Pharmacogenetics of tenofovir clearance among Southern Africans living with HIV

Zinhle Cindi[a], Aida N. Kawuma[a], Gary Maartens[a], Yuki Bradford[b], Simiso Sokhela[c], Nomathemba Chandiwana[c], Willem D. Francois Venter[c], Roeland E. Wasmann[a], Paolo Denti[a], Lubbe Wiesner[a], Marylyn D. Ritchie[d], David W. Haas[e,f] and Phumla Sinxadi[a]

Background **Tenofovir is a component of preferred combination antiretroviral therapy (ART) regimens in Africa. Few pharmacogenetic studies have been conducted on 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 ($P=3.0\times10^{-8}$) and intergenic rs142693425 ($P=1.4\times10^{-8}$), 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. *Pharmacogenetics and Genomics* 33: 79–87 Copyright © 2023 Wolters Kluwer Health, Inc. All rights reserved.**

Pharmacogenetics and Genomics 2023, **33**:79–87

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

[a]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa, [b]Department of Genetics, University of Pennsylvania, Philadelphia, Pennsylvania, USA, [c]Ezintsha, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, [d]Department of Genetics and Institute for Biomedical Informatics, University of Pennsylvania, Philadelphia, Pennsylvania, [e]Department of Medicine, Vanderbilt University Medical Center and [f]Department of Internal Medicine, Meharry Medical College, Nashville, Tennessee, USA

Correspondence to Phumla Sinxadi, MD, PhD, Division of Clinical Pharmacology, Department of Medicine. K45-41 Old Main Building, Groote Schuur Hospital, Observatory, 7925 Cape Town, South Africa
Tel: +27 21 650 4096; e-mail: phumla.sinxadi@uct.ac.za

Received 16 October 2022 **Accepted** 21 January 2023.

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 [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.

TDF, a prodrug of tenofovir, is a substrate for the efflux transporter P-glycoprotein [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 [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

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.pharmacogeneticsandgenomics.com.

DOI: 10.1097/FPC.0000000000000495

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 although neither was statistically significant [20]. Carriers of the *ABCC4* rs1751034 variant had slower tenofovir renal clearance [21]. *CYP3A4* rs35599367 was associated with higher plasma TAF $AUC_{0-24\,h}$ [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-naive 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 utilized 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 (SCIEX, Framingham, Massachusetts, USA) 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 0.5–300 ng/mL. The combined accuracy (%Nom) of the limit of quantification, low, medium and high-quality controls (three 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. Post-genotype quality control was performed by Vanderbilt Technologies for Advanced Genomics Analysis and Research Design and included sex checks, call rates by marker and sample, identity by descent 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 $<1.0 \times 10^{-8}$. Linkage disequilibrium 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 h post-dose. At the time of pharmacokinetic sampling, the median duration on ART was 19.9 months (IQR 19.2–20.7 months). 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 model describing tenofovir pharmacokinetics 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. The model consists of two compartments with clearance, central volume and peripheral volume estimated at 44.7 L/h, 378 L and 356 L, respectively, for a typical 70 kg individual. The model included between-subject variability in clearance of 20.1% (coefficient of variation). In addition, TDF and TAF absorption are described by two separate absorption processes. When given as TDF, tenofovir quickly appears in plasma with an estimated absorption rate constant of 3.04 (1/h). On the other hand, after TAF administration, tenofovir absorption was described using two pathways. A 'fast' pathway with absorption rate constant of 1.45 L/h and a 'slow' pathway in which tenofovir was first absorbed intracellularly and then transitioned from there to the plasma at a half-life of 6.8 days. Allometric scaling with weight was included as a predictor on all clearance and volume parameters. No other covariates were included in the model.

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. The formula below was applied where the individual clearance (Clearance_i) is defined by the typical population parameter value and individual influence (e^{BSVCL_i}).

$$\text{Clearance}_i = \text{Typical} \quad \text{clearance} \quad \text{value.}\ e^{\text{BSVCL}_i}$$

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 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.

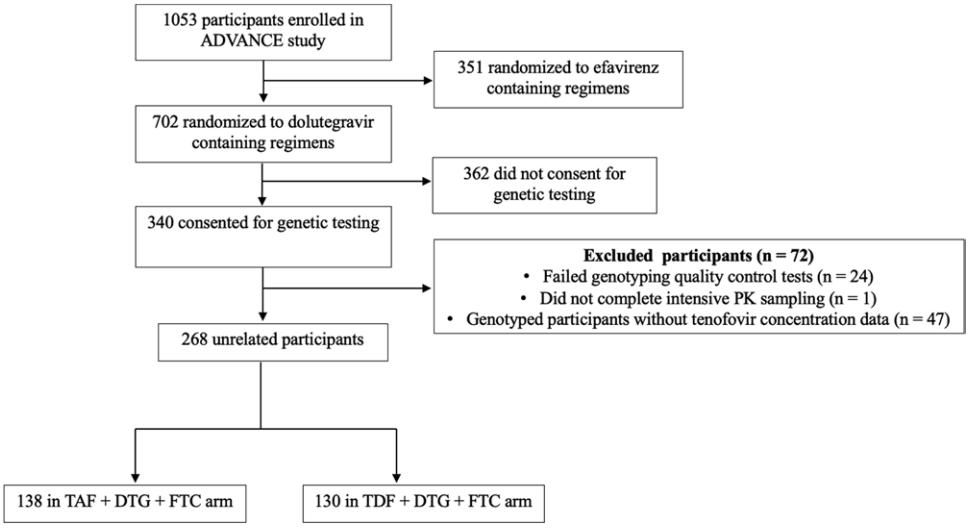
We used as references the Pharmacogenomics Knowledge Base (PharmGKB, accessed 5 April 2022) [31] and the NHGRI-EBI GWAS Catalog (accessed 5 April 2022) [32]. In PharmGKB, 141 polymorphisms were previously associated with at least one drug-related phenotype (pharmacokinetics, efficacy, or toxicity) with 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 was 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 to genetic analyses, 268 were successfully genotyped and had pharmacokinetic data. Participant disposition is presented in Fig. 1. All participants were Black Africans and 62% were females. Study participant characteristics are shown in Table 1.

Fig. 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. TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

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).

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 cutoffs. 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 (cutoff $P < 0.0045$), and with the C allele being associated with increased tenofovir CL_{BSV} (Online supplemental material Figure S1, Supplemental Digital Content 1, *http://links.lww.com/FPC/B455*). 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 were again *IFNL4* rs12979860 ($\beta = 0.1$, $P = 0.003$) (Table 4); which withstood correction for multiple testing (cutoff $P < 0.0045$), with the C allele associated with increased tenofovir CL_{BSV} (Online supplemental material Figure S2, Supplemental Digital Content 2, *http://links.lww.com/FPC/B456*). 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

Table 1 Baseline characteristics for tenofovir alafenamide and tenofovir disoproxil fumarate recipients included in genetic analyses

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

[a]CrCl, creatinine clearance; DTG, dolutegravir; FTC, emtricitabine; IQR, interquartile range; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

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, Supplemental Digital Content 3, *http://links.lww.com/FPC/B457*). 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.

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 (Fig. 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. 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 (Fig. 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.

Discussion

We characterized genetic associations with unexplained variability in tenofovir clearance (CL_{BSV}) in ART-naive participants randomized to TAF or TDF in the ADVANCE study in South Africa. We prioritized evaluation of polymorphisms from prior genetic association studies represented

Table 2 Associations with polymorphisms selected *a priori*

Tenofovir prodrug	Polymorphism	Gene	MAF[a]	Beta	*P* value[b]
TAF					
	rs3740066	*ABCC2*	0.2	−0.1	0.4
	rs3742106	*ABCC4*	0.4	0.03	0.5
	rs11854484	*SLC28A2*	0.2	−0.03	0.6
	rs7311358	*SLOC1B3*	0.4	0.02	0.7
	rs1751034	*ABCC4*	0.4	−0.001	1.0
TDF	rs3740066	*ABCC2*	0.2	−0.1	0.3
	rs1751034	*ABCC4*	0.4	−0.03	0.4
	rs11854484	*SLC28A2*	0.2	0.01	0.8
	rs3742106	*ABCC4*	0.4	−0.002	1.0

[a]MAF, minor allele frequency; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide.
[b]Bonferroni significance threshold was 0.01 for the subset of five polymorphisms selected *a priori*.

Table 3 Lowest *P* values for genetic association with unexplained variability in tenofovir clearance in the tenofovir alafenamide arm

	Polymorphism	Gene	MAF	Beta	*P* value
PharmGKB and GWAS Catalog[a] (n=11 polymorphisms)					
	rs12979860	*IFNL4*	0.4	0.1	0.003*
	rs8099917	Intergenic	0.05	−0.2	0.06
	rs1801133	*MTHFR*	0.09	−0.1	0.07
	rs7412	*APOE*	0.2	−0.05	0.27
	rs396991	*FCGR3A*	0.3	0.04	0.4
PharmGKB but not in GWAS Catalog[b] (n=15 polymorphisms)	rs1051266	*SLC19A1*	0.3	0.1	0.1
	rs4673993	*ATIC*	0.07	−0.1	0.1
	rs1042713	*ADRB2*	0.5	0.1	0.1
	rs7294	*VKORC1*	0.4	0.04	0.3
	rs20455	*KIF6*	0.2	−0.1	0.3
GWAS Catalog[c] (n=82 787 polymorphisms)	rs3850736	*LINC01414*	0.2	0.2	3.5×10^{-5}
	rs2356369	*LINC01414*	0.2	0.2	4.6×10^{-5}
	rs7570090	*RPE*	0.4	−0.2	8.2×10^{-5}
	rs7841320	*LINC01414*	0.3	0.2	9.0×10^{-5}
	rs10032941	*C1QTNF7*	0.3	0.2	1.0×10^{-4}
Genome-wide genotype data[d] (n=8 903 713 polymorphisms)	rs9305223[e]	*LINC01684*	0.3	0.2	3.0×10^{-8}
	rs4816969	*LINC01684*	0.3	0.2	3.7×10^{-8}
	rs2829163[f]	Intergenic	0.4	0.2	4.5×10^{-8}
	rs7282679	Intergenic	0.2	0.2	5.9×10^{-8}
	rs2226443	*LINC01684*	0.3	0.2	6.6×10^{-8}

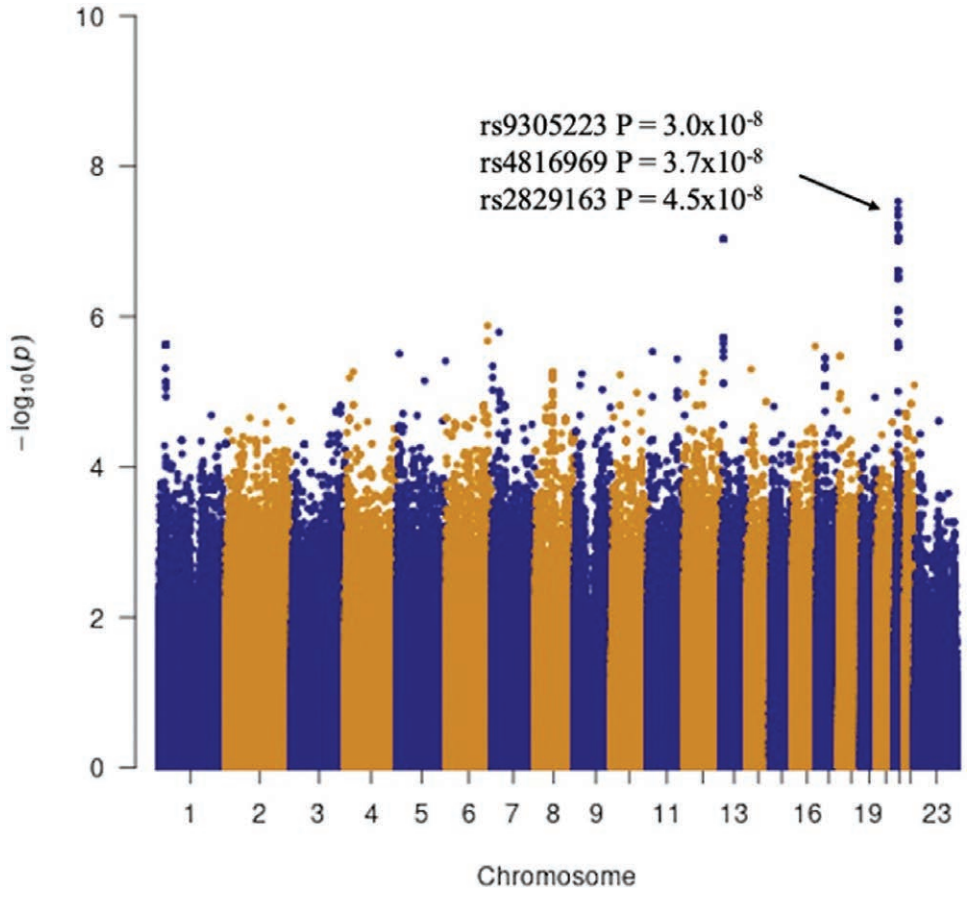
[a]Significance threshold was 4.5×10^{-3} for a subset of 11 polymorphisms.
[b]Significance threshold was 3.3×10^{-3} for a subset of 15 polymorphisms.
[c]Significance threshold was 6.0×10^{-7} for a subset of 82 787 polymorphisms.
[d]Significance threshold was 5.0×10^{-9} for genome-wide analysis.
[e]The rs9305223 polymorphism was in strong Linkage disequilibrium with rs4816969 and rs2226443, $r^2 > 0.9$.
[f]The rs2829163 polymorphism was in Linkage disequilibrium with rs7282679, $r^2 > 0.5$.
*Significant P value.

Table 4 **Lowest *P* values for genetic association with unexplained variability in tenofovir clearance in the tenofovir disoproxil fumarate arm**

	Polymorphism	Gene	MAF	Beta	*P* value
PharmGKB and GWAS Catalog[a] (*n*=11 polymorphisms)	rs12979860	*IFNL4*	0.4	0.1	0.003*
	rs12777823	Intergenic	0.3	−0.05	0.2
	rs1800629	*TNF*	0.2	−0.06	0.2
	rs7412	*APOE*	0.2	−0.05	0.25
	rs10929302	*UGT1A*	0.3	0.02	0.6
PharmGKB but not in GWAS Catalog[b] (*n*=15 polymorphisms)	rs2297595	*DPYD*	0.1	0.1	0.03
	rs3745274	*CYP2B6*	0.4	0.1	0.05
	rs1042713	*ADRB2*	0.5	0.04	0.2
	rs28399499	*CYP2B6*	0.1	−0.1	0.3
	rs1801159	*DPYD*	0.2	0.05	0.3
GWAS Catalog[c] (*n*=82 787 polymorphisms)	rs144511092	Intergenic	0.1	0.3	1.9×10^{-5}
	rs7902657	Intergenic	0.5	0.1	2.5×10^{-5}
	rs4682844	*CCDC12*	0.2	0.2	2.7×10^{-5}
	rs34940374	*GIMAP6*	0.1	0.2	3.4×10^{-5}
	rs7970054	*LRIG3*	0.05	0.3	5.0×10^{-5}
Genome-wide genotype data[d] (*n*=8 903 713 polymorphisms)	rs142693425	Intergenic	0.1	0.3	1.4×10^{-8}
	rs112914324	Intergenic	0.3	0.2	1.6×10^{-8}
	rs11995962	Intergenic	0.1	0.3	2.3×10^{-8}
	rs73151902	Intergenic	0.05	0.4	1.1×10^{-7}
	rs866325353	Intergenic	0.05	0.4	2.1×10^{-7}

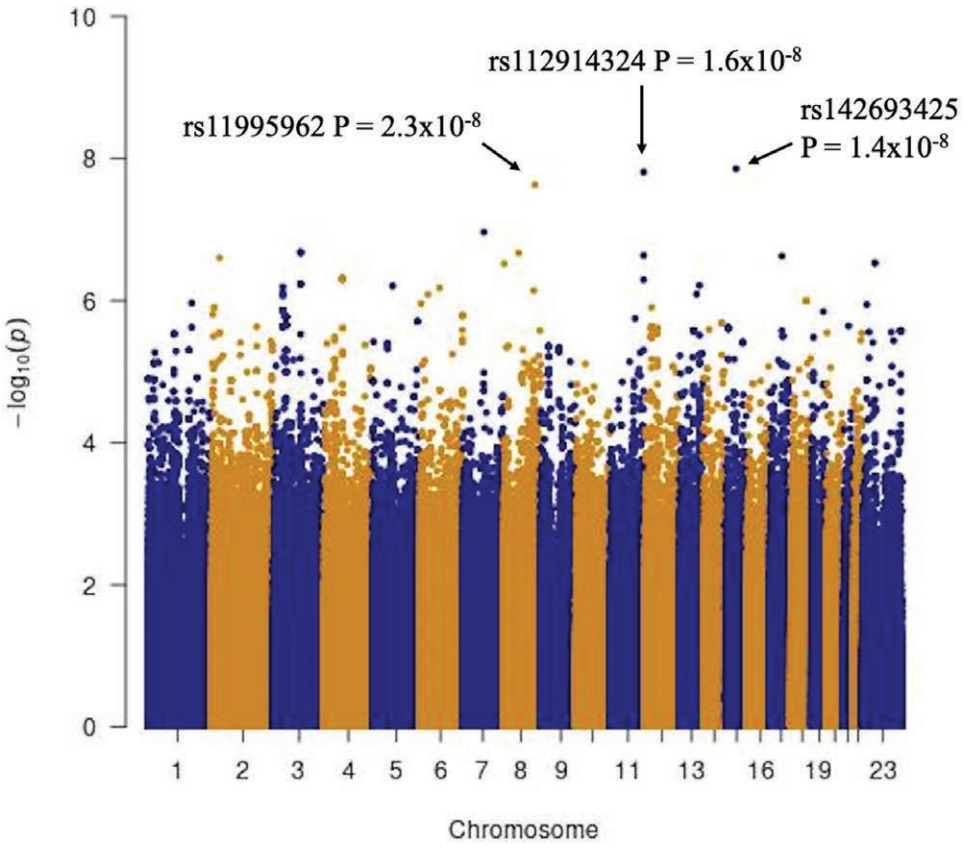
[a]Significance threshold was 4.5×10^{-3} for a subset of 11 polymorphisms.
[b]Significance threshold was 3.3×10^{-3} for a subset of 15 polymorphisms.
[c]Significance threshold was 6.0×10^{-7} for a subset of 82 787 polymorphisms.
[d]Significance threshold was 5.0×10^{-9} for genome-wide analysis.
*Significant *P* value

Fig. 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 $P<5.0\times10^{-8}$. TAF, tenofovir alafenamide.

Fig. 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 $P<5.0\times10^{-8}$. TDF, tenofovir disoproxil fumarate.

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 that, in some humans, encodes the interferon lambda 4 (IFNL4) proteins 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 ($P=3.0\times10^{-8}$) 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 ($P=1.4\times10^{-8}$) 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 $CL_{BSV.}$ This may be due to low minor allele frequencies of these polymorphisms in our dataset. In a small American study, carriers of the *ABCC4* rs1751034 variant had slower tenofovir clearance [21]. In contrast, in a Thai population, carriers of *ABCC4* rs1751034 had more rapid tenofovir clearance (CL/F)

[38]. In a Chinese population, *ABCC2* rs3740066, *ABCC4* rs3742106 and *SLCO1B3* rs7311358 were associated with TAF pharmacokinetics [20]. In a study from Italy, *SLC28A2* rs11854484 CT/TT genotypes were associated with plasma tenofovir exposure [39].

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.

Acknowledgements

Research reported in this publication was funded, in part, by the Fogarty International Center of the National Institutes of Health (NIH), under Award Number D43 TW010559 (to Z.C. and D.W.H.), and the National Institute of Allergy and Infectious Diseases (NIAID) (Award Numbers UM1 AI068634, UM1 AI068636 and UM1 AI106701; to University of Cape Town's Pharmacology Laboratory and L.W.). NIH grant support also included AI110527, AI077505 and TR000445 (to D.W.H.). This work was also funded by the National Research Foundation through the Thuthuka Grant and Black Academic Advancement Programme (113983 and 120647, respectively; to P.S.). A.N.K. was supported by a doctoral training grant from Pharmacometrics Africa NPC. Grant support from USAID, Unitaid and the SAMRC was received for the ADVANCE clinical trial (to S.S., N.C. and W.D.F.V.).

Z.C., Y.B., M.D.R., D.W.H., G.M. and P.S. worked on the genetic association analyses. Z.C., D.W.H. and P.S. contributed to drafting and finalizing the article. A.N.K., R.E.W. and P.D. developed the tenofovir population pharmacokinetics model. L.W. supervised P.K. sample analysis. S.S., N.C., W.D.F.V., A.N.K., R.E.W., M.D.R. and P.D. edited the article.

Conflicts of interest

W.D.F.V. reports grants from ViiV; personal fees and non–financial support from ViiV Healthcare and Gilead Sciences, during the conduct of the study; and personal fees from Mylan, Merk, Adcock-Ingram, Aspen, Abbott, Roche and Johnson & Johnson, outside the submitted work. S.S. reports grants from ViiV Healthcare and Gilead Sciences, during the conduct of the study. N.C. reports grants from ViiV Healthcare and Gilead Sciences, during the conduct of the study. S.S., N.C. and W.D.F.V. received research funding and drug donation for the ADVANCE trial through their institution from ViiV Healthcare and Gilead Sciences. N.C. declares personal fees and non-financial support from Johnson & Johnson, personal fees from Cipla, Frontiers Biotech and Novo Nordisk outside the submitted work. All other authors have nothing to declare. For the remaining authors, there are no conflicts of interest.

References

1 Stats SA. Statistical release P0302. 2021.
2 The AIDSinfo. Global data on HIV epidemiology and response. 2022. http://aidsinfo.unaids.org/. [Accessed 25 May 2021]
3 Republic of South Africa National Department of Health. 2019 ART clinical guidelines for the management of HIV in adults, pregnancy, adolescents, children, infants and neonates. www.health.gov.za/wp-content/uploads/2020/11/2019-art-guideline.pdf. [Accessed 25 May 2021]
4 Mallants R, Van Oosterwyck K, Van Vaeck L, Mols R, De Clercq E, Augustijns P. Multidrug resistance-associated protein 2 (MRP2) affects hepatobiliary elimination but not the intestinal disposition of tenofovir disoproxil fumarate and its metabolites. *Xenobiotica* 2005; **35**:1055–1066.
5 Lee WA, He GX, Eisenberg E, Cihlar T, Swaminathan S, Mulato A, *et al*. Selective intracellular activation of a novel prodrug of the human immunodeficiency virus reverse transcriptase inhibitor tenofovir leads to preferential distribution and accumulation in lymphatic tissue. *Antimicrob Agents Chemother* 2005; **49**:1898–1906.
6 Sax PE, Zolopa A, Brar I, Elion R, Ortiz R, Post F, *et al*. Tenofovir alafenamide vs. tenofovir disoproxil fumarate in single tablet regimens for initial HIV-1 therapy: a randomized phase 2 study. *J Acquir Immune Defic Syndr* 2014; **67**:52–58.
7 Van Aubel RAMH, Smeets PHE, Van Den Heuvel JJMW, Russel FGM. Human organic anion transporter MRP4 (ABCC4) is an efflux pump for the purine end metabolite urate with multiple allosteric substrate binding sites. *Am J Physiol – Ren Physiol* 2005; **288**:F327–F333.
8 Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D, Beniowski M, *et al*. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. *AIDS* 2010; **24**:1667–1678.
9 Morlat P, Vivot A, Vandenhende MA, Dauchy FA, Asselineau J, Déti E, *et al*. Role of traditional risk factors and antiretroviral drugs in the incidence of chronic kidney disease, ANRS CO3 Aquitaine cohort, France, 2004-2012. *PLoS One* 2013; **8**:e66223.
10 McComsey GA, Kitch D, Daar ES, Tierney C, Jahed NC, Tebas P, *et al*. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: AIDS Clinical Trials Group A5224s, a substudy of ACTG. *J Infect Dis* 2011; **203**:1791–1801.
11 Birkus G, Kutty N, He GX, Mulato A, Lee W, McDermott M, *et al*. Activation of 9-[(R)-2-[[(S)-[[(S)-1-(Isopropoxycarbonyl)ethyl]amino] phenoxyphosphinyl]-methoxy]propyl]adenine (GS-7340) and other tenofovir phosphonoamidate prodrugs by human proteases. *Mol Pharmacol* 2008; **74**:92–100.
12 Birkus G, Wang R, Liu X, Kutty N, MacArthur H, Cihlar T, *et al*. Cathepsin a is the major hydrolase catalyzing the intracellular hydrolysis of the antiretroviral nucleotide phosphonoamidate prodrugs GS-7340 and GS-9131. *Antimicrob Agents Chemother* 2007; **51**:543–550.
13 Podany AT, Bares SH, Havens J, Dyavar SR, O'Neill J, Lee S, *et al*. Plasma and intracellular pharmacokinetics of tenofovir in patients switched from tenofovir disoproxil fumarate to tenofovir alafenamide. *AIDS* 2018; **32**:761–765.
14 Van Rompay KKA, Durand-Gasselin L, Brignolo LL, Ray AS, Abel K, Cihlar T, *et al*. Chronic administration of tenofovir to rhesus macaques from infancy through adulthood and pregnancy: summary of pharmacokinetics and

biological and virological effects. *Antimicrob Agents Chemother* 2008; **52**:3144–3160.
15 Aceti A. Pharmacogenetics as a tool to tailor antiretroviral therapy: a review. *World J Virol* 2015; **4**:198.
16 Lazerwith SE, Siegel D, McFadden RM, Mish MR, Tse WC. New antiretrovirals for HIV and antivirals for HBV. In: Samuel C, David R, Simon EW, editors. *Comprehensive medicinal chemistry III*. Elsevier; 2017. pp. 628–664.
17 Rungtivasuwan K, Avihingsanon A, Thammajaruk N, Mitruk S, Burger DM, Ruxrungtham K, *et al*. Influence of ABCC2 and ABCC4 polymorphisms on tenofovir plasma concentrations in Thai HIV-infected patients. *Antimicrob Agents Chemother* 2015; **59**:3240–3245.
18 Manosuthi W, Sukasem C, Thongyen S, Nilkamhang S, Sungkanuparph S. ABCC2*1C and plasma tenofovir concentration are correlated to decreased glomerular filtration rate in patients receiving a tenofovir-containing antiretroviral regimen. *J Antimicrob Chemother* 2014; **69**:2195–2201.
19 Bleasby K, Hall LA, Perry JL, Mohrenweiser HW, Pritchard JB. Functional consequences of single nucleotide polymorphisms in the human organic anion transporter hOAT1 (SLC22A6). *J Pharmacol Exp Ther* 2005; **314**:923–931.
20 Li X, Tan XY, Cui XJ, Yang M, Chen C, Chen XY. Pharmacokinetics of tenofovir alafenamide fumarate and tenofovir in the chinese people: Effects of non-genetic factors and genetic variations. *Pharmgenomics Pers Med* 2021; **14**:1315–1329.
21 Kiser JJ, Aquilante CL, Anderson PL, King TM, Carten ML, Fletcher CV. Clinical and genetic determinants of intracellular tenofovir diphosphate concentrations in HIV-infected patients. *J Acquir Immune Defic Syndr* 2008; **47**:298–303.
22 Cerrone M, Alfarisi O, Neary M, Marzinke MA, Parsons TL, Owen A, *et al*. Rifampicin effect on intracellular and plasma pharmacokinetics of tenofovir alafenamide. *J Antimicrob Chemother* 2019; **74**:1670–1678.
23 Venter WDF, Moorhouse M, Sokhela S, Fairlie L, Mashabane N, Masenya M, *et al*. Dolutegravir plus two different prodrugs of tenofovir to treat HIV. *N Engl J Med* 2019; **381**:803–815.
24 Venter WDF, Sokhela S, Simmons B, Moorhouse M, Fairlie L, Mashabane N, *et al*. Dolutegravir with emtricitabine and tenofovir alafenamide or tenofovir disoproxil fumarate versus efavirenz, emtricitabine, and tenofovir disoproxil fumarate for initial treatment of HIV-1 infection (ADVANCE): week 96 results from a randomised, phase 3, n. *Lancet HIV* 2020; **7**:e666–e676.
25 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**:1215–1215.
26 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**:559–575.
27 Altshuler DL, Durbin RM, Abecasis GR, Bentley DR, Chakravarti A, *et al*. Genomes Project C. A map of human genome variation from population-scale sequencing. *Nature* 2010; **467**:1061–1073.
28 Lee BT, Barber GP, Benet-Pagès A, Casper J, Clawson H, Diekhans M, *et al*. The UCSC Genome Browser database: 2022 update. *Nucleic Acids Res*. 2022;**50**:D1115–D1122.
29 Kawuma A, Wasmann R, Sinxadi P, Sokhela S, Chandiwana N, Venter W, *et al*. Population pharmacokinetics of tenofovir given as either tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF). 30th Population Approach Group Europe (PAGE) 2022 conference; 28 June – 1 July. Ljubljana, Slovenia; 2022.
30 Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; **38**:904–909.
31 Whirl-Carrillo M, Huddart R, Gong L, Sangkuhl K, Thorn CF, Whaley R, Klein TE. An evidence-based framework for evaluating pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther* 2021. doi: 10.1002/cpt.2350
32 Buniello A, Macarthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, *et al*. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res* 2019; **47**:D1005–D1012.
33 Li W, Jiang Y, Jin Q, Shi X, Jin J, Gao Y, *et al*. Expression and gene polymorphisms of interleukin 28B and hepatitis B virus infection in a Chinese Han population. *Liver Int* 2011; **31**:1118–1126.
34 Chihab H, Badre W, Tahiri M, Jadid FZ, Zaidane I, Elfihry R, *et al*. IFNL4 rs12979860 polymorphism influences HBV DNA viral loads but not the outcome of HBV infection in Moroccan patients. *Microbes Infect* 2021; **23**:1048024–1048025.
35 Machmach K, Abad-Molina C, Romero-Sánchez MC, Abad MA, Ferrando-Martínez S, Genebat M, *et al*. IL28B single-nucleotide polymorphism rs12979860 is associated with spontaneous HIV control in white subjects. *J Infect Dis* 2013; **207**:651–655.
36 Sajadi MM, Shakeri N, Talwani R, Howell CD, Pakyz R, Redfield RR, *et al*. IL28B genotype does not correlate with HIV control in African Americans. *Clin Transl Sci* 2011; **4**:282–284.
37 Salgado M, Kirk GD, Cox A, Rutebemberwa A, Higgins Y, Astemborski J, *et al*. Protective interleukin-28B genotype affects hepatitis C virus clearance, but does not contribute to HIV-1 control in a cohort of African–American elite controllers/suppressors. *AIDS* 2011; **25**:385–387.
38 Rungtivasuwan K, Avihingsanon A, Thammajaruk N, Mitruk S, Burger DM, Ruxrungtham K, *et al*. Pharmacogenetics-based population pharmacokinetic analysis of tenofovir in Thai HIV-infected patients. *Pharmacogenomics* 2017; **18**:1481–1490.
39 Calcagno A, Cusato J, Marinaro L, Trentini L, Alcantarini C, Mussa M, *et al*. Clinical pharmacology of tenofovir clearance: a pharmacokinetic/pharmacogenetic study on plasma and urines. *Pharmacogenomics J* 2016; **16**:514–518.