Pharmacogenetic Analysis of the Model-Based Pharmacokinetics of Five Anti-HIV Drugs: How Does This Influence the Effect of Aging?

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Analysis of aging and pharmacogenetics (PGx) on antiretroviral pharmacokinetics (PKs) could inform precision dosing for older human HIV-infected patients. Seventy-four participants receiving either atazanavir/ritonavir (ATV/RTV) or efavirenz (EFV) with tenofovir/emtricitabine (TFV/FTC) provided PK and PGx information. Aging-PGx-PK association and interaction analyses were conducted using one-way analysis of variance (ANOVA), multiple linear regression, and Random Forest ensemble methods. Our analyses associated unbound ATV disposition with multidrug resistance protein (MRP)4, RTV with P-glycoprotein (P-gp), and EFV with cytochrome P450 (CYP)2B6 and MRP4 genetic variants. The clearance and cellular distribution of TFV were associated with P-gp, MRP2, and concentrative nucleoside transporters (CNTs), and FTC parameters were associated with organic cation transporters (OCTs) and MRP2 genetic variants. Notably, p16INK4a expression, a cellular aging marker, predicted EFV and FTC PK when genetic factors were adjusted. Both age and p16INK4a expression interacted with PGx on ATV and TFV disposition, implying potential dose adjustment based on aging may depend on genetic background.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ Previous studies determined an important role of aging on disposition of some ARVs. Gene polymorphisms, especially in metabolic enzymes, are related to variability in ARV PK, but their role in influencing the aging-PK relationship is unknown.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ This study asked whether gene polymorphisms alter the interpretation of the effect of aging on (1) unbound ATV, RTV, and EFV clearance and (2) clearance and cellular distribution of TFV and FTC.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✔ This study updates the previous aging studies, shedding light on the significant effect of the cellular aging biomarker, p16INK4a, on unbound EFV disposition. We also present evidence of PGx-aging interaction in ATV and TFV PKs, implying potential dose adjustment of these two drugs based on aging may be dependent on gene polymorphisms.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✔ The p16INK4a might be an important biomarker to guide ATV dosing for older HIV-infected patients. Evaluating the role of aging in dose adjustment of ATVs should take into account the presence of genetic variants in metabolic enzymes and drug transporters.

Gene polymorphisms play an important role in drug disposition and individualized therapy, as they can contribute to variability in pharmacokinetics (PKs), and may alter the effect of nongenetic factors. In HIV treatment, pharmacogenetic-pharmacokinetic (PGx-PK) relationships have been demonstrated for many antiretrovirals (ARVs) in the general population.1 Their roles in special populations, such as pregnancy2 and pediatrics,3 have also been evaluated. However, the PGx of ARVs in the older HIV-infected population has not been well studied.

Aging is a trend in the HIV-infected population.4 The estimated proportion of patients aged 50 years old, which is the general definition of “older” in the HIV field, was 42% in 2013,5 and is predicted to be 70% by the end of 2035.6 Aging could change drug absorption, distribution, metabolism, and elimination,7 which could alter efficacy and safety profiles. In our previous studies, we have investigated the effects of chronological age and aging surrogates on the PK of five selected ARVs.5,9 Our analysis showed that the clearances of two renally eliminated drugs, tenofovir (TFV) and
emtricitabine (FTC), demonstrated a significant association with creatinine clearance, which is strongly related to chronological age. In addition, we found that the cellular clearances of their active metabolites, tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP), are associated with the expression of a cellular aging biomarker, p16INK4a. On the other hand, three hepatically metabolized drugs, atazanavir (ATV), ritonavir (RTV), and efavirenz (EFV), did not show statistically significant relationships with aging, in either total or unbound drug disposition, although the sample size for these drugs was smaller than that of TFV/FTC.

Identification of influential single-nucleotide polymorphisms (SNPs) could provide PGx information for ARV dosing adjustment, and improve statistical analyses of aging by reducing variability in PK. Additionally, gene polymorphisms can modify the effect of aging on PK, and evidence for such interactions has been shown for non-ARV drugs. For example, less profound changes in systemic clearance of omeprazole between younger and older patients was observed in cytochrome P450 (CYP)2C19 poor metabolizers than in extensive metabolizers. This implies that dose adjustment in older patients might be less important given the dysfunctional CYP2C19 genotype. In another example, an eightfold increase in the mean dose-adjusted plasma concentration of venlafaxine was seen in older compared with younger patients among the CYP2D6 poor metabolizers, but not in the other genotype groups. This suggests that dose adjustment might be necessary in the presence of CYP2D6 dysfunction. Therefore, investigating aging in the context of PGx or aging-PGx interaction is essential to provide precise treatment for older patients.

In addition to the aging-PK associations, as outlined in our previous publications, our studied drugs also serve as reasonable models for aging-PGx-PK investigation, because their disposition profiles cover a broad spectrum of metabolic enzymes and drug transporters. ATV and RTV are CYP3A substrates, and served as two model drugs to probe these enzymes. In addition, the upstream CYP3A expression regulator, pregnane X receptor (PXR), affects ATV clearance. Uridine 5′-diphosphate glucuronosyltransferase (UGT)1A is responsible for ATV glucuronidation and is inhibited by ATV. EFV has a complex metabolic profile, which involves CYP2B, CYP2A, CYP3A, CYP1A, and UGT. It is an inducer or inhibitor of CYP2B, CYP3A, and CYP2C. Transporters in the gut, such as P-glycoprotein (P-gp) and multidrug resistance proteins (MRPs), are presumably important globally to ARVs, which are mostly orally administrated. Organic anion-transporting polypeptides (OATPs), which are enriched in the liver, could be relevant to ATV, RTV, and EFV, and OATs or organic cation transporters (OCTs) in the kidney are important to TFV or FTC. Nucleoside transporters, concentrative nucleoside transporters (CNTs) and equilibrative nucleoside transporters, are believed to transport nucleoside analogs, which could be crucial to the disposition of TFV and FTC and the cellular accumulation of their active metabolites.

In the current study, we aim to re-evaluate the effect of aging on PK on five studied drugs in the context of PGx and investigate the aging-PGx interaction in PK. Using a candidate gene approach to select important SNPs, we also aim to provide new hypotheses for further PGx investigations of ARVs. For phenotype, we have focused on the clearance of TFV, FTC and unbound ATV, RTV and EFV, as well as cellular clearance of TFV-DP and FTC-TP, using the associated individual parameters derived from the previous established population PK models.

**Materials and Methods**

**Clinical trial**

Study conduct has been described in previous publications. Briefly, HIV-infected patients were enrolled from the University of North Carolina Health Care Infectious Diseases Clinic (Chapel Hill, NC) and the Cone Health Regional Center for Infectious Diseases (Greensboro, NC). All the subjects received steady-state tenofovir disoproxil fumarate (TDG)/FTC 300/200 mg with either EFV 600 mg or ATV/RTV 300/200 mg daily. Blood samples were collected either sparsely at four time points (predose, and then 2 h, 4–6 h, and 10–14 h postdose) or intensively at 11 time points for PK analysis. An extra blood sample was collected for those who consented to be involved into the PK analysis. The study protocol was approved by the institutional review boards of both institutions (Clinicaltrials.gov NCT01180075).

**Genotyping**

Samples for this test were obtained from patients who consented to genotyping assays at screening. Genomic DNA was extracted from whole blood using QiAamp DNA Blood Mini Kit (QIAGEN). Genotyping was conducted for selected metabolic enzymes and drug transporters using DMET Plus microarray (Affymetrix, CA). Samples with a call rate <95% were excluded from analysis. The SNPs were excluded if its minor allele frequency was <4% and its missing genotypes were >30%. Deviations from expected proportions based on Hardy-Weinberg equilibrium (exact test, α > 0.05) and linkage disequilibrium (R² > 0.2) analysis were performed in PLINK software version 1.07.

**Phenotypes**

The phenotypes in this study were the post hoc PK parameters. The PK analysis was performed as previously described. Briefly, drug concentrations were measured in our previous publications. Drug concentrations were measured in whole blood using QiAamp DNA Blood Mini Kit (QIAGEN). Genotyping was conducted for selected metabolic enzymes and drug transporters using DMET Plus microarray (Affymetrix, CA). Samples with a call rate <95% were excluded from analysis. The SNPs were excluded if its minor allele frequency was <4% and its missing genotypes were >30%. Deviations from expected proportions based on Hardy-Weinberg equilibrium (exact test, α > 0.05) and linkage disequilibrium (R² > 0.2) analysis were performed in PLINK software version 1.07.

Total and intracellular drug concentrations were measured by validated liquid-chromatography tandem mass spectrometry. Unbound EFV, ATV, and RTV concentrations were analyzed using rapid equilibrium dialysis followed by liquid-chromatography tandem mass spectrometry. Population PK models were developed for each drug using non-linear mixed effects modeling program NONMEM version 7.3 (ICON Development Solutions, Hanover, MD). For the current study, individual PK parameters of interest included clearance of the unbound drug for EFV, ATV, and RTV, clearance of the parent drug for TFV and FTC, and the rate constant of intracellular metabolite conversion and elimination for TFV-DP and FTC-TP. These estimates were obtained using the Bayesian post hoc estimation method based on the final models, including the covariate effects of creatinine.
clearance on TFV and FTC clearance and p16\textsuperscript{NK4a} expression on TFV-DP and FTC-TP disposition.

Statistical analysis
A candidate gene approach was used to perform this analysis. The candidate genes were chosen if shown to be relevant to studied drug disposition pathways. Variables were log-transformed to achieve normality when appropriate. In the univariate analysis, the association analysis between genotypes and PK parameters was conducted using one-way analysis of variance (ANOVA). Then, SNPs with a nominally significant \( P \) value were included in multivariate analysis using the stepwise selection procedure in both forward and backward directions. Model selection was based on Akaike information criteria, involving demographic covariates of interest, including chronological age, p16\textsuperscript{NK4a} expression level, race, sex, and body mass index (BMI). Missing genotypes were imputed using IMPUTE2 with the 1000 Genomes Phase III panel.\textsuperscript{19,20} For race, patients who were other, unknown, and nonreported races were grouped as one category ("others") due to low counts. Additionally, the effect of creatinine clearance and treatment arms were evaluated for TFV and FTC. Comparison of demographics between two treatment arms was conducted using Mann-Whitney U test or Kruskal-Wallis test. These statistical analyses were performed using R version 3.2 (R-project.org). A \( P \) value < 0.05 was considered statistically significant.

To investigate the interaction between aging and gene polymorphism, we used distinct linear regression models (R function \texttt{lm}, package stats version 3.4.0; www.r-project.org)\textsuperscript{21} to determine if there is an effect on drug clearance due to the interaction between chronological age and gene polymorphisms (SNPs), and between p16\textsuperscript{NK4a} expression and gene polymorphisms. An F-test (R function \texttt{ANOVA}, package stats version 3.4.0)\textsuperscript{21} was used to compare the model without interaction effects to the one with interaction effects. Only significant predictors from the best fitting model were retained in the final model, with the cutoff for significance being \( P < 0.05 \) for each predictor. The factors involved in each interaction effect were always included in the model. Multiple testing corrections were performed across all drugs using the Benjamini-Hochberg method with a false discovery rate of \( q < 0.05 \) for each predictor. Decreased RTV CL\textsubscript{u/F} was associated with variants \texttt{ABCC4} (rs3745274), \texttt{ABCB1} (rs1045642), \texttt{SLC28A2}, \texttt{ABCC4} \_c.*38T>G (rs3742106). Notably, after adjusting for the effects of these SNPs, p16\textsuperscript{NK4a} expression was found to be significantly associated with EFV CL\textsubscript{u/F}. A doubling of p16\textsuperscript{NK4a} expression was shown to be related to 315 L/h (31% of the median) decrease in EFV unbound CL\textsubscript{u/F}.

Linear regression analysis
The \( P \) values for univariate analysis are shown in Table 2. Twenty-one SNPs were found to be significantly associated with the PK parameters. The SNP basic information, genotype frequency, effects on phenotypes, and SNP function are summarized in Table 3.\textsuperscript{15,28-31} No significant SNPs were found for the TFV-DP CL\textsubscript{out}. The FTC-TP CL\textsubscript{in} showed the same statistical results as the FTC CL/F, because during the model construction the FTC-TP CL\textsubscript{in} was described as a fixed fraction of FTC CL/F.\textsuperscript{9} Therefore, TFV-DP CL\textsubscript{out} and FTC-TP CL\textsubscript{in} were excluded from further analysis. Associations between demographics and PK parameters were consistent with the previous population PK analysis. Briefly, BMI was associated with RTV clearance. Age and creatinine clearance were correlated, and shown to be related to CL/F of both TFV and FTC. The p16\textsuperscript{NK4a} expression was associated with FTC CL/F and FTC CL\textsubscript{out}.

In the multiple linear regression, \texttt{ABCC4} \_c.*879T>C (rs1059751) was associated with increased ATV CL\textsubscript{u/F}, with no demographics being statistically significant. Decreased RTV CL\textsubscript{u/F} was associated with the mutation at \texttt{ABCB1} \_c.3435 (rs1045642). BMI remained significant with RTV CL\textsubscript{u/F}. In addition, race was found to be predictive of RTV CL\textsubscript{u/F}; African Americans had significantly lower apparent clearance of RTV than the other populations. Decreased EFV CL/F was related to variants \texttt{CYP2B6*6} (rs3745274), \texttt{ABCC4} \_c.3348A>G/Del (rs1751034), and \texttt{ABCC4} \_c.*38T>G (rs3742106). Notably, after adjusting for the effects of these SNPs, p16\textsuperscript{NK4a} expression was found to be significantly associated with EFV CL\textsubscript{u/F}. A doubling of p16\textsuperscript{NK4a} expression was shown to be related to 315 L/h (31% of the median) decrease in EFV unbound CL\textsubscript{u/F}.

The polymorphisms at \texttt{ABCB1} \_c.-129 (rs3213619) and \texttt{SLC28A1} \_c.1561 (rs2242046) were significantly associated with decreased TFV CL/F, whereas \texttt{SLC28A2} \_c.531T>C (rs8023604) showed the opposite effect. Creatinine
### Table 1
Overview of demographics of studied population and the pharmacokinetics and pharmacogenetics of the studied drugs

|                | ATV  | RTV  | EFV  | TFV  | FTC  |
|----------------|------|------|------|------|------|
| No. of subjects| 25   | 49   | 74   |      |      |
| Age (years)    |      |      |      |      |      |
| African        | 15 (60%) | 30 (61%) | 45 (61%) |      |      |
| White          | 8 (32%) | 16 (33%) | 24 (32%) |      |      |
| Others         | 2 (8%)  | 3 (8%)  | 5 (7%)   |      |      |
| Race           |      |      |      |      |      |
| Sex            |      |      |      |      |      |
| Female         | 10 (40%) | 12 (24%) | 22 (30%) |      |      |
| Male           | 15 (60%) | 37 (76%) | 52 (70%) |      |      |
| CrCl (L/h)     | 96.6 (66.8, 228) | 112 (43.3, 200) | 109 (43.3, 228) |      |      |
| BMI (kg/m²)    | 29.8 (20.2, 40.4) | 27.1 (17.6, 44.3) | 28.6 (17.6, 44.3) |      |      |
| Frailty status |      |      |      |      |      |
| Nonfrail       | 16 (64%) | 37 (76%) | 53 (72%) |      |      |
| Pre-frail      | 8 (32%) | 10 (20%) | 18 (24%) |      |      |
| Fail           | 1 (4%)  | 2 (4%)  | 3 (4%)   |      |      |
| p16INK4a expression⁴ | 2.20 (0.158, 3.91) | 1.92 (-1.14, 2.81) | 2.01 (-1.14, 3.91) |      |      |

| PK parameters and medians (ranges) | CLu/F: 93.6 (18.2, 355) | CLu/F: 965 (69.5, 2380) | CLu/F: 1010 (417, 3280) | TFV CL/F: 46.8 (16.6, 115) | FTC CL/F: 17.9 (4.54, 27.4) |
|------------------------------------|--------------------------|------------------------|--------------------------|----------------------------|----------------------------|
|                                    | TFV-CL/F: 0.00235 (0.000814, 0.00874) | FTC CL/F: 0.00214 (0.000869, 0.00511) | FTC-TP CLout: 0.0292 (0.0978, 0.141) |      |      |
| No. of SNPs | 40 | 37 | 44 | 52 | 60 |
| Studied genes | Genes for metabolic enzymes | PXR | PXR | PXR | - | - |
|                | Genes for transporters | ABCB1 | ABCB1 | ABCB1 | ABCC1 | - |
|                | SLCO1B1 | ABCB1 | ABCB4 | ABCB2 | ABCB2 | ABCC2 |
|                | - | ABCB4 | ABCB3 | ABCC2 | ABCC3 | ABCC1 |
|                | - | ABCG2 | ABCG4 | ABCC5 | ABCC2 | ABCG2 |
|                | - | SLC22A11 | SLC22A2 | SLC22A2 | SLC22A3 | SLC22A4 |
|                | - | SLC22A2 | SLC22A2 | SLC22A2 | SLC22A2 | SLC22A2 |
|                | - | SLC22A1 | SLC28A1 | SLC28A2 | SLC28A2 | SLC28A3 |
|                | - | SLC28A2 | SLC28A2 | SLC28A2 | SLC28A2 | SLC28A3 |
|                | - | SLC28A2 | SLC28A2 | SLC28A2 | SLC28A2 | SLC28A3 |
|                | - | SLC28A2 | SLC28A2 | SLC28A2 | SLC28A2 | SLC28A3 |

ATV, atazanavir; BMI, body mass index; CL/F, apparent clearance; CLu/F, apparent unbound drug clearance; CrCL, creatinine clearance; CYP, cytochrome P450; EFV, efavirenz; F, undetermined bioavailability; FTC, emtricitabine; FTC-TP, emtricitabine triphosphate; PK, pharmacokinetic; PXR, pregnane X receptor; RTV, ritonavir; SNP, single-nucleotide polymorphism; TFV, tenofovir; TFV-DP, tenofovir diphosphate; UGT, uridine 5′-diphosphate glucuronosyltransferase.

Clearance is in liter/hour (L/h).

⁴The expression of p16INK4a is presented as logarithm (base 2) of gene expression. Continuous variables are in median (range). Categorical variables are in count (percentage).

Clearance remained significant, whereas age was removed from the model. Sex was related to TFV CL/F after adjusting for gene polymorphism, with women demonstrating lower TFV clearance than men. The ABCB1_c.1725+38G>A (rs2235013), SLC28A2_c.374G>C (rs10519020), and ABCB2_c.3563T>A (rs1722723) were retained in the model for TFV-CL/F. For FTC CL/F, the significant predictors were SLC22A3_c.360C>T>G (rs668871), SLC22A2_c.1506A>G (rs316003), ABCB2_c.4488C>T (rs8187707), creatinine clearance, and p16INK4a expression; the effect of age was eliminated. The ABCB2_c.1249G>A (rs2273697) and p16INK4a expression were the two significant predictors in the FTC CLout linear model. Results from the multiple linear regressions are summarized in Table 4.

### Interaction analysis
To analyze interactions, we used two distinct linear regression models to determine if there is an interaction effect...
between chronological age and genetic polymorphisms, and between p16<sup>NK4a</sup> expression and genetic polymorphisms. Most of the significant SNPs in the previous multiple linear regressions were confirmed in this analysis, which was without other demographic variables (Table 5). Notably, we found significant evidence of the interaction effects of age and ABCC4<sup>−38T</sup>C (rs1059751) with ATV CL<sub>u/F</sub>. This effect was also found in the model with p16<sup>NK4a</sup> expression. In addition, both models showed very strong significant evidence of the interaction between age or p16<sup>NK4a</sup> expression and SLC28A2<sup>−734G</sup>C (rs10519020) with TFV-DP CL<sub>in</sub>.

In addition to the analysis with traditional statistical approaches, we performed machine learning analysis to evaluate the variable interactions and the overall importance of each of the variables with the PK parameters. Although the Random Forest method did not find consistent interactions, we used it to determine variable importance. Although the Random Forest method did not find consistent interactions, we used it to determine variable importance. The three most important predictors for ATV CL<sub>u/F</sub> were ABCB1<sup>−1249G</sup>A (rs2273697), sex, and BMI. For EFV CL<sub>u/F</sub>, they were creatinine clearance, p16<sup>NK4a</sup> expression, and age. For FTC-TP CL<sub>out</sub>, the three most important predictors were ABCC2<sup>−1249G</sup>A (rs2273697), and SLC28A1<sup>−1149A</sup>G (rs2305367). The three most important predictors for each drug are listed in Table 6.

**DISCUSSION**

In this study, we investigated the effects of aging and gene polymorphisms on key PK parameters of five ARVs. Some participant characteristics were significant predictors after adjusting for genetic factors, demonstrating the importance and potential of accounting for PGx in PK analysis. The results from the interaction analysis brought to light interactions between genotype and aging (both chronological and cellular p16<sup>NK4a</sup> expression). The multiple analyses methods used reinforced several of our findings.

Our analysis showed that increased apparent clearance of unbound ATV is associated with ABCC4<sup>−38T</sup>C. ATV is highly metabolized in the liver; this variant might lead to decreased efflux<sup>32</sup> of ATV out of the hepatic cells, thus increased ATV metabolism due to accumulation. There is a nominal association with rs4149057 in SLC01B1, the gene that also showed marginal significance in a genomewide association study of ATV PK.<sup>33</sup> Possibly due to limited

| Table 2 | The P values in the univariate analysis |
| Factors | ATV CL<sub>u/F</sub> | RTV CL<sub>u/F</sub> | EFV CL<sub>u/F</sub> |
| Age | 0.74 | 0.64 | 0.55 |
| p16 expression | 0.20 | 0.43 | 0.57 |
| Race | 0.40 | 0.95 | 0.22 |
| BMI | 0.66 | 0.025<sup>4</sup> | 0.64 |
| Sex | 0.10 | 0.75 | 0.07 |
| Frailty status | 0.074 | 0.21 | 0.69 |
| CrCL | – | – | – |
| Treatment arm | – | – | – |
| SNP | SLCO1B1<sup>c</sup>.571T<sup>c</sup>-C: 0.036 | ABCC4<sup>c</sup>.879T<sup>c</sup>-C: 0.040 | CYP2B6<sup>c</sup>−15631G<sup>c</sup>-T: 0.00025 |
| | | | ABCB4<sup>c</sup>.3435C<sup>c</sup>-T: 0.001 |
| | | | CYP1A2<sup>c</sup>.3860G<sup>c</sup>-A: 0.011 |
| | | | CYP2B6<sup>c</sup>−14593C<sup>c</sup>-G: 0.047 |
| Factors | TFV CL<sub>F</sub> | TFV-DP CL<sub>in</sub> | FTC CL<sub>F</sub> | FTC-TP CL<sub>out</sub> |
| Age | <0.001<sup>**</sup> | 0.43 | <0.001<sup>**</sup> | 0.50 |
| p16 expression | 0.78 | 0.86 | 0.020<sup>**</sup> | <0.001<sup>***</sup> |
| Race | 0.34 | 0.49 | 0.14 | 0.47 |
| BMI | 0.11 | 0.26 | 0.19 | 0.83 |
| Sex | 0.20 | 0.17 | 0.54 | 0.23 |
| Frailty status | 0.58 | 0.30 | 0.11 | 0.22 |
| CrCL | <0.001<sup>***</sup> | 0.28 | <0.001<sup>***</sup> | 0.63 |
| Treatment arm | 0.11 | 0.28 | 0.15 | 0.86 |
| SNP | ABCB1<sup>c</sup>.1297T<sup>c</sup>-C: 0.013 | ABCC1<sup>c</sup>.1725+38A<sup>c</sup>-G<sup>c</sup>: 0.0064 | SLC22A2<sup>c</sup>.3561T<sup>c</sup>-G<sup>c</sup>: 0.038 | ABCC2<sup>c</sup>.1249G<sup>c</sup>-A: 0.0046 |
| | SLC28A1<sup>c</sup>.1561G<sup>c</sup>-A: 0.015 | SLC22A2<sup>c</sup>.734G<sup>c</sup>-C<sup>c</sup>: 0.0900 | SLC22A2<sup>c</sup>.1506G<sup>c</sup>-A: 0.015 | SLC28A1<sup>c</sup>.1149A<sup>c</sup>-G: 0.0038 |
| | SLC28A2<sup>c</sup>.531T<sup>c</sup>-C: 0.0060 | ABCC2<sup>c</sup>.3561T<sup>c</sup>-G<sup>c</sup>: 0.038 | ABCC2<sup>c</sup>.4488C<sup>c</sup>-T: 0.020 |

<sup>ATV</sup>, atazanavir; <sup>BM</sup>I, body mass index; <sup>CL</sup> F, apparent clearance; <sup>CL</sup> in, clearance into the cell; <sup>CL</sup> out, clearance out of the cell; <sup>CL</sup> u/F, apparent unbound drug clearance; <sup>CrCL</sup>, creatinine clearance; <sup>EFV</sup>, efavirenz; <sup>F</sup>, undetermined bioavailability; <sup>FTC</sup>, emtricitabine; <sup>FTC-TP</sup>, emtricitabine triphosphate; <sup>RTV</sup>, ritonavir; <sup>SNP</sup>, single-nucleotide polymorphism; <sup>TFV</sup>, tenofovir; <sup>TFV-DP</sup>, tenofovir diphosphate.

<sup>**</sup> <i>P</i> < 0.05; <sup>***</sup> <i>P</i> < 0.001.
| Studied drug | dbSNP RS | Associated gene/protein | c. | Base change | Function | Genotype frequency | Phenotype | Previously reported phenotype | Physiological reasonable? | Physiological reason |
|--------------|----------|-------------------------|----|-------------|----------|-------------------|-----------|-------------------------------|--------------------------|----------------------|
| ATV          | rs1059751 | ABCC4/MRP4              | 13 | 879T>C      | 3'-UTR   | T/T-T/C: 23 (82%) C/C: 2 (8%) | ↑ CL/F     | Decreased function            | Y                        | Decreased efflux from liver to system |
| RTV          | rs1045642 | ABCB1/P-gp              | 7  | 3435C>T     | I145I    | C/C: 14 (66%) C/T-T/T: 11 (44%) | ↓ CL/F     | Decreased expression          | Y                        | Increased bio-availability |
| EFV          | rs3745274 | CYP2B6/CYP2B6           | 19 | 15631G>T    | Q72H     | G/G+G/T: 41 (84%) T/T: 6 (12%) Missing: 2 (4%) | ↓ CL/F     | 6, a decreased function       | Y                        | Decreased metabolism |
| TFV          | rs3213619 | ABCB1/P-gp              | 7  | -129T>C     | 5'-UTR   | T/T: 61 (82%) T/C: 13 (18%) | ↓ CL/F     | Decreased function            | Y                        | Increased bio-availability |
| TFV-DP       | rs2235013 | ABCB1/P-gp              | 7  | 1725-38G>A  | Intrinsic | G/G: 24 (27%) G/A+G/A: 50 (73%) | ↓ CLin     | Decreased function            | N°                       | Presumably decreased cellular uptake |
|              | rs10519020| SLC28A2/CNT2            | 15 | 734G>C      | S245T    | G/G+G/C: 72 (97%) C/C: 2 (3%) | ↑ CLin     | Decreased function            | N°                       | Decreased cellular uptake |
|              | rs17222723| ABCC2/MRP2              | 10 | 3563T>A     | V1188E   | T/T: 64 (86%) T/A: 10 (14%) | ↑ CLin     | Decreased function            | Y                        | Decreased efflux |

(Continued)
| Gene/protein | Base change | Function | Genotype frequency | Physiological reason | Physiological phenotype |
|-------------|-------------|----------|--------------------|----------------------|------------------------|
| FTC         | rs668871    | FT/C     | CT+1/T: 7%         | Increased expression | CL/F Increased excretion |
| SLC22A3     | 6360C>T     | OCT3     | G/G: 77%           |                        |                        |
|             |             |          | C/T: 23%           |                        |                        |
|             |             |          | G: 81%             |                        |                        |
|             |             |          | C: 19%             |                        |                        |
|             |             |          | Newborn            | Absent                |                        |
|             |             |          | Adult              | Increased             |                        |
|             |             |          |                   | renal                  |                        |
|             |             |          |                   | excretion              |                        |
|             |             |          |                   |                       |                        |
| RTV         | rs316003    | OCT2     | AG: 29%            |                        | CL/F Decreased function |
|             |             |          | C/G: 48%           |                        |                        |
|             |             |          | C/C: 23%           |                        |                        |
|             |             |          | T/G: 15%           |                        |                        |
|             |             |          | Missing: 5%        |                        |                        |
|             |             |          |                   |                       |                        |
| ABCC2       | rs8187707   | MRP2     | TC: 94%            | Decreased function     | CL/F Decreased function |
|             |             |          | C/C: 92%           |                        |                        |
|             |             |          | T/T: 8%            |                        |                        |
|             |             |          | Missing: 1%        |                        |                        |
|             |             |          |                   |                       |                        |
| ABCC2       | rs2273697   | FTC-TP   | G/G: 61%           | Increased function     | CLout/F Increased efflux |
|             |             |          | A/A: 32%           |                        |                        |
|             |             |          | A/G: 5%            |                        |                        |
|             |             |          | Missing: 2%        |                        |                        |
|             |             |          |                   |                       |                        |
| SLC28A1     | 1149A>C     | CNT1     | G/G: 36%           | Decreased           | CL/F Decreased function |
|             |             |          | G/C: 36%           |                       |                        |
|             |             |          | C/C: 28%           |                       |                        |
|             |             |          | Missing: 1%        |                       |                        |
|             |             |          |                   |                       |                        |

**Legend:**
- **FTC:** Emtricitabine
- **ATV:** Atazanavir
- **RTV:** Ritonavir
- **EFV:** Efavirenz
- **TFV:** Tenofovir
- **rs:** dbSNP reference single-nucleotide polymorphism ID
- **CL/F:** Apparent clearance
- **CLout/F:** Clearance out of the cell
- **Clin:** Clearance into the cell
- **CLin:** Clearance into the cell
- **CLout:** Clearance out of the cell
- **Pg:** P-glycoprotein
- **P-gp:** P-glycoprotein
- **MRP:** Multidrug resistance protein
- **OATP:** Organic anion-transporting polypeptide
- **UGT1A1:** UDP-glucuronosyltransferase-1A1
- **CDK2:** Cyclin-dependent kinase 2
- **p16INK4a:** Inhibitor of cyclin-dependent kinase 4

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Sample size, our study was not able to detect the UGT1A1 genotype, such as UGT1A1 *28, as a significant predictor of ATV PK.

The PGx profile of RTV is poorly studied. In our analysis, we found that *ABCB1* c.3435C>T is significantly associated with decreased CL/F, as previously reported. RTV is a substrate of P-gp; the presence of variant might increase the bioavailability of RTV, thus decreases the CL/F. In the univariate analysis, CYP1A2*1C-3860G>A (CYP1A2 *1C, decreased protein function) is associated with decreased RTV CL/F, whereas CYP2B6 14593C>G (CYP2B6 *1C, conflicting protein function) shows the opposite effect. BMI remains a significant predictor and is associated with higher RTV CL/F. Additionally, we found that race plays a role, with African Americans showing lower RTV clearance than other races. However, the effect of “other” races is not interpretable due to the lack of information of the actual racial background. We found no association between race and the three SNPs mentioned above (data not shown), implying that race might predict RTV CL/F independent of these three SNPs. Our finding suggests that RTV drug disposition is influenced by several factors in a mixed manner, potentially explaining the RTV observed high-PK variability.

Our analyses replicated the demonstrated effect of CYP2B6 15631G>T (rs3745274) to decrease EFV clearance. In addition, we found *ABCC4* c.3348A>G and *ABCC4* c.311G>A, both of which decrease MRP4 function, were associated with decreased EFV CL/F. This might be due to increased bioavailability from MRP4 dysfunction. Interestingly, we found that after accounting for these SNPs, decreased EFV CL/F was significantly related to higher p16INK4a expression, suggesting that elimination of EFV is associated with cellular aging. The underlying mechanism still needs to be elucidated, although previous work demonstrated that the expression of CYP2B6 is negatively regulated through a CDK2 signaling pathway involving p16INK4a in a hepatic carcinoma cell line, HepG2. Our analysis shows that EFV CL/F decreases by 31% of the median with twofold increase in p16INK4a expression. Further analysis with larger sample size is needed to validate this finding and inform the potential application of this biomarker to dosing guidance in older HIV-infected patients.

The significant SNPs in the univariate analysis independently predict TFV and FTC CL/F, given that all of them are retained in the final model. We found that TFV CL/F is associated with three variants on P-gp, CNT1, and CNT2, respectively. The ABCB1 c.1297C>T (rs3213619) is shown to decrease CL/F, which might be associated with increased bioavailability through the gut due to decreased P-gp function. The SLC28A1 c.1561G>A (rs2242046) also decreases CL/F, and this may be related to the decreased renal excretion of TFV resulting in increased CNT1 function, which transports more drugs back to the nephrons. There is no information about SLC28A2 c.531T>C (rs8023604) function in the literature, but we hypothesize this SNP might decrease CNT2 function, in turn increasing drug excretion to the urine. The three SNPs associated with FTC elimination all increase CL/F. The SLC22A3 c.360C>T might be associated with increased OCT3 expression, which increases renal excretion of drugs. The *ABCC2* c.4488C>T
Cellular uptake of TFV-DP (CL\text{in}) is significantly associated with both TFV and FTC CL/F, and, expectedly, the effect of age was eliminated in multivariate analysis, due to the collinearity of the two variables. However, p16\text{INK}4a expression was significantly associated with FTC CL/F, but not TFV CL/F; potentially, pathways sensitive to cellular aging might play a role in FTC elimination.

Cellular uptake of TFV-DP (CL\text{in}) is significantly associated with three SNPs on P-gp, CNT2, and MRP2 genes, respectively. TFV is not a substrate of P-gp, thus this association might be due to the bioavailability of tenofovir disoproxil fumarate, the prodrug of TFV, during the absorption phase rather than the cellular distribution. The SLC28A2_c.734G>C (rs10519020) is shown to increase TFV-DP CL\text{in}, implying this SNP may increase CNT2 function. However, there is no known information about this SNP, and the function prediction is the opposite of our finding. The ABCC2_c.3563T>A (rs17222723) is related to increased CL\text{in}, which makes some sense given that this SNP is associated with decreased function of MRP2.

ATV, atazanavir; BMI, body mass index; c., chromosome number; CL/F, apparent clearance; CL\text{in}, clearance into the cell; CL\text{out}, clearance out of the cell; CL\text{u/F}, apparent unbound drug clearance; CNT, concentrative nucleoside transporter; CrCL, creatinine clearance; EFV, efavirenz; F, undetermined bioavailability; FTC, emtricitabine; FTC-TP, emtricitabine triphosphate; MRP, multidrug resistance protein; OCT, organic cation transporter; P-gp, P-glycoprotein; PK, pharmacokinetic; RTV, ritonavir; TFV, tenofovir; TFV-DP, tenofovir diphosphate.

Note: Only factors that are retained in the final linear regression model are shown in this table. Drug clearance was modeled on the log scale. The expression of p16\text{INK}4a is presented as logarithm (base 2) of gene expression. Protein names related to the genes are presented in the parentheses.
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Table 5 Aging-PGx interaction-focused linear regression analysis

| PK parameters | Interaction model with | Factor | P value | Q value | Effect size |
|---------------|------------------------|--------|---------|---------|-------------|
| ATV CL/F      | Age                    | ABCC4.c.*879T>C | 3.60E-02 | 4.02E-02 | -1.33E+03   |
|               | Age:ABCC4.c.*879T>C   | 2.07E-02 | 2.77E-02 | 3.29E+01 |
|               | p16Rks4a expression    | p16Rks4a expression:ABCC4.c.*879T>C | 1.44E-02 | 2.28E-02 | 1.18E+02   |
| RTV CL/F      | Age                    | ABCB1.c.3345C>T | 3.47E-03 | 7.32E-03 | -6.29E+02   |
|               | CYP2B6*1C,14593C>G     | 5.75E-03 | 1.03E-02 | 6.83E+02 |            |
|               | p16Rks4a expression    | ABCB1.c.3345C>T | 1.63E-03 | 6.18E-03 | -6.99E+02   |
|               | CYP2B6*1C,14593C>G     | 2.07E-02 | 2.62E-02 | 5.71E+02 |            |
| EFV CL/F      | Age                    | CYP2B6*16531G>T | 3.77E-04 | 1.19E-03 | -6.92E-01   |
|               | ABCC4.c.*38T>G         | 4.77E-04 | 1.29E-03 | -8.73E-01 |
|               | ABCC4.c.3345A>G/Del    | 2.58E-02 | 3.06E-02 | -4.94E+01 |
|               | p16Rks4a expression    | p16Rks4a expression | 6.60E-03 | 1.27E-02 | -2.41E+01   |
|               | CYP2B6*16531G>T        | 3.04E-04 | 1.44E-03 | -6.86E-01 |
|               | ABCC4.c.*38T>G         | 2.48E-04 | 1.44E-03 | -8.66E-01 |
|               | ABCC4.c.3345A>G/Del    | 2.79E-03 | 6.74E-03 | -8.16E-01 |
| TFV CL/F      | Age                    | SLC28A2.c.531T>C | 2.55E-07 | 1.62E-06 | -1.11E+00   |
|               | SLC28A1.c.1561G>A      | 1.63E-02 | 2.38E-02 | -1.47E+01 |
|               | p16Rks4a expression    | SLC28A2.c.531T>C | 3.94E-02 | 4.86E-02 | 1.34E+01   |
|               | SLC28A1.c.1561G>A      | 1.63E-02 | 2.38E-02 | -1.47E+01 |
| TFV-DP CLin   | Age                    | SLC28A2.c.734G>C | 8.28E-10 | 1.57E-08 | 3.58E-02   |
|               | Age: SLC28A2.c.734G>C  | 8.45E-09 | 8.03E-08 | -6.20E-04 |
|               | ABCB1.c.1725T>38G>A    | 2.19E-02 | 2.77E-02 | -3.89E-04 |
|               | p16Rks4a expression    | SLC28A2.c.734G>C | 3.29E-11 | 6.26E-10 | 1.68E-02   |
|               | p16Rks4a expression: SLC28A2.c.734G>C | 4.99E-09 | 4.74E-08 | -6.14E-03 |
| FTC CL/F      | Age                    | ABCC2.c.4488C>T | 2.08E-04 | 7.91E-04 | -1.65E-01   |
|               | SLC22A2.c.1506A>G      | 1.45E-04 | 6.90E-04 | 6.29E+00  |
|               | SLC22A1.c.360G>T/G     | 2.45E-03 | 5.83E-03 | 3.72E+00  |
|               | p16Rks4a expression    | p16Rks4a expression: ABCC2.c.4488C>T | 1.86E-02 | 2.52E-02 | -1.81E+00   |
|               | ABCC2.c.4488C>T        | 2.17E-03 | 6.74E-03 | 6.32E+00  |
|               | SLC22A2.c.1506A>G      | 2.84E-03 | 6.74E-03 | 4.00E+00  |
|               | SLC22A1.c.360G>T/G     | 9.33E-03 | 1.61E-02 | 3.65E+00  |
| FTC-TP CLout  | Age                    | ABCC2.c.1249G>A | 1.63E-02 | 2.38E-02 | 1.13E-02   |
|               | p16Rks4a expression    | p16Rks4a expression: ABCC2.c.1249G>A | 6.96E-03 | 1.27E-02 | 9.23E+03   |

ATV, atazanavir; c., chromosome number; CL/F, apparent clearance; CLin, clearance into the cell; CLout, clearance out of the cell; CL/F, apparent unbound drug clearance; EFV, efavirenz; F, undetermined bioavailability; FTC, emtricitabine; FTC-TP, emtricitabine triphosphate; PGx, pharmacogenetics; RTV, ritonavir; TFV, tenofovir; TFV-DP, tenofovir diprophosphate.

The P values, Q values, and effect sizes shown from interaction models containing gene polymorphisms (single-nucleotide polymorphisms [SNPs]) and age as factors or SNPs and p16INK4a expression as factors. Only factors that were significant after a multiple testing correction at the false discovery rate of q < 0.1 are shown. Drug clearance was modeled on the log scale for drug EFV. The expression of p16INK4a is presented as logarithm (base 2) of gene expression. A colon between two variables indicates interaction between the two.

Two individuals with the SLC28A2 variant in the cohort, limiting the power of the current study. Other demographic variables were not included in the linear regression models, due to the small sample size. Hence, we performed Random Forest analysis that included all the available variables. Random Forest has been shown to have improved power over regression approaches to detect interactions in genetic association studies.40 However, due to the large number of predictors as compared with the limited sample size, the interaction effects of aging and polymorphisms may have been suppressed by other, stronger predictors in the Random Forest analysis.

Our study has some limitations. First, the small sample size, especially the ATV/RTV treatment arm, has limited our conclusions. We might have ruled out some important SNPs because of the low allele frequency. The low numbers of participants also compromised the distribution of some studied factors and the statistical power of our analysis; particularly, we lack participants with the frailty phenotype, limiting our ability to investigate frailty. Second, due to the large number of variables, we conducted the interaction analysis only involving aging (both chronological and cellular) and genetic factors, potentially excluding the interaction of other demographic factors with genetic factors. The interaction analyses were limited by low numbers of participants also compromised the distribution of some studied factors and the statistical power of our analysis; particularly, we lack participants with the frailty phenotype, limiting our ability to investigate frailty. Second, due to the large number of variables, we conducted the interaction analysis only involving aging (both chronological and cellular) and genetic factors, potentially excluding the interaction of other demographic factors with genetic factors. The interaction analyses were limited by low numbers of the minor alleles for the SNPs studied. The estimated effect sizes should be used to motivate well-powered follow-up study.
Drug clearance was modeled on the log scale for drug EFV. The expression of ritonavir; TFV, tenofovir; TFV-DP, tenofovir diphosphate; P-gp, P-glycoprotein; PK, pharmacokinetic; RTV, efavirenz; F, undetermined bioavailability; FTC, emtricitabine; FTC-TP, emtricitabine transporter; CrCL, creatinine clearance; CYP, cytochrome P450; EFV, etravirine; BMI, body mass index; c., chromosome number; CL/F, apparent clearance; CLint, clearance into the cell; CLout, clearance out of the cell; Clp, apparent unbound drug clearance; CNT, concentative nucleo-side transporter; CrCL, creatinine clearance; CYP, cytochrome P450; EFV, efavirenz; F, undetermined bioavailability; FTC, emtricitabine; FTC-TP, emtricitabine triphosphate; MRP, multidrug resistance protein; OATP, organic anion-transporting polypeptide; P-gp, P-glycoprotein; PK, pharmacokinetic; RTV, ritonavir; TFV, tenofovir; TFV-DP, tenofovir diphosphate.

Drug clearance was modeled on the log scale for drug EFV. The expression of p16INK4a is presented as logarithm (base 2) of gene expression. Protein names related to the genes are presented in the parentheses.

Our study introduces new markers to better understand the pharmacokinetics of ARVs, and facilitate further studies with focus on precision treatments, especially those containing drugs with similar disposition profiles with our studied ARVs, for the growing older HIV-infected population.

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Conflict of Interest. The authors declared no conflict of interest.

Table 6 Top three predictors for drug PK in the Random Forest analysis

| PK parameters | Top three predictors |
|---------------|---------------------|
| ATV CL/F      | ABCC4_c:879T>C      (MRP4) |
|               | SLC01B1_c:571T>C    (OATP1B1) |
|               | Sex                 |
| RTV CL/F      | ABCB1_c:3435C>T     (P-gp) |
|               | CYP1A2*1C>3880G>A   (CYP1A2) |
|               | BMI                 |
| EFV CL/F      | CYP2B6*6, 15631G>T  (CYP2B6) |
|               | ABCB4_c:3348A>G     (MRP4) |
|               | ABCB4_c:311G>A      (MRP4) |
| TFV CL/F      | SLC28A1_c:1581G>A  (CNT1) |
|               | Age                 |
|               | CrCL                |
| TFV-DP CLin   | ABCB1_c:1725+38G>A  (P-gp) |
|               | Sex                 |
|               | BMI                 |
| FTC CL/F      | Age                 |
|               | CrCL                |
|               | p16INK4a expression |
| FTC-TP CLout  | ABCB2_c:1249G>A    (MRP2) |
|               | SLC28A1_c:1149A>G   (CNT1) |
|               | p16INK4a expression |

ATV, atazanavir; BMI, body mass index; c., chromosome number; CL/F, apparent clearance; CLint, clearance into the cell; CLout, clearance out of the cell; Clp, apparent unbound drug clearance; CNT, concentative nucleoside transporter; CrCL, creatinine clearance; CYP, cytochrome P450; EFV, efavirenz; F, undetermined bioavailability; FTC, emtricitabine; FTC-TP, emtricitabine triphosphate; MRP, multidrug resistance protein; OATP, organic anion-transporting polypeptide; P-gp, P-glycoprotein; PK, pharmacokinetic; RTV, ritonavir; TFV, tenofovir; TFV-DP, tenofovir diphosphate.

Drug clearance was modeled on the log scale for drug EFV. The expression of p16INK4a is presented as logarithm (base 2) of gene expression. Protein names related to the genes are presented in the parentheses.
18. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 599–610 (2007).
19. Howie, B.N., Donnelly, P. & Marchini, J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet. 5, e1000529 (2009).
20. Howie, B., Marchini, J. & Stephens, M. Genotype imputation with thousands of genomes. G2 (Bethesda) 1, 457–470 (2011).
21. R Development Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria (2016).
22. Liaw, A. & Wiener, M. Classification and regression by randomForest. R News 2, 18–22 (2002).
23. Chelala, C., Khan, A. & Lemoine, N.R. SNPnexus: a web database for functional annotation of newly discovered and public domain single nucleotide polymorphisms. Bioinformatics 25, 655–661 (2009).
24. Dayem Ullah, A.Z., Lemoine, N.R. & Chelala, C. SNPnexus: a web server for functional annotation of novel and publicly known genetic variants (2012 update). Nucleic Acids Res. 40 (Web Server issue), W65–W70 (2012).
25. Dayem Ullah, A.Z., Lemoine, N.R. & Chelala, C. A practical guide for the functional annotation of genetic variations using SNPnexus. Brief Bioinform. 14, 437–447 (2013).
26. Ward, L.D. & Kellis, M. HaplReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 40 (Database issue) D930–D934 (2012).
27. Holzinger, E.R. et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. Pharmacogenet. Genomics 22, 585–587 (2012).
28. Holzinger, E.R. et al. Functional and genetic diversity in the concentrative nucleoside transporter, CNT1, in human populations. Pharmacogenomics 13, 110–120 (2013).
29. Elens, L., Yombi, J.C., Lison, D. & Wallemacq, P. Genetic and epigenetic regulation of the organic cation transporter 3, SLC22A3. Brief Bioinform. 14, 1229–1241 (2013).
30. Elens, L., Yombi, J.C., Lison, D. & Wallemacq, P. Genetic and epigenetic regulation of the organic cation transporter 3, SLC22A3. Pharmacogenomics 14, 1598–1597 (2013).
31. Elens, L. et al. Functional defect caused by the 4544G>A SNP in ABCC2: potential impact for drug cellular disposition. Pharmacogenet. Genomics 21, 884–893 (2011).
32. Likononsakul, S. et al. A single-nucleotide polymorphism in ABCC4 is associated with tenofovir-related beta-2-microglobulinuria in Thai patients with HIV-1 infection. PLoS One 11, e0147724 (2016).
33. Johnson, D.H. et al. Genome-wide association study of atazanavir pharmacokinetics and hyperbilirubinemia in AIDS Clinical Trials Group protocol AS202. Pharmacogenet. Genomics 24, 195–203 (2014).
34. Anderson, P.L. et al. Atazanavir pharmacokinetics in genetically determined CYP3A5 expressors versus non-expressors. J. Antimicrob. Chemother. 64, 1071–1079 (2009).
35. Thorn, C.F., Akilivu, E., Klein, T.E. & Atman, R.B. PharmGKB summary: very important pharmacogenome information for CYP1A2. Pharmacogenet. Genomics 22, 73–77 (2012).
36. Mega, J.L. et al. Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. Circulation 119, 2553–2560 (2009).
37. Kiser, J.J., Aquilante, C.L., Anderson, P.L., King, T.M., Carter, M.L. & Fletcher, C.V. Clinical and genetic determinants of intracellular tenofovir diphosphate concentrations in HIV-infected patients. J. Acquir. Immune Defic. Syndr. 47, 298–303 (2008).
38. Sánchez, A. et al. Population pharmacokinetic/pharmacogenetic model for optimization of efavirenz therapy in Caucasian HIV-infected patients. Antimicrob. Agents Chemother. 55, 5314–5324 (2011).
39. Rungtivaswan, K. et al. Influence of ABC2C and ABC24 polymorphisms on tenofovir plasma concentrations in Thai HIV-infected patients. Antimicrob. Agents Chemother. 59, 3240–3245 (2015).
40. Suganani, J., Osabe, M., Kurosawa, M., Kitamura, N., Ikari, A. & Miwa, M. Induction of UGT1A1 and CYP2B6 by an antimitogenic factor in HepG2 cells is mediated through suppression of cyclin-dependent kinase 2 activity: cell cycle-dependent expression. Drug Metab. Dispos. 38, 177–186 (2010).
41. Abraham, J.E. et al. Replication of genetic polymorphisms reported to be associated with taxane-related sensory neuropathy in patients with early breast cancer treated with paclitaxel. Clin. Cancer Res. 20, 2466–2475 (2014).
42. Gray, J.H. et al. Functional and genetic diversity in the concentrative nucleoside transporter, CNT1, in human populations. Mol. Pharmacol. 65, 512–519 (2004).
43. Ray, A.S. et al. Mechanism of active renal tubular efflux of tenofovir. Antimicrob. Agents Chemother. 50, 3297–3304 (2006).
44. Izzedine, H. et al. Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. J. Infect. Dis. 194, 1461–1469 (2006).
45. Dablin, A. et al. A pharmacogenetic candidate gene study of tenofovir-associated Fanconi syndrome. Pharmacogenet. Genomics 25, 82–92 (2015).
46. Motsinger, A.A., Ritchie, M.D. & Reif, D.M. Novel methods for detecting epistasis in pharmacogenomics studies. Pharmacogenomics 8, 1229–1241 (2007).

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