Genomics (VANTAGE). Post genotype quality control was performed by Vanderbilt Technologies for Advanced Genomics Analysis and Research Design (VANGARD). All quality control steps were performed using PLINK version 1.9.²⁴ Genotyping efficiency per participant was >95% for all samples. Markers with genotyping efficiency <95% were censored, as were those with minor allele frequency (MAF) <5%. We excluded 21 samples with overall genotyping call rates <95%. After quality control, data were imputed to 1000 genomes after transforming to genome build 37 using liftOver and stratification by chromosome to parallelize the imputation process.^{25,26} For each chromosome in each phase, 100% concordance with genotyped data was assessed. Imputed polymorphisms with imputation scores less <0.7, genotyping call rates <95%, or MAF <0.05 were excluded. To address population stratification, we performed data reduction using

multidimensional scaling (MDS) implemented in PLINK, which produces a k-dimensional representation of structure. Associations between percentage weight gain from baseline to week 48 and genes relevant to dolutegravir, TAF, and/or TDF metabolism were characterized.

We focused on genes that encode proteins relevant to dolutegravir and tenofovir disposition. Dolutegravir is primarily metabolized by uridine diphosphate glucuronosyl- transferase 1A1 (UGT1A1) and less so by cytochrome P450 (CYP) 3A4.²⁷ Dolutegravir is also a substrate for ATP-binding cassette transporters B1 (encoded by *ABCB1*) and G2 (encoded by *ABCG2*).²⁸ In some studies, polymorphisms in *SCL22A6*, *ABCC2*, and *ABCC4* were associated with higher tenofovir concentrations^{29–32} although a subsequent study did not replicate associations between tenofovir concentrations and *ABCC* family polymorphisms.³³ Both TAF and TDF are substrates of P-glycoprotein and human breast cancer resistance protein.³⁴ TDF is a substrate of the OAT1 and OAT3 organic anion transporters³⁵ and is minimally metabolized by CYP3A4.³⁶ We also considered genome-wide associations.

Association Analyses

Multivariable linear regression models were used to characterize associations between polymorphisms relevant to dolutegravir, TAF, and TDF with percentage weight gain from study baseline to week 48. Covariates included baseline age, self-reported sex, and concomitant NRTI, as well as the first 3 MDS coordinates to adjust for population stratification.²⁴ We report the regression coefficient (β) for additive associations with polymorphisms, where positive β values indicate an association with greater weight gain. From the NHGRI-EBI GWAS Catalog (accessed 26 November 2020),³⁷ we identified 86 polymorphisms previously associated with obesity trait in the general population at $P < 5.0 \times 10^{-8}$ in at least 1 published study. Of these, 77 polymorphisms were represented in our

genotype data. The Bonferroni method was used to determine significance threshold, with $P = 5.0 \times 10^{-8}$ for genome-wide analyses, and 0.05 divided by the number of polymorphisms tested in targeted polymorphism and gene analyses.

Results

Characteristics of Participants

Among the 340 (92%) participants who consented for genetic analyses, 314 were successfully genotyped and had genome-wide genotype data. Participant disposition is

presented in **Figure 1**. Baseline

characteristics of study

participants are shown in **Table**

1. All participants were Black

South Africans, and most were

women. Baseline characteristics

were similar between those who

consented and those who did not

consent for genotyping (Table 1).

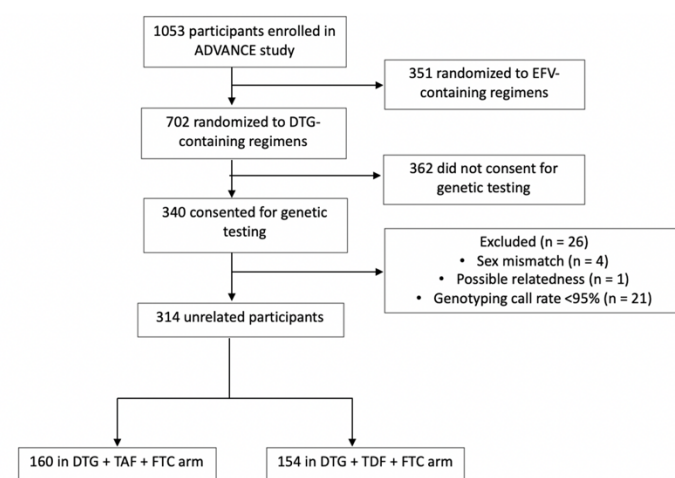


Figure 1. Disposition of study participants. Of 1053 participants enrolled in the ADVANCE study, 314 who were randomized to dolutegravir-containing regimens were evaluable for genetic associations.

Table 1. Baseline characteristics in all dolutegravir recipients

	Participants with Genetic consent		Participants without Genetic consent
	DTG, TAF, and FTC n = 154	DTG, TDF, and FTC n = 166	DTG, TAF or TDF, and FTC n = 362
Age in years, (IQR)	32 (27 to 38)	32 (27 to 37)	32 (26 to 37)
Sex			
Male, n (%)	65 (37.4%)	61 (36.8%)	154 (42.5%)
Female, n (%)	109 (62.6%)	105 (63.3%)	208 (57.5%)
Baseline BMI, kg/m ² (IQR)	23.6 (20.6 to 26.5)	23.0 (20.0 to 27.1)	23.1 (20.1 to 27.0)
Weight gain at week 48, kg (IQR)	5.8 (2.4 to 9.4)	2.5 (0.0 to 5.6)	2.9 (0.2 to 7.6)
Baseline CD4 count (IQR)	326 (170 to 502)	272 (159 to 424)	295 (173 to 451)
Baseline viral load (IQR)	28,968 (6 180 to 75,314)	25,703 (6 408 to 75,619)	22,064 (5 973 to 73,796)
Viral load suppression (<50 c/mL) at week 48, n (%)	164 (95.9%)	157 (96.3%)	262 (92.6%)

Abbreviations: IQR, Interquartile range, DTG, dolutegravir, FTC, emtricitabine

Associations With Dolutegravir-Relevant Polymorphisms and Genes

We first focused on dolutegravir, characterizing associations in polymorphisms that have previously been associated with dolutegravir metabolism or transport (selected *a priori* for this analysis) and percentage weight gain from baseline to week 48 among all dolutegravir recipients, by multivariable linear regression analysis. Results for all 314 dolutegravir recipients are presented in **Table 2**. The lowest P-value for association was *ABCG2* rs13137622 ($\beta = -1.24$, $P = 0.03$), which was not significant after correcting for multiple testing (cut-off $P < 1.0 \times 10^{-3}$).

Table 2. Association among 314 dolutegravir recipients between percentage weight gain at week 48 and selected polymorphisms relevant to dolutegravir

SNP ^a	Gene	MAF	Beta	P-value ^c
rs13137622	<i>ABCG2</i>	0.46	-1.24	0.03
rs2725252	<i>ABCG2</i>	0.19	-1.29	0.06
rs1042640	<i>UGT1A1</i>	0.18	1.34	0.07
rs10929302	<i>UGT1A1</i>	0.29	1.01	0.09
rs28401781	<i>ABCB1</i>	0.25	-1.12	0.09
rs2246709	<i>CYP3A4</i>	0.31	-0.94	0.12
rs1976391	<i>UGT1A1</i>	0.41	0.84	0.13
rs887829 ^b	<i>UGT1A1</i>	0.41	0.81	0.15
rs2231137	<i>ABCG2</i>	0.06	1.73	0.16
rs10011796	<i>ABCG2</i>	0.25	-0.92	0.16
rs12505410	<i>ABCG2</i>	0.1	-1.1	0.24
rs4148738	<i>ABCB1</i>	0.14	-0.89	0.25
rs4124874	<i>UGT1A1</i>	0.37	0.92	0.26
rs3789243	<i>ABCB1</i>	0.38	0.66	0.27
rs1922242	<i>ABCB1</i>	0.45	0.59	0.28
rs10276036	<i>ABCB1</i>	0.15	-0.81	0.28
rs1045642	<i>ABCB1</i>	0.89	0.93	0.35
rs3842	<i>ABCB1</i>	0.28	0.55	0.36
rs2235015	<i>ABCB1</i>	0.36	0.47	0.41
rs3213619	<i>ABCB1</i>	0.17	0.55	0.47
rs10248420	<i>ABCB1</i>	0.35	0.4	0.5
rs2235067	<i>ABCB1</i>	0.15	-0.42	0.6
rs10267099	<i>ABCB1</i>	0.1	0.49	0.6
rs1128503	<i>ABCB1</i>	0.06	0.59	0.61
rs2740574	<i>CYP3A4</i>	0.25	-0.3	0.62
rs11983225	<i>ABCB1</i>	0.21	-0.26	0.72
rs3735451	<i>CYP3A4</i>	0.17	0.24	0.76
rs4646440	<i>CYP3A4</i>	0.08	-0.28	0.78
rs4728709	<i>ABCB1</i>	0.39	0.15	0.79
rs4148740	<i>ABCB1</i>	0.19	-0.17	0.81
rs2242480	<i>CYP3A4</i>	0.14	0.19	0.82

rs7699188	<i>ABCG2</i>	0.44	-0.12	0.83
rs3114020	<i>ABCG2</i>	0.15	-0.17	0.83
rs8330	<i>UGT1A1</i>	0.4	0.12	0.84
rs10280101	<i>ABCB1</i>	0.2	-0.11	0.88
rs4646437	<i>CYP3A4</i>	0.13	0.09	0.92
rs2235047	<i>ABCB1</i>	0.23	-0.05	0.94
rs10929303	<i>UGT1A1</i>	0.37	-0.03	0.95
rs7787082	<i>ABCB1</i>	0.27	-0.02	0.97
rs2235040	<i>ABCB1</i>	0.17	-0.02	0.98

^a SNP, single nucleotide polymorphism.

^b *UGT1A1* rs887829 T allele is known to be in strong linkage disequilibrium (LD) with the Gilbert trait decrease expression allele, *UGT1A1**28.

^c Significance threshold was 1.0×10^{-3} for the subset of 40 polymorphisms.

We next assessed 1729 polymorphisms in *ABCB1*, *ABCG2*, *CYP3A4*, and *UGT1A1* (± 50 kB for each gene) for association with percentage weight gain among all dolutegravir recipients, by multivariable linear regression analysis. The 2 lowest P-values in each gene are presented in **Table S1**. The lowest P-value overall was *ABCG2* rs4148149 ($\beta = -1.96$, $P = 7.0 \times 10^{-4}$), which did not achieve the Bonferroni cut-off of $P < 2.9 \times 10^{-5}$.

Associations with Tenofovir-Relevant Polymorphisms and Genes

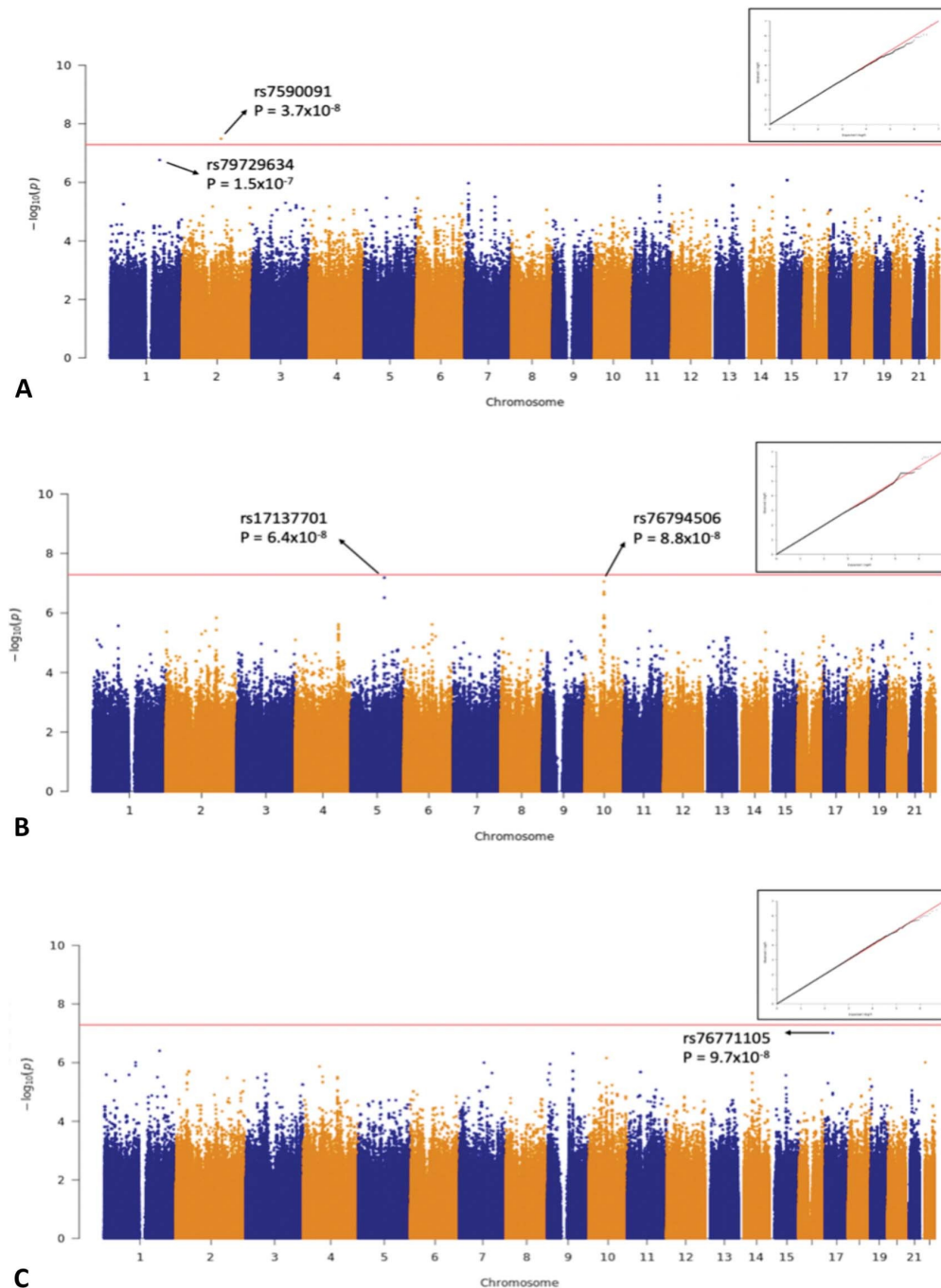
We next focused on tenofovir, characterizing associations in polymorphisms that have previously been associated with tenofovir disposition (selected a priori for this analysis) and percentage weight gain from baseline to week 48 among all tenofovir recipients (i.e., TDF and TAF), by multivariable linear regression analysis. Results for all 314 tenofovir recipients are presented in **Table S2**. The lowest P-value for association was *ABCC4* rs3742106 ($\beta = 1.59$, $P = 0.04$), which did not achieve the Bonferroni cut-off of $P < 3.0 \times 10^{-3}$. We next assessed all 3945 polymorphisms in *ABCC2*, *ABCC4*, *ABCC10*, *SLC22A2*, *SLC22A6*, and *SLC22A11* (± 50 kB for each gene) for association with percentage weight gain among all tenofovir recipients, by multivariable linear regression analysis. The 2 lowest P-values in

each gene are presented in **Table S3**. The lowest P-value for association overall was *ABCC10* rs67861980 ($\beta = 1.82$, $P = 1.0 \times 10^{-2}$), which did not achieve the Bonferroni cut-off of $P < 1.3 \times 10^{-5}$.

Genome-Wide Associations with Percent Weight Gain at 48 Weeks

Associations between genetic polymorphisms and percentage weight gain from baseline to week 48 were explored by multivariable linear regression analysis in all participants, followed by separate regression models for those randomized to TDF and TAF. Among all 314 dolutegravir recipients, the lowest P-value for association was *TMEM163* rs7590091 ($P = 3.7 \times 10^{-8}$), presented in **Figure 2A**. In analyses limited to the 160 TAF recipients, the 2 lowest P-values for association were *LOC105379130* rs17137701 ($P = 6.4 \times 10^{-8}$) and rs76794506 in an intergenic region on chromosome 10 ($P = 8.8 \times 10^{-8}$), presented in **Figure 2B**. In analyses limited to the 154 TDF recipients, the lowest P-values for association were *LOC105371716* rs76771105 ($P = 9.7 \times 10^{-8}$), presented in **Figure 2C**. Only *TMEM163* rs7590091 was genome-wide significant after correcting for multiple testing. There was no evidence of genomic inflation of each analysis, based on QQ-plots.

Of the 86 polymorphisms identified in the GWAS Catalog, 77 were represented in our genotype data. These are presented in **Table S4**. The lowest P-value in our analysis was for *ETV5* rs1516725 ($\beta = -1.58$, $P = 0.01$). None of the 77 polymorphisms were significant after correcting for multiple testing.



C
Figure 2. Genome-wide associations with weight gain from baseline to week 48 in ADVANCE study participants. Manhattan plots and QQ-plot for genome-wide associations with percentage weight gain from baseline to week 48. Analyses controlled for age, sex, first 3 MDS coordinates, and, in the dolutegravir analysis, concomitant NRTI. Horizontal red lines indicate a genome-wide significance threshold of $P < 5.0 \times 10^{-8}$. **A**, Associations among 314 participants in the dolutegravir arm. A polymorphism in *TMEM163* (rs7590091) achieved genome-wide significance ($P = 3.7 \times 10^{-8}$). **B**, Associations among 160 participants in the TAF arm. Two polymorphisms, *LOC105379130* rs17137701 and rs76794506 in an intergenic region on chromosome 10, had P-values approaching statistical significance ($P = 6.4 \times 10^{-8}$ and $P = 8.8 \times 10^{-8}$, respectively). **C**, Associations among 154 participants in the TDF arm. The polymorphism *LOC105371716* rs76771105 had the lowest P-value ($P = 9.7 \times 10^{-8}$).

Discussion

In our analysis for association with percentage weight gain, and 59 selected polymorphisms relevant to dolutegravir and tenofovir disposition, *ABCG2* rs13137622 and *ABCC4* rs3742106 had the lowest P-values, respectively, but were not significant after Bonferroni correction. In targeted analysis involving 1729 polymorphisms in genes suggested to affect dolutegravir disposition, *ABCG2* rs4148149 had the lowest P-value, whereas in analysis of 3945 polymorphisms in genes suggested to affect tenofovir disposition, *ABCC10* rs67861980 had the lowest P-value. In genome-wide association analysis for each study drug (dolutegravir, TAF, and TDF), we found no associations between polymorphisms relevant to dolutegravir and tenofovir disposition and weight gain. For dolutegravir, only *TMEM163* rs7590091 achieved genome-wide significance ($\beta = 3.3$, $P = 3.7 \times 10^{-8}$). For TAF and TDF, no polymorphism achieved genome-wide significance.

This is the first study to explore associations between dolutegravir or tenofovir-associated weight gain and polymorphisms in genes relevant to their disposition, including *ABCG2* and *ABCC4*. In studies of *ABCG2*-null mice, *ABCG2* deficiency did not affect dolutegravir metabolism or excretion.³⁸ In addition, polymorphisms in the *ABCC* family have previously been associated with proximal kidney tubule cell dysfunction, including *ABCC2* rs717620, *ABCC4* rs3742106, *ABCC10* rs2125739, and *ABCC10* rs9349256^{39–42} with possibly greater risk of renal dysfunction among HIV-positive patients of African descent than of European descent.⁴² In our study, *ABCC4* rs3742106 ($P = 0.05$) and *ABCC10* rs2125739 ($P = 0.77$) were not significantly associated with weight gain. The lack of association between *ABCG2* or *ABCC* family polymorphisms and weight gain suggests a relationship that may not be driven by plasma drug concentrations.

In genome-wide analyses, *TMEM163* rs7590091 was significantly associated with weight gain. *TMEM163* encodes transmembrane protein 163⁴³ a zinc-binding protein that has been reported to interact with the ion channel transient receptor potential mucolipin-1 (TRPML1), which may help maintain zinc homeostasis in a cell-type specific manner.⁴⁴ A previous study observed a reduction in *TMEM163* mRNA and protein expression levels in mucopolidosis type IV (MLIV) fibroblast cells which correlated with increased lysosomal zinc levels.⁴⁴ It has been reported that variants of zinc transported proteins on pancreatic β -cells are associated with type 2 diabetes mellitus.^{45,46} Two other *TMEM163* polymorphisms, rs6723108 and rs998451, have been associated with reduced fasting plasma insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR), suggesting that *TMEM163* might modulate susceptibility to type 2 diabetes mellitus by affecting insulin secretion.⁴⁷ The *TMEM163* rs6723108 polymorphism has also been associated with waist circumference.⁴⁷ However, a relationship between *TMEM163* and dolutegravir has not been previously reported. This possible association warrants replication. In analyses focused on polymorphisms previously associated with obesity trait in the general population, the lowest P-value was with *ETV5* rs1516725 ($\beta = -1.58$, $P = 0.01$). The gene *ETV5* encodes a transcription factor. Polymorphisms in *ETV5* have been associated with body mass index and bipolar disorder,⁴⁸ and inhibition of *ETV5* homolog in *Drosophila melanogaster* induced bipolar disorder and obesity-related phenotypes.⁴⁹

Mechanisms underlying greater weight gain with dolutegravir and with TAF are unknown. One hypothesis for the greater weight gain observed with TAF compared with TDF is that this reflects TDF-induced depletion of mitochondrial DNA in adipocytes, or some other off-target metabolic effect. In addition, TDF may cause loss of appetite. In a clinical trial of patients in the United States randomized to initiate efavirenz-containing ART, lesser weight

gain occurred among *CYP2B6* slow metabolizers who were also randomized to receive concomitant TDF, but not those randomized to receive concomitant abacavir.²¹

Our study had limitations. We only considered individual polymorphisms. It is possible that other approaches, such as the polygenetic risk score, may identify associations not apparent in our analyses. We did not consider mitochondrial genetics or epigenetics. Although associations with pharmacogenetic variants can often be identified with small sample sizes, because of large effect sizes, a larger study would have greater power to detect associations, especially in the TDF and TAF subgroup analyses. Fifty two percent of participants from the main ADVANCE study did not consent to genotyping, resulting in a smaller sample size and reduced power to detect associations. We are encouraged that baseline characteristics were similar between the 2 groups, suggesting that our results are likely generalizable to the larger ADVANCE study population.

In summary, we identified potential associations between human genetic polymorphisms and percentage weight gain during the first 48 weeks of ART initiation with dolutegravir plus emtricitabine and either TAF or TDF. No polymorphisms in genes relevant to dolutegravir, TAF, or TDF were significantly associated with weight gain after correcting for multiple testing. In a genome-wide association analysis among dolutegravir recipients, a polymorphism in *TMEM163* achieved genome-wide significance. This association needs to be replicated in other cohorts before a causal link can be made with weight gain.

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Supplementary material

Table S1: Associations among 314 dolutegravir recipients between percentage weight gain at week 48 all polymorphisms in genes relevant to dolutegravir

SNP^a	Gene^b	Beta	P-value^c
rs4148149	<i>ABCG2</i>	-1.96	7.0×10^{-4}
rs12641369	<i>ABCG2</i>	-1.88	0.00104
rs28898593	<i>UGT1A1</i>	-2.76	0.005
rs11695770	<i>UGT1A1</i>	-1.59	0.006
rs17131983	<i>ABCB1</i>	3.45	0.006
rs78903144	<i>ABCB1</i>	-2.80	0.008
rs4986911	<i>CYP3A4</i>	1.87	0.102
rs28988604	<i>CYP3A4</i>	-1.60	0.103

^a SNP: Single nucleotide polymorphism.

^b We considered polymorphisms in *ABCB1*, *ABCG2*, *CYP3A4* and *UGT1A1* $\pm$ 50 kB in each gene.

^c Significance threshold was 2.9×10^{-5} for the subset of 1729 SNPs. For each gene, we show the two SNPs with the lowest P-values.

Table S2: Association among 314 tenofovir (TAF or TDF) recipients between percentage weight gain at week 48 and selected polymorphisms relevant to tenofovir

SNP^a	Gene	MAF^b	Beta	P-value^c
rs3742106	<i>ABCC4</i>	0.42	1.59	0.05
rs8187707	<i>ABCC2</i>	0.09	-2.58	0.05
rs3740066	<i>ABCC2</i>	0.15	-1.99	0.06
rs17222723	<i>ABCC2</i>	0.09	-2.54	0.07
rs11231809	<i>SLC22A11</i>	0.06	-2.14	0.15
rs11568655	<i>ABCC4</i>	0.08	1.69	0.25
rs1751034	<i>ABCC4</i>	0.36	0.86	0.27
rs2487663	<i>ABCC10</i>	0.04	-0.74	0.3
rs2274405	<i>ABCC4</i>	0.36	0.75	0.37
rs7080681	<i>ABCC2</i>	0.13	0.66	0.54
rs1557070	<i>ABCC4</i>	0.3	-0.3	0.72
rs1059751	<i>ABCC4</i>	0.19	-0.31	0.74
rs2273697	<i>ABCC2</i>	0.14	-0.22	0.84
rs8187710	<i>ABCC2</i>	0.26	-0.14	0.87
rs3127573	<i>SLC22A2</i>	0.2	0.15	0.87
rs11568695	<i>ABCC4</i>	0.14	-0.14	0.9
rs2274407	<i>ABCC4</i>	0.2	-0.12	0.9
rs2274406	<i>ABCC4</i>	0.31	0.1	0.91
rs899494	<i>ABCC4</i>	0.26	-0.03	0.97

^a SNP: Single nucleotide polymorphism.

^b MAF: Minor allele frequency.

^c Significance threshold was 3.0×10^{-3} for the subset of 19 SNPs.

Table S3: Associations between percentage weight gain at week 48 in 314 tenofovir (TAF or TDF) recipients and all polymorphisms in genes relevant to tenofovir

SNP^a	Gene^b	Beta	P-value^c
rs67861980	<i>ABCC10</i>	1.82	0.01
rs116643428	<i>ABCC4</i>	-2.25	0.03
rs61965967	<i>ABCC4</i>	1.80	0.03
rs751875927	<i>ABCC10</i>	-2.07	0.04
rs55804858	<i>ABCC2</i>	-2.83	0.05
rs113513789	<i>ABCC2</i>	-2.58	0.05
rs76078067	<i>SLC22A11</i>	1.49	0.18
rs149563293	<i>SLC22A11</i>	-2.30	0.19
rs371552166	<i>SLC22A6</i>	0.51	0.54
rs10792366	<i>SLC22A6</i>	0.47	0.55
rs315991	<i>SLC22A2</i>	-0.34	0.66
rs1482124455	<i>SLC22A2</i>	-0.31	0.69

^a SNP: Single nucleotide polymorphism.

^b We considered polymorphisms in *ABCC2*, *ABCC4*, *ABCC10*, *SLC22A2*, *SLC22A6* and *SLC22A11* $\pm$ 50 kb for each gene.

^c Significance threshold was 1.3×10^{-5} for the subset of 3945 SNPs. We selected the top two SNPs with the lowest P-values for each gene.

Table S4: Associations between percentage weight gain at week 48 in 314 tenofovir (TAF or TDF) recipients and all polymorphisms in SNPs previously associated with obesity

SNP^a	Gene	MAF^b	Beta	P-value^c
rs1516725	<i>ETV5</i>	0.26	-1.58	0.01
rs10423928	<i>GIPR</i>	0.19	1.68	0.02
rs476828	<i>MC4R</i>	0.42	1.14	0.05
rs16933812	<i>PAX5</i>	0.21	-1.28	0.06
rs538656	<i>MC4R</i>	0.36	1.11	0.06
rs2568958	<i>NEGR1</i>	0.44	1.03	0.07
rs3101336	<i>NEGR1</i>	0.44	1.03	0.07
rs9816226	<i>ETV5</i>	0.22	-1.19	0.08
rs10182181	<i>ADCY3, POMC</i>	0.08	-1.71	0.10
rs1993709	<i>NEGR1</i>	0.11	-1.24	0.13
rs1800437	<i>GIPR</i>	0.12	1.08	0.20
rs7989336	<i>HS6ST3</i>	0.37	0.97	0.20
rs11109072	<i>RMST</i>	0.23	-0.80	0.20
rs7503807	<i>RPTOR</i>	0.48	0.67	0.23
rs1957894	<i>NRXN3</i>	0.24	-0.79	0.24
rs12446554	<i>LOC105371116</i>	0.11	-1.04	0.26
rs12446632	<i>LOC105371116</i>	0.11	-1.04	0.26
rs564343	<i>PACSI1</i>	0.20	0.94	0.27
rs11639988	<i>GPRC5B</i>	0.22	-0.67	0.31
rs2531995	<i>ADCY9</i>	0.09	0.90	0.32
rs10938397	<i>GNPDA2</i>	0.23	-0.67	0.33
rs13130484	<i>GNPDA2</i>	0.23	-0.67	0.33
rs7579427	<i>NR</i>	0.09	0.53	0.35
rs2370983	<i>NRXN3</i>	0.10	0.82	0.36
rs1424233	<i>NR</i>	0.27	0.52	0.39
rs2060457	<i>TDH</i>	0.12	0.70	0.41
rs1514177	<i>TNNI3K</i>	0.34	-0.51	0.41
rs2605100	<i>LYPLAL1</i>	0.05	1.00	0.43
rs11088859	<i>NCAM2</i>	0.10	0.72	0.44
rs56094641	<i>FTO</i>	0.05	0.93	0.45
rs2943650	<i>IRS1</i>	0.38	0.43	0.45
rs10493544	<i>NR</i>	0.11	-0.64	0.49
rs10189761	<i>TMEM18</i>	0.15	0.53	0.49
rs12463617	<i>TMEM18</i>	0.19	0.73	0.52
rs7132908	<i>FAIM2</i>	0.12	0.32	0.53

rs35669975	<i>FAM155A</i>	0.20	-0.63	0.54
rs6711012	<i>TMEM18</i>	0.09	0.57	0.55
rs7498665	<i>SH2B1</i>	0.28	-0.34	0.57
rs11208659	<i>LEPR</i>	0.41	-0.29	0.62
rs2206277	<i>TFAP2B</i>	0.13	-0.40	0.63
rs1514174	<i>TNNI3K</i>	0.24	-0.30	0.65
rs4735692	<i>HNF4G</i>	0.37	0.25	0.66
rs17024258	<i>GNAT2</i>	0.08	0.45	0.66
rs13041126	<i>LOC105372666</i>	0.20	0.29	0.67
rs7185735	<i>FTO</i>	0.45	0.23	0.67
rs9299	<i>HOXB5</i>	0.20	0.30	0.67
rs10875976	<i>BCDIN3D-AS1</i>	0.23	0.26	0.69
rs633715	<i>SEC16B</i>	0.08	0.41	0.70
rs17782313	<i>MC4R</i>	0.25	0.24	0.71
rs9568856	<i>OLFM4</i>	0.46	-0.28	0.71
rs8028313	<i>MAP2K5</i>	0.27	0.23	0.73
rs10871777	<i>MC4R</i>	0.26	0.22	0.73
rs2307111	<i>C5orf37</i>	0.10	0.43	0.74
rs887912	<i>FANCL</i>	0.07	-0.36	0.75
rs7184597	<i>RABEP2</i>	0.00	1.09	0.75
rs2112347	<i>C5orf37</i>	0.43	-0.23	0.76
rs13078807	<i>CADM2</i>	0.02	-0.57	0.76
rs734597	<i>TFAP2B</i>	0.07	0.32	0.78
rs17817449	<i>FTO</i>	0.38	-0.13	0.82
rs17150703	<i>MSRA, TNKS</i>	0.01	0.52	0.84
rs11152213	<i>MC4R</i>	0.23	0.14	0.84
rs6567160	<i>NR</i>	0.20	-0.13	0.84
rs987237	<i>TFAP2B</i>	0.08	0.21	0.85
rs11624704	<i>PRKCH</i>	0.07	0.17	0.87
rs539515	<i>NR</i>	0.24	0.09	0.88
rs7141420	<i>NRXN3</i>	0.48	0.08	0.88
rs7138803	<i>NR</i>	0.18	-0.10	0.89
rs6731302	<i>FANCL</i>	0.12	0.12	0.89
rs9941349	<i>FTO</i>	0.13	0.05	0.91
rs8050136	<i>FTO</i>	0.41	0.05	0.93
rs11042023	<i>RPL27A</i>	0.39	0.07	0.93
rs8043757	<i>FTO</i>	0.40	0.03	0.96
rs7826222	<i>MSRA</i>	0.02	-0.08	0.97

rs10968576	<i>LINGO2</i>	0.13	0.01	0.99
rs1412239	<i>LINGO2</i>	0.13	0.01	0.99
rs1421085	<i>FTO</i>	0.06	0.36	1.07
rs1558902	<i>FTO</i>	0.06	0.36	1.07

^a SNP: Single nucleotide polymorphism.

^b MAF: Minor allele frequency.

^c Significance threshold was 6.5×10^{-4} for the subset of 77 SNPs.

Chapter 6

Conclusions

The ADVANCE Randomized Controlled Trial (RCT) was designed as a 96-week, phase 3, investigator-led, open-label, randomized trial in South Africa, in which a triple-therapy combination of emtricitabine and dolutegravir plus either of two tenofovir prodrugs (tenofovir alafenamide (TAF) or tenofovir disoproxil fumarate (TDF)) was compared against the then local standard-of-care regimen of TDF–emtricitabine–efavirenz. The findings in the ADVANCE RCT showed that the dolutegravir-based therapy was as effective as the efavirenz-based therapy in achieving virological suppression. However, weight gain was markedly increased in the dolutegravir-based arms, especially in the arm including TAF. In this thesis, we report associations between genetic polymorphisms, dolutegravir and tenofovir exposure, and weight gain among participants enrolled in the dolutegravir-containing arms of ADVANCE. We focused on three main objectives:

First, in **Chapter 3**, we investigated the pharmacogenetics of dolutegravir plasma exposure among 284 participants. Our primary outcome of interest was the unexplained variability in dolutegravir area under the concentration-time curve (AUC_{VAR}) and secondarily the unexplained variability in dolutegravir clearance (CL_{BSV}). We reported independent associations between AUC_{VAR} and polymorphisms from the *UGT1A1* locus, rs887829 ($P = 1.8 \times 10^{-4}$) and rs28899168 ($P = 0.02$). The rs887829 is in strong linkage disequilibrium with *UGT1A1**28. Our findings were consistent with other findings that reported associations with *UGT1A1**28 and the maximum dolutegravir plasma concentration,¹ AUC_{0-24h} ,² and dolutegravir concentrations.³ We also found the rs887829 polymorphism to be strongly associated with CL_{BSV} and when the rs887829 polymorphism is included in the population pharmacokinetics model as a covariate, a novel polymorphism (rs28899168) was found to be

independently associated with AUC_{VAR} . To our knowledge, associations between rs28899168 and dolutegravir plasma exposure have not been reported.

We used bilirubin as a positive control for our genetic association study as previous findings have reported associations between serum bilirubin levels and polymorphisms in the *UGT1A* locus,⁴ and variants of the *UGT1A1**28 polymorphism have been associated with the Gilbert trait.⁵ Both rs887829 ($P = 8.6 \times 10^{-13}$) and rs28899168 ($P = 7.7 \times 10^{-8}$) polymorphisms were also associated with $\log_{10}$ bilirubin concentrations. To our knowledge, this study is the first to investigate genetic associations with dolutegravir plasma exposure among Southern Africans and our warrant replication in other cohorts.

Second, in **Chapter 4**, we investigated the pharmacogenetics of tenofovir clearance among 268 participants. We used linear regression models, which were stratified by study arm (TDF vs. TAF). No associations were found between genetic polymorphisms selected *a priori* (based on previous association with tenofovir exposure). We found the same association between tenofovir clearance and *IFNL4* rs12979860 when we looked at polymorphisms previously associated with any drug-related phenotype ($P = 0.003$ in both study arms). The *IFNL4* protein is involved in immune response to viral infections and the rs12979860 polymorphism has previously been associated with resolution of hepatitis B infection in participants treated with interferon-based therapy.^{6,7} In addition, the rs12979860 polymorphism has been associated with spontaneous HIV control,⁷ but this association could not be replicated in other studies.^{8,9} To our knowledge, this is the first study to show an association between *IFNL4* rs12979860 and tenofovir pharmacokinetics. It is unclear how this gene would affect tenofovir disposition.

Genome-wide, the lowest P-values for the unexplained variability in tenofovir clearance in the TAF and TDF arms were with intergenic polymorphisms *LINC01684* rs9305223 ($P = 3.0$

$\times 10^{-8}$) and rs142693425 ($P = 1.4 \times 10^{-8}$), respectively. Four more genome-wide significant intergenic polymorphisms (2 in each study arm) were identified. However, it is unclear how these intergenic polymorphisms could affect tenofovir disposition.

Third, in **chapter 5**, we investigated genetic associations with weight gain among 314 participants who initiated dolutegravir- and tenofovir-containing regimens. We reported genome-wide associations with *TMEM* rs7590091 ($P = 3.7 \times 10^{-8}$) and weight gain at week 48. We found no genetic associations between weight gain and polymorphisms selected *a priori* in genes relevant to dolutegravir and tenofovir disposition.

TMEM163 encodes transmembrane protein 163,¹⁰ a zinc-binding protein that has been reported to interact with the ion channel transient receptor potential mucolipin-1 (TRPML1), which may help maintain zinc homeostasis in a cell-type specific manner.¹¹ There is no direct association between polymorphisms from *TMEM163* and weight gain. However, one study reported associations between reduced fasting plasma insulin levels and homeostasis model assessment of insulin resistance and two polymorphisms from *TMEM163* rs6723108 and rs998451. It is suggesting that by affecting insulin secretion, TMEM163 may modulate susceptibility to type 2 diabetes mellitus.¹² In addition, the *TMEM163* rs6723108 has also been associated with waist circumference.¹² These reports suggest a plausible association between TMEM163 and weight gain. However, no association has been reported between *TMEM163* and dolutegravir. To our knowledge, this is the first study to investigate genetic associations with weight gain among Southern Africans who initiated dolutegravir- and tenofovir-containing regimens.

Our studies had several limitations. First, we only considered individual polymorphisms. It is possible that other approaches, such as the polygenic risk score, may identify associations not apparent in our analyses. Second, we did not consider mitochondrial genetics or

epigenetics. Although associations with pharmacogenetic variants can often be identified with small sample sizes, but because of large effect sizes, a larger study would have greater power to detect associations. Third, we could not replicate all previously described polymorphisms in our data because some of these polymorphisms are infrequent in Africans or they did not meet our imputation quality threshold. Fourth, our sample size was small as fewer than anticipated participants consented to genetic testing. However, these were the largest pharmacogenetics studies of dolutegravir and tenofovir in an African population.

Implications for clinical research

In **Chapter 3**, we replicated an expected association between *UGT1A* rs887829 and dolutegravir AUC_{VAR}. However, the novel independent association between *UGT1A* rs28899168 and dolutegravir AUC_{VAR} should be replicated in larger cohorts.

In **Chapter 4**, we found an association between the unexplained variability in tenofovir clearance and *IFNL4* rs12979860 when looking at polymorphisms previously associated with any drug-related phenotype for both TAF and TDF. In genome-wide analyses, we identified associations between intergenic polymorphisms and the unexplained variability in tenofovir clearance in both TAF and TDF arms. It is unclear mechanistically how these polymorphisms could affect tenofovir disposition. However, these associations should be replicated in other cohorts to validate pharmacogenetic significance.

In **Chapter 5**, we observed a novel association between *TMEM163* rs7590091 and weight gain at week 48. This improved our understanding of polymorphisms affecting percentage weight gain in patients initiating dolutegravir and tenofovir containing regimens. This requires replication in other larger cohorts.

In summary, we identified several potential genetic associations with dolutegravir exposure, tenofovir clearance and weight gain. The novel associations between dolutegravir AUC_{VAR}

and *UGT1A* rs28899168, tenofovir clearance and *IFNL4* rs12979860, and weight gain and *TMEM163* rs7590091 require independent replication. The data will be uploaded on dbgap to allow access to other researchers, who may wish to combine with other datasets.

Implications for clinical practice

All three studies are the first to describe genetic associations with plasma dolutegravir and tenofovir exposure in an African population. This is important especially with the widespread use of dolutegravir in Africa.

We replicated associations between *UGT1A* rs887829 T/T (poor metabolizer) genotypes with higher dolutegravir exposure and with log₁₀ bilirubin concentrations. Genetic testing can be considered before therapy initiation to identify poor metabolizers, who may be at risk for toxicity given the widespread use of dolutegravir in Africa. In addition, the association between *UGT1A* rs887829 with dolutegravir exposure may be considered for inclusion in the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline as part of genetic testing for dolutegravir use.

For tenofovir clearance, the *IFNL4* protein is involved in immune response to viral infections and is known to be associated with spontaneous HIV control. This finding may not have clinical practice implications as it is unclear how this protein would mechanistically affect the pharmacokinetics of tenofovir, and there are no apparent drug metabolism or transport genes nearby on chromosome 19.

For weight gain, a novel association in *TMEM163* gene was found. *TMEM163* may affect insulin secretion which may modulate susceptibility to type 2 diabetes mellitus thereby, increasing the risk for weight gain. In clinical practice, if an affordable genetic test with a quick turnaround time was available for screening, then there should be close monitoring of weight and checking for insulin resistance in those who screen positive for *TMEM163*

polymorphism. Implementing a balanced diet and exercise in those who have gained weight on dolutegravir and TAF would play an important role in mitigating weight gain. This is crucial as weight gain associated with ART is markedly high in women and patients of African descent.

This thesis contributes new knowledge in pharmacogenetics research, where participants of African descent are still highly underrepresented. The research was done on dolutegravir and tenofovir (TAF and TDF) and has relevance to the HIV population globally.

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