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No relationship between drug transporter genetic variants and tenofovir plasma concentrations or changes in glomerular filtration rate in HIV-infected adults

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Host genetics of drug transporters located in the renal tubule may contribute towards tenofovir disoproxil fumarate (TDF)-associated kidney disease susceptibility^{1, 2}. Genetic variants in the ATP-binding cassette (ABC) transporters *ABCC2* (1249 G>A) and *ABCC4* (3463A>G) have been associated with a greater decline in creatinine clearance (CrCL) over 96 weeks of TDF-based treatment³. High plasma tenofovir (TFV) trough concentrations have been correlated with a decrease in glomerular filtration rate⁴. A recent study in Thailand reported that tenofovir ‘mid-dose’ plasma concentrations were significantly lower in *ABCC2* -24 CT/TT carriers compared to CC carriers after 24 weeks of TDF/3TC/EFV treatment and independently associated with decreased glomerular rate after 48 weeks⁵. We investigated the association between drug transporter genetic polymorphisms implicated in tenofovir excretion and/or associated nephrotoxicity with TFV plasma concentrations and changes in kidney glomerular filtration rate in antiretroviral naive HIV-infected adults initiating TDF as part of a NNRTI-based regimen.

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This was a retrospective analysis of adults enrolled in an observational cohort study in Thailand [NCT00433030]. Criteria for treatment initiation were: CDC clinical stage B/C or CD4 <250 cells/mm³. Tenofovir-DF was prescribed 300 mg once-daily. Patients were follow-up monthly during the first 3 months of treatment and then 3-monthly thereafter. A single random blood sample was collected at each visit and plasma frozen at -70°C. The exact time of last drug intake and blood draw were recorded. Creatinine clearance was calculated using the Cockcroft–Gault equation to estimate glomerular filtration rate (eGFR). Local institutional review boards approved the protocol and signed informed consent was obtained from all subjects.

Human genomic DNA isolated from EDTA cell pellets stored at -20°C. Ten Single Nucleotide Polymorphisms (SNPs) linked with TFV excretion and/or associated nephrotoxicity were genotyped: ABCC2: -24C>T (rs717620), 1249G>A (rs2273697), 3563 T>A (rs8187694), 3972C>T (rs3740066) and 4544 G>A (rs8187710); ABCC4: 3463 A>G (rs1751034); ABCC10: G>A (rs9349256), 2843 T>C (rs2125739) and SLC22A2: -1604 T>C (rs3127573), G>A, (rs316009). Primers/probes were designed by the TaqMan® “Assays-by-Design®” SNP Genotyping Assay Service (Applied Biosystems, CA, USA) and SNPs identified using Real-time PCR.

Plasma TFV concentrations were measured using a validated reversed-phase high-performance liquid chromatography assay⁶. This assay was internally validated and the average accuracy was 99–102% and precision (inter/intra-assay) was <5% of the coefficient of variation. Population means and variances of TFV pharmacokinetic parameters were estimated using non-linear mixed effects regression (Monolix v4.2, <http://www.lixoft.eu>)⁷. Individual patient characteristics including weight, sex, age, body mass index, serum creatinine and CrCL were evaluated for their inclusion in the model using a stepwise forward inclusion and backward elimination procedure. Validity of the model was evaluated using a visual predictive check. Post-hoc subject specific pharmacokinetic parameters were used to estimate individual TFV trough concentrations (C₂₄) and area-under the concentration time curve (AUC_{0-24h}).

With ~240 subjects, we had 90% power to detect a difference in TFV C₂₄ of 0.02 µg/mL between combined variants (homo- and heterozygous) and homozygosity of the common allele provided at least 6% (~14 subjects) were in the combined variant group (using a two-sided test, with a standard deviation of 0.02 µg/mL). Differences in TFV C₂₄ between variants (homo- and heterozygous) and homozygosity of the common allele were assessed by Wilcoxon rank-sum tests (due to non-normal C₂₄ distribution). To control the false discovery rate (FDR) q-values were determined⁸. CrCL was assumed normally distributed so t-tests were used to compare differences in change in CrCL between variants and homozygosity of the common allele (adjusted for baseline CrCL level). All statistical tests performed were two-sided using STATA (v11.1).

Study Population

238 HIV-infected adults (58% female) were included. At treatment initiation, the median (interquartile range) age was 36 years (31–42), weight 52 kg (48–59), serum creatinine

(SCr) 0.8 mg/dL (0.7–0.9), creatinine clearance (CrCL) 89 mL/min (74–105), HIV-1 RNA viral load 4.8 log₁₀ copies/mL (4.3–5.2) and CD4 count 144 cell/mm³ (91–208). Antiretroviral regimens were either TDF/emtricitabine/efavirenz (n=191, 80%) or TDF/emtricitabine/nevirapine (n=47, 20%).

Tenofovir population pharmacokinetic model

1,123 plasma samples from 238 patients were included. Median (range) number of samples per patient was 5 (1–6) and median time after dose was 13 hours (0.17–26.75). TFV plasma concentrations were best described by a two-compartment model with the absorption and the distribution rate constants equal. Residual variability was described using a proportional error model. For the final model, tenofovir CL/F (L/hr) was estimated using the equation $CL/F = TVCL \times (CrCL/89)^{0.534}$ [TVCL- typical population CL/F value and 89 was the median CrCL in this study]. Population estimates (interindividual variability) for tenofovir CL/F, Vc/F, Q and Vp/F were 51.2 L/hr (0.19), 244 L (1.25), 121 L/hr, and 2430 L, respectively. Proportional residual variability was 0.23. All relative standard errors were <31% and <10% for structural and variability parameters, respectively. The median (IQR) TFV plasma AUC_{0-24h} and C₂₄ were 2.62 (1.72–3.90) µg.hr/mL and 0.064 (0.038–0.14) µg/mL, respectively, after 1 year of treatment. These values were similar after 3 years of treatment.

Transporter Genetic Polymorphisms and TFV Concentrations

Minor Allele Frequency for *ABCC2* -24C>T, 1249 G>A, 3563 T>A, 3972 C>T, and 4544 G>A were 0.200, 0.042, 0.004, 0.227, 0.004, respectively; *ABCC4* 3463 A>G was 0.187; *ABCC10* G>A (rs9349256) and 2843 T>C were 0.498 and 0.076, respectively; and *SLC22A2* -1604 T>C and G>A (rs316009) were 0.095 and 0.034, respectively.

For each SNP, no significant difference in TFV C₂₄ was observed between homozygosity of the common allele, hetero- and homozygous variant genotypes after 1 and 3 years of TDF treatment. Hetero- and homozygous variants were combined and compared to patients with homozygosity of the common allele. However, there was no significant difference in TFV C₂₄ after 1 and 3 years of treatment for any of the SNPs (Figure 1). Weak trends towards lower TFV C₂₄ with *ABCC2* -24 CT/TT variants at 1 year (p=0.06) and higher TFV C₂₄ with *ABCC4* 3463 AG/GG variants at 1 year (p=0.07) were observed but removed with the FDR analysis (q=0.25). No association between TFV AUC_{0-24h} and SNPs were found.

Transporter Genetic Polymorphisms and Changes in Creatinine Clearance

No relationship between the individual SNPs (or combining hetero- or homozygous variants vs. the common allele) and change in CrCL from baseline and after 1 and 3 years of treatment was observed. The WHO recommends TDF/emtricitabine/efavirenz for first line antiretroviral therapy for adults⁹. It is important to identify patients with a higher risk of developing TDF-associated kidney dysfunction. Low body weight was an independent risk factor of renal dysfunction [>25% decrease of eGFR from baseline] in Thai¹⁰ and Japanese patients¹¹. Perhaps it is reasonable to speculate that individuals with lower body weight may be exposed to higher TFV concentrations and consequently at higher risk of renal drug

toxicity. However, the tenofovir AUC_{0-24h} and C₂₄ observed were similar to those reported in non-Asian populations¹². The allele frequency of the SNPs were similar to US and Europe populations, except for *ABCC2* 1249G>A which was lower (4% vs. 22%)^{13, 14}.

We found no significant associations between the drug transporter polymorphisms tested and TFV trough concentrations after 1 and 3 years of treatment. This result is in contrast to a recent study also performed in Thailand which reported significantly lower TFV concentrations in *ABCC2* -24 CT/TT carriers after 24 weeks of TDF/3TC/EFV treatment [CC vs. CT/TT, $p=0.018$]⁵. Although the larger sample size of our cohort (2-fold) may support the lack of an association it is possible that the shorter duration of TDF-treatment and different TFV measurements (i.e. C₂₄ vs mid-dose) may explain this difference. A study in 30 HIV-infected adults in the USA found *ABCC2* -24 CT/TT carriers excreted 19% more TFV than CC genotype and *ABCC4* 3463 A>G carriers had higher tenofovir AUC (↑32%) than AA genotype¹⁴. More data in larger diverse populations are needed to determine the strength of these associations and TFV plasma concentrations.

Also, none of the SNPs assessed were associated with mean change in CrCL after 3 year of TDF-treatment. The *ABCC2* -24 CC genotype was independently associated with decreased glomerular rate after 48 weeks of TDF-treatment (although not at 96 weeks) in a prior study in Thailand⁵. Baseline age, body weight and serum creatinine were similar between studies; however, there was a higher proportion of women and higher baseline CD4 count (114 vs. 42 cells/mm³) in the present study. Different formulas were also used to estimate glomerular filtration (Cockcroft–Gault vs. Modification of Diet in Renal Disease). Other studies have also reported contrasting results regarding the association between transporter polymorphisms and changes in eGFR. *ABCC2* 1249 G>A and *ABCC4* 3463A>G reported to be associated with a greater decline in CrCL over 96 weeks³ were not confirmed in a larger study¹⁵. In these studies concomitant lopinavir/ritonavir use may have been a factor as lopinavir/ritonavir increases TFV exposure, likely via drug-interactions with transporters in the renal tubule^{2, 16}. Transporter genetic polymorphisms may have a better predictive value for kidney dysfunction with ritonavir-boosted protease inhibitors than NNRTI-based regimens.

Overall, transporter polymorphisms implicated to be involved in TFV excretion and/or associated nephrotoxicity were not associated with plasma TFV trough concentrations or changes in glomerular filtration rate after 3 years of a first-line NNRTI-based treatment.

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Figure 1.

Transporter polymorphisms and predicted plasma tenofovir C₂₄ (µg/ml) after 1 and 3 years of treatment. The middle bar indicates the median. Numbers of subjects are indicated below each group. Wilcoxon rank-sum test were used to compare tenofovir C₂₄ between combined variant and homozygosity of the common allele (p-value). Comparisons were adjusted for multiple testing using the method of Benjamini-Hochberg to control for the False Discovery Rate (q-value). Data presented for SNPs with a MAF >0.004.