Pharmacogenomics of Plasma Tenofovir Clearance and Change in Creatinine Clearance

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Introduction

- Tenofovir disoproxyl fumarate (TDF) is a component of recommended first-line regimens for HIV-1 infection
- Some patients on TDF experience declines in creatinine clearance (CrCl)
- Renal tubular secretion of tenofovir is mediated by efflux transporters including ABCC4
- Declines in CrCl associated with HIV-1 protease inhibitors may reflect ABCC4 inhibition
- Replication of associations between single nucleotide polymorphisms (SNPs) and adverse renal effects with TDF-containing regimens is limited

Objectives

- To investigate SNPs associated with plasma tenofovir clearance among patients randomized to tenofovir-containing regimens
- To evaluate the association between SNPs and changes in CrCl among patients randomized to TDF versus abacavir

Study subjects

- In ACTG study A5202, individuals were randomized to once-daily regimens of either TDF/emtricitabine or abacavir/lamivudine, with either open-label atazanavir plus ritonavir, or efavirenz.
- Serum CrCl was determined before entry, at entry, at weeks 4, 8, 16 and 24, and every 12 weeks thereafter

Tenofovir assays, plasma sampling and pharmacokinetic (PK) model development

- PK samples included those collected between weeks 4 and 24
- Steady-state plasma concentrations of tenofovir were measured using tandem mass spectrometry detection
- A nonlinear mixed effects model was used to model plasma tenofovir determinations
- Final structural PK model was assessed by successful convergence and goodness-of-fit plots

Genetic polymorphisms

- Genome-wide genotype data were obtained using Illumina Human 1M-Duo
- For tenofovir PK association analyses, we considered 18 PharmGKB SNPs from 11 candidate genes
- For CrCl change analyses, we considered 77 SNPs associated with renal phenotypes in the GWAS Catalog

Imputing genotypes

- Imputation was performed at Dr. Marylyn Ritchie's lab (Penn State University)
- Post-QC data were imputed to 1000 Genomes after converting to genome build 37
- ShapeIt2 was used to check strand alignment and to phase data
- IMPUTE2 algorithm was used to impute additional genotypes
- Imputed genotypes were included if posterior probabilities > 0.9
- Imputed SNPs were dropped for imputation scores < 0.3, genotyping call rates < 98% and MAF < 0.05

Pharmacokinetic association analyses

- Only subjects randomized to TDF-containing arms who had complete clinical and genotype data were included
- Multivariable linear regression analyses were performed on all subjects and separately by race/ethnicity; adjusted for age, sex, BMI, concomitant antiretroviral (ARV) use, baseline CrCl, self-reported race and first two principal components (PCs), as appropriate
- Meta-analysis of race-specific models was performed
- Known *UGT1A1* SNP associations with bilirubin were tested for QC purposes

CrCl association analyses

- Subjects randomized to TDF or abacavir arms and outcomes within 200 days after randomization were included
- *Time-dependent* CrCl: generalized least squares regression, including all main effects, and two- and three-way interactions of time, treatment arm (TDF or abacavir), and genotype. Adjusted for sex, age, BMI, self-reported race, concomitant ARV, baseline CrCl, and first two PCs
- As above, race/ethnicity-specific models were fit and meta-analysis was performed
- As a sensitivity analysis, we repeated analyses as above, using *6-month CrCl change* as the phenotype

Statistical software and technical aspects

- PLINK version 1.07 and R version 3.0.1
- Smartpca in EIGENSTRAT used to generate PCs to infer and adjust for genetic ancestry
- Random effects p-values reported
- Bonferroni correction to determine significance thresholds

Results

Tenofovir PK association analyses

- From a 2-compartment model, median plasma tenofovir clearance was 46.8 L/hr (IQR 39.7 to 53.7).
- rs12662869 in *SLC17A1* was associated with increased tenofovir clearance in GWAS analysis with imputed SNPs
- No candidate SNPs were associated with tenofovir clearance

CrCl association analyses

- Median change in CrCl from baseline to 6 months was -0.5 ml/min (IQR -10.7 to +10.8) and 2.2 (IQR -9.9 to +13.2) in the TDF and abacavir arms, respectively
- After Bonferroni correction, no SNP was associated with time-dependent CrCl
- In candidate SNP analyses stratified by population, rs3127573 in *SLC22A2* was significantly associated with a positive 6-month CrCl change among African Americans ($P = 3.3x10^{-5}$)

Acknowledgements

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Table. Baseline characteristics of study subjects

Variable	TDF/FTC (n = 501)	ABC/3TC (n = 548)
Male; No. (%)	434 (87)	472 (86)
Self-reported race/ethnicity; n (%)		
White	243 (49)	243 (44)
Black	149 (30)	189 (36)
Hispanic	100 (20)	102 (18)
Other	9 (2)	14 (2)
Concomitant antiretroviral; n (%)		
Atazanavir/r	246 (49)	269 (49)
Efavirenz	255 (51)	279 (51)
Age in years; median (IQR)	39.0 (31.0, 45.0)	38.0 (31.0, 45.0)
BMI in kg/m^2; median (IQR)	24.8 (22.3, 27.9)	24.8 (22.3, 27.8)
Baseline CrCl in ml/min; median (IQR)	116.0 (99.8, 135.5)	116.6 (99.2, 138.0)

TDF/FTC = tenofovir disoproxil fumarate with emtricitabine; ABC/3TC = abacavir with lamivudine.

Figure 1. Data management and QC

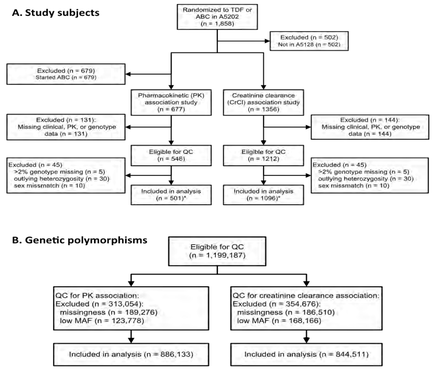


Figure 2. Q-Q plot for SNPs in meta-analysis of plasma tenofovir clearance

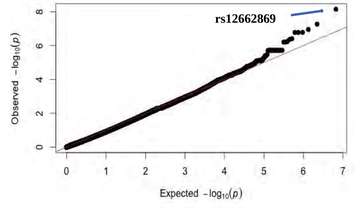
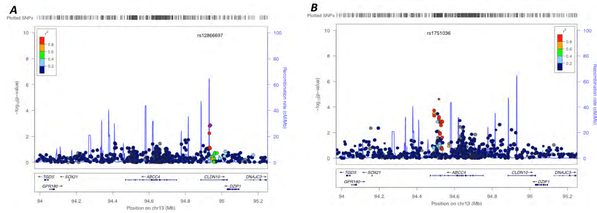


Figure 3. Locus zoom plot of *ABCC4* (±500KB) for tenofovir PK (A) and CrCl change (B) by meta-analysis



Lowest p-value SNPs are represented by purple diamond. ***Panel A*** represents tenofovir PK analyses – the lowest p-value SNP in this region is rs12866697. ***Panel B*** represents change in CrCl analyses – the lowest p-value SNP in this region is rs1751036. The 12 SNPs with the lowest p-values for change in CrCl are rs7330330, rs7331488, rs4148540, rs2766475, rs1678387, rs1678409, rs1678365, rs1189466, rs1751043, rs943289, rs1189435, and rs1189434.

Conclusions

- *SLC17A1* was significantly associated with tenofovir clearance (rs12662869, $P = 7.1x10^{-9}$); additional SNPs in strong LD with rs12662869 were among the top SNPs detected
- No SNPs achieved genome-wide significance ($P < 5.0 \times 10^{-8}$) for association with change in CrCl
- No SNPs were associated in targeted analyses with candidate SNPs
- A SNP near *ABCC4*, rs12866697 located in *CLDN10*, was the top SNP in the *ABCC4* region (± 500 KB)
- The sensitivity analysis of the 212 candidate SNPs identified SNP rs3127573 as significant in the CrCl associations – our results are in the same direction as previous studies that have shown associations of some *SLC22A2* SNPs with renal phenotypes.
- Renal toxicity associated with tenofovir in A5202 was modest, so this analysis did not include extreme phenotypes
- It is possible that other markers of renal tubular function are more affected by genotype
- The potential relevance of *SLC17A1* to tenofovir disposition warrants further study

References

1. The Panel on Clinical Practices for Treatment of HIV Infection. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Available at: http://www.aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. Accessed Updated February 21, 2014.
2. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, Hurley L, et al. Impact of Tenofovir on Renal Function in HIV-Infected Antiretroviral Naive Patients. J Acquir Immune Defic Syndr 2009; **53**:62-9.
3. Ray AS, Cihlar T, Robinson KL, Tong L, Vela JE, Fuller MD, et al. Mechanism of active renal tubular efflux of tenofovir. Antimicrob Agents Chemother 2006; **50**:3297-304.
4. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; **16**:31-41.
5. Haas DW, Wilkinson GR, Kuritzkes DR, Richman DD, Nicotera J, Mahon LF, et al. A multi-investigator/institutional DNA bank for AIDS-related human genetic studies: AACTG Protocol A5128. HIV Clin Trials 2003; **4**:287-300.
6. Pereyra F, Jia X, McLaren PJ, Telenti A, de Bakker PI, Walker BD, et al. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. Science 2010; **330**:1551-7.
7. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; **81**:559-75.
8. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet. **2009**; 5:e1000529.
9. Johnson DH, Venuto C, Ritchie MD, Morse GD, Daar ES, McLaren PJ, Haas DW. Genomewide association study of atazanavir pharmacokinetics and hyperbilirubinemia in AIDS Clinical Trials Group protocol A5202. Pharmacogenet Genomics 2014; **24**: 195-203.
10. Breiderhoff T, Himmerkus N, Stuiver M, Mutig K, Will C, Meij IC, et al. Deletion of claudin-10 (Cldn10) in the thick ascending limb impairs paracellular sodium permeability and leads to hypermagnesemia and nephrocalcinosis. Proc Natl Acad Sci U S A 2012; **109**:14241-6.