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Evaluating the association of single-nucleotide polymorphisms with tenofovir exposure in a diverse prospective cohort of women living with HIV

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Abstract

Higher exposure to tenofovir (TFV) increases the risk for kidney function decline, but the impact of genetic factors on TFV exposure is largely unknown. We investigated whether single-nucleotide polymorphisms (SNPs, $n = 211$) in 12 genes are potentially involved in TFV exposure.

Participants ($n = 91$) from the Women's Interagency HIV Study, underwent a 24 h intensive pharmacokinetic sampling of TFV after witnessed dose and TFV area under the time–

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CONFLICT OF INTEREST

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concentration curves (AUCs) were calculated for each participant. SNPs were assayed using a combination of array genotyping and Sanger sequencing. Linear regression models were applied to logarithmically transformed AUC. Those SNPs that met an *a priori* threshold of $P < 0.001$ were considered statistically associated with TFV AUC. *ABCG2* SNP rs2231142 was associated with TFV AUC with rare allele carriers displaying 1.51-fold increase in TFV AUC (95% confidence interval: 1.26, 1.81; $P = 1.7 \times 10^{-5}$). We present evidence of a moderately strong effect of the rs2231142 SNP in *ABCG2* on a 24 h TFV AUC.

INTRODUCTION

Treatment for HIV has dramatically improved in the last 20 years, but lifelong antiretroviral treatment is still required. Ideally, such treatment would represent a sufficient exposure—that is, an exposure to an adequate drug level to suppress HIV replication while minimizing drug toxicity. A barrier to optimal drug exposure is an incomplete understanding of the factors that contribute to inter-individual variability in drug concentration. There are a variety of factors that affect drug exposure, but pharmacogenetics represents an inherent determinant of exposure that may offer an opportunity to understand an individual's potential for achieving viral suppression and/or developing toxicity.

Pharmacokinetics collectively refers to the activation, bioavailability, absorption, distribution, metabolism and elimination of a given drug; all of which are key factors in determining an individual's exposure to a compound after administration. A variety of pharmacokinetic studies for different antiretroviral medications demonstrated that increasing drug exposure is commonly associated with toxicity.^{1–10} Tenofovir disoproxil fumarate (TDF) is a nucleotide-analog reverse transcriptase inhibitor with broad activity against HIV and is currently co-formulated in a number of pill combinations. TDF is considered to be a World Health Organization essential medication and is a preferred first-line agent in the treatment and prevention of HIV.^{11,12} One primary concern in the use of TDF is the risk of decline in kidney function over time. Recent studies suggest that variability in tenofovir (TFV, the active metabolite) pharmacokinetics can partially explain the risk of decline in kidney function^{2,13} and although some work has been done to understand the factors that affect TFV exposure,^{14–23} the genetic factors that contribute to TFV exposure are less well characterized.

Pharmacogenetics refers to genetic factors that determine pharmacokinetics and specific adverse responses. Such factors could inform precision therapeutics; that is, maximizing benefit while minimizing toxicity taking into consideration an individual's risk/benefit and dosing profile. With respect to TDF in the treatment of HIV, pharmacogenetic studies have focused on relatively acute toxicity or genotypes associated with intracellular concentrations, but few studies have investigated how single-nucleotide polymorphisms (SNPs) may affect tenofovir (TFV) exposure.^{18,24–30} Thus, the primary aim of this study, in a large prospective diverse cohort of women living with HIV, was to examine how SNPs in TFV pharmacogenes ultimately impact TFV exposure as measured by 24 h TFV area under the time–concentration curves (AUCs). The primary hypothesis was that genes with SNPs

specifically implicated in the transport of organic anions, would result in higher TFV exposure.

MATERIALS AND METHODS

Study design and population

The study design is cross-sectional, evaluating the association between the TFV AUC and candidate gene SNPs that were selected for inclusion based on previous published association with TFV toxicity, metabolism or organic anion transport. The Women's Interagency HIV Study (WIHS) is a large, multicenter, prospective cohort study of HIV-infected women and at-risk HIV uninfected women in the United States,^{31,32} operational since 1993. The WIHS is highly representative of the US women living with HIV in terms of age, race/ethnicity, socioeconomic status, concomitant medications and comorbid medical conditions. We previously described the 'WIHS Intensive Pharmacokinetics Study',^{33,34} which enrolled 480 HIV-infected women on different antiretroviral regimens, from 2004 to 2008, for 12 to 24 h sampling of various antiretroviral plasma levels after administration of a dose witnessed by study team members. For the current study, eligible WIHS participants were adult women (≥ 18 years of age) living with HIV, consented to the study (including separate written informed consent for the WIHS study, the genetic study and the intensive pharmacokinetic study), who had used TDF for at least 6 months before pharmacokinetic evaluation. They had previously undergone 24 h intensive pharmacokinetic sampling and had samples available for SNP testing. Laboratory measurements, physical exams, demographic information, adherence data and several other characteristics were obtained every 6 months on participants as long as they remained in the cohort. Follow-up of the cohort is ongoing. Institutional review boards at all participating institutions approved the study, consent and protocol materials, and written informed consent was obtained from each study participant.

Intensive pharmacokinetic protocol methods

Pharmacokinetic protocols were conducted in clinical research centers or other facilities associated with collaborating WIHS sites. The TFV measurement procedure has been previously described,¹⁴ but important details are included.

Plasma samples were drawn over 24 h for drug levels under conditions of actual use (including diet and concomitant medications). Participants were seen for the pharmacokinetics visit within 6 weeks of their core WIHS visit and data were collected at both visits on weight, comorbidities, HIV RNA level, CD4 cell counts, medication use and renal function. All participants received their standard dose of TDF (300 mg orally once daily) and drug levels were measured in specimens collected at 0, 4, 8, 15, 18 and 24 h after a dose witnessed by study personnel. Calculation of AUC is outlined below.

Laboratory procedures

Plasma levels of TFV were determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS) with TDF-d6 as the internal standard.³⁵ The plasma sample was pretreated with trifluoroacetic acid for protein precipitation before injecting into the Micromass

Quattro Ultima LC-MS/MS system as previously described.^{14,35} The assay was validated from 10 to 1000 ng ml⁻¹ of TFV with a coefficient of variation of 15% for quality control samples at low, medium and high concentrations.

Covariates

The following baseline characteristics were included as covariates in all the multivariable models as continuous variables: age (for every 10-year increment) and body mass index (for every 10 percent change, measured in kg m⁻²). The following baseline characteristics were included as covariates in all the multivariable models as categorical variables: race (self-reported African American or not), estimated glomerular filtration rate ($\geq$ or $<$ 70 ml min⁻¹ per 1.73 m², estimated using the serum creatinine) and concurrent ritonavir use (yes or no). Finally, ancestry informative markers were used to estimate individual level biogeographic ancestry and to minimize bias from stratification.³⁶ Visual inspection of scatter plots of orthogonal principal components were used to distinguish the major racial/ethnic groups in the sample (that is, Caucasian, African and Hispanic). The first three principal components were selected to adjust for potential confounding due to population stratification, by including them in all the multivariate regression models. The ancestry informative markers and their principal components were available for all the participants.

Nucleic acid extraction

Genomic DNA was extracted previously for all of the participants recruited for the intensive pharmacokinetic studies. The DNA samples were quantified by spectrophotometry and normalized to a concentration of 50 ng μ l⁻¹.

Gene and SNP selection

A comprehensive systematic literature search identified genes implicated in TDF absorption, distribution, metabolism and excretion. A custom array was designed to interrogate nine absorption, distribution, metabolism and excretion ‘pharmacogenes’ (that is, ATP-binding cassette transporter (*ABC*) *B1*, *ABCC2*, cytochrome (*CYP*) *2B6*, *CYP2C19*, *CYP2D6*, *CYP3A4/A5*, solute carrier transporter (*SLC22A6*), UDP glucuronosyltransferase-1 A1 (*UGT1A1*)). Genotyping was undertaken using the GoldenGate genotyping platform (Illumina, San Diego, CA, USA). GoldenGate genotyping array data were processed according to standard protocols using GenomeStudio (Illumina). Signal intensity profiles and resulting genotype calls for each SNP was visually inspected and confirmed in a blinded manner. Tagging SNPs (tagSNPs; defined as an efficient subset of SNPs available within a given gene region that are in high-linkage disequilibrium with unmeasured SNPs) were selected from across coding and noncoding regions of each gene to capture the majority of the genetic variability surrounding each gene. TagSNP selection was performed using Snagger,³⁷ which selects tagSNPs that are informative across the racial and ethnic groups. In addition, SNPs in three additional pharmacogenes specific to TFV absorption, distribution, metabolism and excretion or pharmacodynamics (that is, *ABCC4*, *ABCG2*, adenylate kinase isoenzyme 1 (*AKI*)) were selected based on literature review. Of the additional literature-driven SNPs from among the three additional candidate genes, each was measured by Sanger DNA sequencing. The *CYP2B6* ‘Metabolizer’ haplotype was constructed as described previously.³⁸

DNA sequencing

Three SNPs were typed by Sanger sequencing. Polymerase chain reaction (PCR) primers were designed using Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/>) to amplify a region containing the variation. The resulting PCR product was treated with shrimp alkaline phosphatase and exonuclease I enzymes (ExoSAP-IT PCR cleanup kit, Affymetrix, Santa Clara, CA, USA) using the standard product protocol. The treated PCR product served as the template for the sequencing reaction with BigDye Terminator (Applied Biosystems, Foster City, CA, USA). The sequencing reaction was cleaned with X-Terminator (Applied Biosystems) and analyzed on the 3730xl DNA Analyzer (Applied Biosystems). The resulting sequencing data were viewed using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA) to perform genotype calling. The final concentrations of the PCR components were 2.5 mM MgCl₂, 0.1 mM dNTPs, 0.025 units of Platinum Taq polymerase (Invitrogen, Carlsbad, CA, USA), 2% DMSO, 1 × PCR Buffer, 200 μM PCR primers and 10 ng of DNA template. The 2 μl reaction was run with the following conditions: 95 °C for 5 min, (94 °C for 20 s, 65 °C for 20 s (0.5 °C decrease per cycle), 72 °C for 45 s; 14 cycles), (94 °C for 20 s, 58 °C for 20 s, 72 °C for 45 s; 35 cycles), 72 °C for 10 min. The sequencing reaction consists of final concentrations of sequencing buffer, BigDye Terminator mix (Applied Biosystems), 500 μM sequencing primer and PCR product template. The running conditions for the sequencing reaction were: 96 °C for 1 min, (96 °C for 10 s, 55 °C for 5 s, 60 °C for 4 min; 25 cycles). Custom array genotyping provided 211 SNPs that passed all quality control criteria described below. TagSNPs were required to be common (defined as a minor allele frequency ≥ 0.05). SNPs with call rates < 95% or SNPs which deviated from Hardy–Weinberg expectations ($P < 0.001$) were excluded. Finally, SNPs with less than three observations in a given genotypic group (for example, heterozygous, homozygous rare) were excluded.

Outcome

AUCs were used to estimate TFV exposure over the 24 h dosing interval; these were calculated for each individual using the trapezoidal rule.³⁹ Any observations with TFV concentrations below the lower limit of quantification (10 ng ml⁻¹) were replaced by 0 ng ml⁻¹ (10 individuals at baseline and one individual for a subsequent level).

Statistical analysis

Linear regression modeling using the SAS mixed procedure with robust standard errors was applied to logarithmically transformed AUC, and predictors' coefficients were back-transformed to produce estimated multiplicative effects on AUCs. Genetic association analyses were conducted in the following manner. The first set of models (Supplementary Table 1) show the effect of each individual SNP on log-transformed AUC over dose when combined with non-genetic factors previously shown to influence exposure. Four genetic models were used to assess each SNP: unstructured, additive, dominant and recessive. The genetic model that best fit the data, minimizing the P -value, was selected for each SNP. A criterion for selection was the presence of at least three observations in each genotypic group. For race/ethnicity, both genetic (ancestry informative markers) and non-genetic parameters were included. In models that examined the effect of each individual SNP on

log-transformed AUC/dose (when controlling for non-genetic factors previously shown to influence exposure), only one SNP met the *a priori* *P*-value threshold ($\alpha = 0.001$). This SNP was then included in a model and all SNPs were then re-screened to identify the next SNP that met this criterion until no additional SNP is retained in the model. No additional SNPs met the *a priori* criterion ($\alpha = 0.001$), and therefore evaluation of models with multiple SNPs was not pursued further. The linearity assumption was evaluated in models with non-genetic factors.¹⁴ An $\alpha = 0.001$ was implemented as a multiple testing penalty for the following reasons. First, the genes that were selected for study have a higher *a priori* probability of being associated with TFV AUC due to evidence of involvement in TFV metabolism. Second, the SNPs spanning each of the genes are not independent (that is, highly correlated). Therefore, an $\alpha = 0.001$ was reasoned to be an appropriately conservative threshold. All analyses were conducted using Stata (version 11.2, College Station, TX, USA) and SAS (version 9.4, SAS Institute, Cary, NC, USA). The figure was generated using R (version 3.2.3, Vienna, Austria).

RESULTS

The AUC results from a larger study in this cohort ($n = 101$) have been previously summarized.¹⁴ Ten individuals from this cohort did not have samples for genetic analyses, thus data from 91 individuals were included in the present analysis. The median TFV AUC among the 91 participants was $3408 \mu\text{g h ml}^{-1}$ (range 1026–9356 $\mu\text{g h ml}^{-1}$). The median age of participants was 44.5 years (range 22.9–64.9 years). The participants were mostly African American ($n = 55$, 60.4%) with a median body mass index of 27 kg m^{-2} (range 15–62 kg m^{-2}). Of the 240 SNPs that were assessed in the 91 participants, 29 failed quality control measures, leaving 211 for analysis in each participant (Supplementary Table 1). One SNP in *ABCG2* (which encodes for a membrane transporter), rs2231142, was associated with TFV AUC assuming a dominant model, with rare allele carriers (that is, AA and CA as compared with CC homozygotes) having 1.51-fold increase in TFV AUC (95% confidence interval: 1.26, 1.81; $P = 1.7 \times 10^{-5}$). The estimated fold-effect for each SNP is included in Supplementary Table 1. For rs2231142, 14 of 91 individuals carried one of the rare allele, and one individual was homozygous for the rare allele, and therefore no other model was considered. Table 1 summarizes the results of the multivariable model controlling for age (per decade), body mass index (per 10 percent increase), African American race, ritonavir use, and whether eGFR is less than 70 ml min^{-1} per 1.73 m^2 . Similar results were obtained when treating eGFR as a continuous variable ($P = 0.06$ for eGFR). Figure 1 displays a boxplot of the distribution of TFV AUC by number of alleles (0 vs 1 or 2) for rs2231142. Given that rs2231142 met the threshold for inclusion in the model, we subsequently re-assessed each SNP in a model with rs2231142. Controlling for rs2231142, no SNP met the *a priori* inclusion threshold to be included in the final model, but the two SNPs that had the smallest *P*-values are noteworthy for the resultant change in fold-effects for rs2231142 when they were included in the model. The first SNP, rs1128503 from the *ABCB1* gene, which had $P = 0.0024$ in a dominant model, resulted in an increase in the estimated fold-effect for *ABCG2* rs2231142 (fold-effect 1.64, 95% confidence interval: 1.38, 1.96; $P = 3.2 \times 10^{-7}$). The second SNP (*ABCB1* rs10236274), which had $P = 0.0036$ in a dominant model, also resulted in an increase in the fold-effect for *ABCG2* rs2231142 (fold-effect 1.62, 95%

confidence interval: 1.34, 1.94; $P = 1.6 \times 10^{-6}$). The linkage disequilibrium between *ABCB1* rs1128503 and rs10236274 is weak (that is, $r^2 = 0.013$, $D' = 0.49$), indicating that each SNP may represent different risk alleles. Finally, identical multivariate models were run replacing $C_{\min}$ and $C_{\max}$ (standardized by dose) for AUC as the outcome. These multivariate models also controlled for age (per decade), body mass index (per 10 percent increase), African American race, ritonavir use and whether eGFR was less than 70 ml min⁻¹ per 1.73 m². For $C_{\max}$, the presence of at least one rs2231142 rare allele remained statistically significant (fold-effect 1.62, (1.28, 2.0), 0.0001), as it did for $C_{\min}$ (fold-effect 1.44, (1.17, 1.77), 0.0009).

DISCUSSION

In this cross-sectional analysis, nested in a cohort study, we present a comprehensive analysis of SNPs previously associated with acute TFV toxicity, metabolism of TFV or in the transport of organic anions. We were able to identify a single SNP in the *ABCG2* gene that, when present, was associated with a 1.51-fold increase in TFV exposure as measured by AUC. To our knowledge, this SNP has not been previously implicated in TFV pharmacokinetics or toxicity and therefore represents a potentially novel mechanism for how TFV exposure may vary between individuals taking TFV-based antiretroviral therapy.

TFV is commonly prescribed for both the treatment and prevention of HIV. Several studies have identified a variety of clinical factors associated with increased exposure to TFV.^{14–20,22,40–42} After including clinically relevant factors in our model of TFV AUC, the genetic factors accounted for the largest effects—a noteworthy finding since genetic polymorphisms often result in more subtle effects on drug exposure than we have found here. In addition, the previously observed influence of higher eGFR on increasing TFV AUC^{14,21,42} was not found to be significant in the multivariate models in this study. Taken together, these findings indicate that genetic effects could be more pronounced in the presence of other factors that affect drug exposure. The changes in eGFR may be upstream mediators of an underlying genetic effect, but the lack of significance for eGFR in this study was likely related to the sampling of a subgroup from the larger cohort. Of note, we observed a similar phenomenon (that is, a biological interaction between the presence of a polymorphism and clinical factors that affect target drug exposure) in an intensive pharmacokinetic pharmacogenetic study of efavirenz in a similarly diverse sample of HIV positive women.³⁴ Additional study of the interplay between pharmacokinetics, genetics and concurrent morbidities is warranted.

Prior studies have sought to elucidate the pharmacogenetic factors related to TFV exposure and activity, but these have largely focused on acute kidney toxicity, including genetic polymorphisms associated with Fanconi's syndrome and proximal tubulopathy, proteinuria and changes in glomerular filtration.^{24,28–30,43–47} We were not able to reach statistical significance for any of these previously identified SNP associations with our more precise, intensive pharmacokinetic assessment of TFV exposure. This is not surprising in that prior studies looked at acute toxicity events and this study sought to understand the impact of genetic polymorphisms on TFV AUC. This possibly indicates that TFV exposure may not be as important of a factor in acute kidney injury as other metrics such as intracellular TFV

concentrations. It should be noted that this analysis was based in an observational cohort, in which treatment is determined by the individual participant's provider; and as study assessments occur twice annually, acute toxicity is not likely to be directly observed, but chronic change in renal function after longer treatment exposure is detected. Thus, this study indicates that chronic TFV toxicity, which is associated with the extent of drug exposure,^{2,13} may be distinct from acute renal toxicity. The polymorphisms identified in prior studies were from genes involved in anion transport at the level of the kidney, similar to *ABCG2*.

The *ABCG2* gene is located on chromosome 4 and encodes for a protein that is found on the apical side of the proximal renal tubular cell and, as a member of the ABC family of transporters, is involved in the transport of anions into the urine. The specific *ABCG2* gene SNP associated with higher TFV exposure in the current study, rs2231142, has previously been associated with a genetic predisposition for increased circulating uric acid and gout.^{48–52} *ABCG2* rs2231142 is the result of a missense mutation and is hypothesized to result in loss of function that reduces transport of uric acid from inside the renal proximal tubular cell into the urine, producing higher circulating uric acid levels.⁴⁹ This mechanism has been demonstrated *in vitro* for both uric acid as well as for several chemotherapeutic agents^{49,53} and provides a plausible mechanism of action for increased TFV concentration in serum as well.⁵⁴

There are a number of strengths to this study. We were able to obtain a robust measure of TFV exposure in a cohort of diverse women living with HIV. Pharmacokinetic exposure is often estimated using single measures, but capturing a 24 h pharmacokinetic profile and directly determining AUC may overcome the individual variability that limits interpretation of single measures of antiretroviral concentration.⁵⁵ In addition, we had data on most SNPs in several genes that, *a priori*, could reasonably be associated with TFV pharmacokinetics. Evaluating such a large number of SNPs enabled us to perform the most comprehensive assessment of TFV pharmacogenetics conducted to date, while also controlling for established clinical factors that are associated with TFV AUC.¹⁴ We also considered SNPs that have been implicated in acute renal toxicity with TFV use, allowing us to assess whether these same factors influence more chronic renal injury, indirectly through AUC.

There are limitations to the interpretation of the results of this study. Nineteen polymorphisms assessed in this study did not have sufficiently high allele frequencies to estimate their association with TFV exposure (see Supplementary Table 1). Although understanding these associations is important for developing a complete understanding of the pharmacogenetics of TFV, alleles with very low frequency are less likely to have major clinical impact with respect to determining drug exposure. We did account for multiple comparisons in this study by setting an *a priori* threshold of significance at $\alpha = 0.001$. Although some might have opted for a threshold determined by a traditional significance threshold ($\alpha = 0.05$) divided by the number of comparisons (211 SNPs $\times$ up to 4 models per SNP = up to 844 comparisons), yielding a threshold of $\alpha = 0.0000592$, doing so would not have impacted the identification of the SNP found in this study. Twenty-four hour AUC measurement does not guarantee that an individual was at steady-state concentration as individuals may have initiated medication in the days leading up to the research study day (and not reported this to the study staff). If this occurred, independently of the SNPs

evaluated here, the additional random variation would be expected to have attenuated associations with AUC. Furthermore, a single TFV AUC measurement does not reflect day-to-day variation and such variation is influenced by diet, concomitant medications, adherence and substance use. As a whole genome approach to evaluating polymorphisms was not possible, there may be other important factors that have not been identified beyond this targeted approach based on the current state of the literature. In addition, rare variants were not tested and such variants may be important to consider in pharmacokinetic variability. Notably, intensive pharmacokinetic sampling is challenging in most clinical settings, is expensive to conduct and requires tremendous dedication on the part of patients and providers. Such sampling makes our results difficult to generalize to other settings, but may be most helpful in establishing the mechanism by which SNPs affect TFV exposure. Finally, a new formulation of tenofovir is now available, called tenofovir alafenamide fumarate, and it is likely that some of the genetic factors identified for TFV will extend to tenofovir alafenamide fumarate, but this will require a dedicated study which will be undertaken as tenofovir alafenamide fumarate becomes more commonly used in the treatment of HIV.

In conclusion, we present here evidence of a novel moderately strong association between the *ABCG2* rs2231142 and increased TFV exposure as measured by 24-hour AUC in a large prospective cohort of women living with HIV. Understanding how this SNP may lead to elevated AUC will be imperative. More importantly, understanding whether rs2231142 is an upstream determinant that drives renal toxicity from TFV use, either mediated through TFV exposure or through an independent pathway, will be fundamental in elucidating the mechanism by which TFV leads to kidney injury over time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Umeh OC, Currier JS. Sex differences in pharmacokinetics and toxicity of antiretroviral therapy. *Expert Opin Drug Metab Toxicol* 2006; 2: 273–283. [PubMed: 16866613]
2. Baxi SM, Scherzer R, Greenblatt RM, Minkoff H, Sharma A, Cohen M et al. Higher tenofovir exposure is associated with longitudinal declines in kidney function in women living with HIV. *AIDS* 2016; 30: 609–618. [PubMed: 26558723]
3. Arab-Alameddine M, Decosterd LA, Buclin T, Telenti A, Csajka C. Antiretroviral drug toxicity in relation to pharmacokinetics, metabolic profile and pharmacogenetics. *Expert Opin Drug Metab Toxicol* 2011; 7: 609–622. [PubMed: 21500966]
4. Burger D, Hugen P, Reiss P, Gyssens I, Schneider M, Kroon F et al. Therapeutic drug monitoring of nelfinavir and indinavir in treatment-naïve HIV-1-infected individuals. *AIDS* 2003; 17: 1157–1165. [PubMed: 12819517]
5. Collin F, Chene G, Retout S, Peytavin G, Salmon D, Bouvet E et al. Indinavir trough concentration as a determinant of early nephrolithiasis in HIV-1-infected adults. *Ther Drug Monit* 2007; 29: 164–170. [PubMed: 17417069]
6. Ezinga M, Wetzels JF, Bosch ME, van der Ven AJ, Burger DM. Long-term treatment with tenofovir: prevalence of kidney tubular dysfunction and its association with tenofovir plasma concentration. *Antivir Ther* 2014; 19: 765–771. [PubMed: 24584104]
7. Gutierrez F, Navarro A, Padilla S, Anton R, Masia M, Borrás J et al. Prediction of neuropsychiatric adverse events associated with long-term efavirenz therapy, using plasma drug level monitoring. *Clin Infect Dis* 2005; 41: 1648–1653. [PubMed: 16267739]
8. Kappelhoff BS, van Leth F, Robinson PA, MacGregor TR, Baraldi E, Montella F et al. Are adverse events of nevirapine and efavirenz related to plasma concentrations? *Antivir Ther* 2005; 10: 489–498. [PubMed: 16038474]
9. Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *AIDS* 2001; 15: 71–75. [PubMed: 11192870]
10. Wang J, Kou H, Fu Q, Han Y, Qiu Z, Zuo L et al. Nevirapine plasma concentrations are associated with virologic response and hepatotoxicity in Chinese patients with HIV infection. *PLoS ONE* 2011; 6: e26739. [PubMed: 22066007]
11. WHO. Guideline on When to Start Antiretroviral Therapy and on Pre-Exposure Prophylaxis for HIV. WHO: Geneva, Switzerland, 2015.
12. WHO. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. WHO: Geneva, Switzerland, 2013.
13. Scherzer REM, Li Y, Choi AI, Deeks SG, Grunfeld C, Shlipak MG. Association of tenofovir exposure with kidney disease risk in HIV infection. *AIDS* 2012; 26: 867–875. [PubMed: 22313955]
14. Baxi SM, Greenblatt RM, Bacchetti P, Scherzer R, Minkoff H, Huang Y et al. Common clinical conditions - age, low BMI, ritonavir use, mild renal impairment affect tenofovir pharmacokinetics in a large cohort of HIV-infected women. *AIDS* 2014; 28: 59–66. [PubMed: 24275255]
15. Gervasoni C, Meraviglia P, Landonio S, Baldelli S, Fucile S, Castagnoli L et al. Low body weight in females is a risk factor for increased tenofovir exposure and drug-related adverse events. *PLoS ONE* 2013; 8: e80242. [PubMed: 24312465]
16. Hill A, Khoo S, Back D, Pozniak A, Boffito M. Should the dose of tenofovir be reduced to 200–250 mg/day, when combined with protease inhibitors? *J Int AIDS Soc* 2014; 17: 19583. [PubMed: 25394089]

17. Kearney BP, Mathias A, Mittan A, Sayre J, Ebrahimi R, Cheng AK. Pharmacokinetics and safety of tenofovir disoproxil fumarate on coadministration with lopinavir/ritonavir. *J Acquir Immune Defic Syndr* 2006; 43: 278–283. [PubMed: 17079992]
18. Kiser JJAC, 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. [PubMed: 18398970]
19. Kiser JJCM, Aquilante CL, Anderson PL, Wolfe P, King TM, Delahunty T et al. The effect of lopinavir/ritonavir on the renal clearance of tenofovir in HIV-infected patients. *Clin Pharmacol Ther* 2008; 83: 265–272. [PubMed: 17597712]
20. Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, Kinai E et al. Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients. *PLoS ONE* 2011; 6: e22661. [PubMed: 21799928]
21. Poizot-Martin I, Solas C, Allemand J, Obry-Roguet V, Pradel V, Bregigeton S et al. Renal impairment in patients receiving a tenofovir-cART regimen: impact of tenofovir trough concentration. *J Acquir Immune Defic Syndr* 2013; 62: 375–380. [PubMed: 23196828]
22. Rodriguez-Novoa S, Labarga P, D'Avolio A, Barreiro P, Albalade M, Vispo E et al. Impairment in kidney tubular function in patients receiving tenofovir is associated with higher tenofovir plasma concentrations. *AIDS* 2010; 24: 1064–1066. [PubMed: 20299966]
23. Szczech LA. Renal dysfunction and tenofovir toxicity in HIV-infected patients. *Top HIV Med* 2008; 16: 122–126. [PubMed: 18838746]
24. Izzedine HHJ, Villard E, Goyenville C, Dominguez S, Ghosn J, Valantin MA et al. Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. *J Infect Dis* 2006; 194: 1481–1491. [PubMed: 17083032]
25. Rodríguez-Nóvoa SLP, Soriano V, Egan D, Albalade M, Morello J, Cuenca L et al. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. *Clin Infect Dis* 2009; 48: e108–e116. [PubMed: 19400747]
26. Rodriguez-Novoa SLP, Soriano V. Pharmacogenetics of tenofovir treatment. *Pharmacogenomics* 2009; 10: 1675–1685. [PubMed: 19842939]
27. Elens LTD, Panin N, Courtoy P, Lison D, Demoulin JB, Haufroid V. Functional defect caused by the 4544G4 > A SNP in ABCC2: potential impact for drug cellular disposition. *Pharmacogenet Genomics* 2011; 21: 884–893. [PubMed: 22027652]
28. Pushpakom SPLN, Rodríguez-Nóvoa S, Labarga P, Soriano V, Albalade M, Hopper-Borge E et al. Genetic variants of ABCC10, a novel tenofovir transporter, are associated with kidney tubular dysfunction. *J Infect Dis* 2011; 204: 145–153. [PubMed: 21628669]
29. Kohler JJHS, Green E, Abuin A, Ludaway T, Russ R, Santoianni R et al. Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. *Lab Invest* 2011; 91: 852–858. [PubMed: 21403643]
30. Nishijima TKH, Higasa K, Takano M, Tsuchiya K, Hayashida T, Oka S et al. Single nucleotide polymorphisms in ABCC2 associate with tenofovir-induced kidney tubular dysfunction in Japanese patients with HIV-1 infection: a pharmacogenetic study. *Clin Infect Dis* 2012; 55: 1558–1567. [PubMed: 22955427]
31. Barkan SE, Melnick SL, Preston-Martin S, Weber K, Kalish LA, Miotti P et al. The Women's Interagency HIV Study. WIHS Collaborative Study Group. *Epidemiology* 1998; 9: 117–125. [PubMed: 9504278]
32. Bacon MC, von Wyl V, Alden C, Sharp G, Robison E, Hessel N et al. The Women's Interagency HIV Study: an observational cohort brings clinical sciences to the bench. *Clin Diagn Lab Immunol* 2005; 12: 1013–1019. [PubMed: 16148165]
33. Gandhi M, Benet LZ, Bacchetti P, Kalinowski A, Anastos K, Wolfe AR et al. Nonnucleoside reverse transcriptase inhibitor pharmacokinetics in a large unselected cohort of HIV-infected women. *J Acquir Immune Defic Syndr* 2009; 50: 482–491. [PubMed: 19408353]
34. Gandhi M, Greenblatt RM, Bacchetti P, Jin C, Huang Y, Anastos K et al. A single-nucleotide polymorphism in CYP2B6 leads to > 3-fold increases in efavirenz concentrations in plasma and hair among HIV-infected women. *J Infect Dis* 2012; 206: 1453–1461. [PubMed: 22927450]

35. Delahunty T, Bushman L, Robbins B, Fletcher CV. The simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopically labeled internal standards. *J Chromatogr B Anal Technol Biomed Life Sci* 2009; 877: 1907–1914.
36. Halder I, Shriver M, Thomas M, Fernandez JR, Frudakis T. A panel of ancestry informative markers for estimating individual biogeographical ancestry and admixture from four continents: utility and applications. *Hum Mutat* 2008; 29: 648–658. [PubMed: 18286470]
37. Edlund CK, Lee WH, Li D, Van Den Berg DJ, Conti DV. Snagger: a user-friendly program for incorporating additional information for tagSNP selection. *BMC Bioinformatics* 2008; 9: 1–13. [PubMed: 18173834]
38. Haas DW, Gebretsadik T, Mayo G, Menon UN, Acosta EP, Shintani A et al. Associations between CYP2B6 polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in African americans. *J Infect Dis* 2009; 199: 872–880. [PubMed: 19239339]
39. Yeh KC, Kwan KC. A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximation. *J Pharmacokinet Biopharm* 1978; 6: 79–98. [PubMed: 650423]
40. Kearney BP, Yale K, Shah J, Zhong L, Flaherty JF. Pharmacokinetics and dosing recommendations of tenofovir disoproxil fumarate in hepatic or renal impairment. *Clin Pharmacokinet* 2006; 45: 1115–1124. [PubMed: 17048975]
41. Kiser JJ, Fletcher CV, Flynn PM, Cunningham CK, Wilson CM, Kapogiannis BG et al. Pharmacokinetics of antiretroviral regimens containing tenofovir disoproxil fumarate and atazanavir-ritonavir in adolescents and young adults with human immunodeficiency virus infection. *Antimicrob Agents Chemother* 2008; 52: 631–637. [PubMed: 18025112]
42. Le MP, Landman R, Koulla-Shiro S, Charpentier C, Sow PS, Diallo MB et al. Tenofovir plasma concentrations related to estimated glomerular filtration rate changes in first-line regimens in African HIV-infected patients: ANRS 12115 DAYANA substudy. *J Antimicrob Chemother* 2015; 70: 1517–1521. [PubMed: 25583749]
43. Calza LTF, Tedeschi S, Piergentili B, Manfredi R, Colangeli V, Viale P. Tenofovir-induced renal toxicity in 324 HIV-infected, antiretroviral-naïve patients. *Scand J Infect Dis* 2011; 43: 656–660. [PubMed: 21453205]
44. Dahlin A, Wittwer M, de la Cruz M, Woo JM, Bam R, Scharen-Guivel V et al. A pharmacogenetic candidate gene study of tenofovir-associated Fanconi syndrome. *Pharmacogenet Genomics* 2015; 25: 82–92. [PubMed: 25485598]
45. Likanonsakul S, Suntisuklappon B, Nitiyanontakij R, Prasithsirikul W, Nakayama EE, Shioda T et al. A Single-Nucleotide Polymorphism in ABCC4 Is Associated with Tenofovir-Related Beta2-Microglobulinuria in Thai Patients with HIV-1 Infection. *PLoS ONE* 2016; 11: e0147724. [PubMed: 26807589]
46. 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. [PubMed: 24788661]
47. Giacomet V, Cattaneo D, Vigano A, Nannini P, Manfredini V, Ramponi G et al. Tenofovir-induced renal tubular dysfunction in vertically HIV-infected patients associated with polymorphisms in ABCC2, ABCC4 and ABCC10 genes. *Pediatr Infect Dis J* 2013; 32: e403–e405. [PubMed: 24067562]
48. Stark K, Reinhard W, Grassl M, Erdmann J, Schunkert H, Illig T et al. Common polymorphisms influencing serum uric acid levels contribute to susceptibility to gout, but not to coronary artery disease. *PLoS ONE* 2009; 4: e7729. [PubMed: 19890391]
49. Woodward OM, Kottgen A, Coresh J, Boerwinkle E, Guggino WB, Kottgen M. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. *Proc Natl Acad Sci USA* 2009; 106: 10338–10342. [PubMed: 19506252]
50. Dehghan A, Kottgen A, Yang Q, Hwang SJ, Kao WL, Rivadeneira F et al. Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. *Lancet* 2008; 372: 1953–1961. [PubMed: 18834626]

51. Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M et al. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. *PLoS Genet* 2009; 5: e1000504. [PubMed: 19503597]
52. Zhang L, Spencer KL, Voruganti VS, Jorgensen NW, Fornage M, Best LG et al. Association of functional polymorphism rs2231142 (Q141K) in the ABCG2 gene with serum uric acid and gout in 4 US populations: the PAGE Study. *Am J Epidemiol* 2013; 177: 923–932. [PubMed: 23552988]
53. Polgar O, Robey RW, Bates SE. ABCG2: structure, function and role in drug response. *Expert Opin Drug Metab Toxicol* 2008; 4: 1–15. [PubMed: 18370855]
54. Neumanova Z, Cervený L, Ceckova M, Staud F. Interactions of tenofovir and tenofovir disoproxil fumarate with drug efflux transporters ABCB1, ABCG2, and ABCC2; role in transport across the placenta. *AIDS* 2014; 28: 9–17. [PubMed: 24413260]
55. Nettles RE, Kieffer TL, Parsons T, Johnson J, Cofrancesco J Jr, Gallant JE et al. Marked intraindividual variability in antiretroviral concentrations may limit the utility of therapeutic drug monitoring. *Clin Infect Dis* 2006; 42: 1189–1196. [PubMed: 16575741]

Figure 1. Boxplot displaying the distribution of 24 h tenofovir area under the time–concentration curves by number of alleles of *ABCG2* rs2231142 (common homozygotes as compared with heterozygotes and rare homozygote). *ABCG2*, ATP-binding cassette transporter G2.

Table 1.
Factors associated with 24 h tenofovir area under the time–concentration curves exposure in 91 women with HIV

Factor	Univariate fold-effect on AUC (95% CI), P-value	Multivariate ^a fold-effect on AUC (95% CI), P-value	Distribution of factor in sample.
African American race	1.00 (0.81, 1.23), 0.97	0.95 (0.72, 1.26), 0.73	55 (60.4%)—no 36 (39.6%)—yes
eGFR < 70 ml min ⁻¹ per 1.73 m ²	1.50 (1.02, 2.2), 0.04	1.30 (0.90, 1.89), 0.16	82 (90.1%)—no 9 (9.9%)—yes
Principal component 1	1.01 (0.94, 1.08), 0.83	1.09 (1.00, 1.19), 0.044	
Principal component 2	0.92 (0.85, 0.99), 0.02	0.95 (0.90, 1.00), 0.038	
Principal component 3	1.04 (0.97, 1.11), 0.30	1.02 (0.97, 1.07), 0.50	
Age (per decade)	1.24 (1.13, 1.37), 0.000026	1.14 (1.03, 1.26), 0.013	Median (min, max)—44.5 (22.9, 64.9)
Body mass index (per 10% increase)	0.95 (0.92, 0.98), 0.0014	0.95 (0.93, 0.98), 0.0033	Median (min, max)—27 (15, 62)
Ritonavir use	1.17 (0.96, 1.44), 0.12	1.26 (1.07, 1.49), 0.0056	36 (39.6%)—no 55 (60.4%)—yes
<i>ABCG2</i> rs2231142 (one or two rare alleles present as compared with common allele homozygotes)	1.49 (1.19, 1.86), 0.0007	1.51 (1.26, 1.81), 0.000017	77 (84.6%)—0 dose 14 (15.4%)—1 or 2 dose

Abbreviations: *ABCG2*, ATP-binding cassette transporter G2; AUC, area under the time–concentration curve; CI, confidence interval; eGFR, estimated glomerular filtration rate.

^a R^2 for the model was 0.40. Statistically significant associations ($P < 0.05$) are rendered in bold.