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ABCC4 3348 T>C SNP Affects Tenofovir Urinary Output in HIV-positive Patients

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ABSTRACT

Background: Several proteins have been involved in tenofovir transport both in the intestinal lumen and at the proximal tubular renal cells; single nucleotide polymorphisms (SNPs) in *ABCC2* and *ABCC10* have been associated with kidney tubular dysfunction along with tenofovir exposure. No study has so far assessed the influence of genetic profile on urinary and plasmatic tenofovir pharmacokinetic parameters.

Methodology: Adult HIV-positive patients on tenofovir-containing HAARTs since at least six months, presenting estimated creatinine clearance (eCRCL) above 60 ml/min and signing an informed consent were included. Twelve hours tenofovir plasma (pC_{12}) and urinary concentration (uC_{12}) were measured through HPLC/MS-MS methods. SNPs in the following genes were obtained through real-time PCR: *ABCB1, ABCC2, ABCC4, ABCC10, AK1, SLC22A6, SLC29A1, SLC28A2*. Results are expressed as median (IQR); non-parametric tests were used for all analysis.

Results: 194 patients (70.1% male) were enrolled. Age, BMI and eCRCL were 45.2 years (39-51), 23.7 kg/m^2 (21.8-26.0) and 91.7 ml/min (80-109) respectively. Tenofovir pC_{12} and uC_{12} were respectively 68 ng/mL (50-97) and 25722 ng/mL (14437-38598) with a U/P ratio of 398 (230-610). Tenofovir pC_{12} correlated with age (rho=0.21, p=0.03) and eCRCL (rho=-0.22, p=0.002) while uC_{12} with BMI (rho=-0.15, p=0.04); U/P ratio correlated with age (rho=-0.17, p=0.02) and borderline with eCRCL (rho=0.13, p0.07). Urine-to-plasma tenofovir ratio was lower in *ABCC4 3348* (rs1751034) CC carriers [87.5 (58-347) vs. 396 (235-558), p=0.008], in *ABCC10 2759* (rs2125739) TT carriers [386 (214-38) vs. 451 (314-743), p=0.028], in *ABCB1 3435* (rs1045642) TT carriers [311 (150-444) vs. 406 (238-671), p=0.024], in *SLC28A2 124* (rs11854484) CT/TT carriers [386 (214-505) vs. 438 (247-683), p=0.042]. At multivariate linear regression analysis (including BMI, ethnicity, gender, tenofovir duration, PI use, ABCB1 3435 SNPs, ABCC10 2759 SNPs) age (p=0.001, 95CI 3.2-16.4) and *ABCC4 3348* CC genotype (p=0.041, 95CI 14.5-679) were independent predictors of tenofovir urinary output.

Conclusions: Age and single nucleotide polymorphism in the *ABCC4* gene are independent predictors of tenofovir urinary output: this finding confirms the relationship between genetic factors and tenofovir exposure in the suggested mechanism underlying proximal tubular dysfunction. Given the interplay between genetic and pharmacokinetic factors in determining renal tubular damage, treatment tailoring in tenofovir-treated patients deserves further clinical evaluation.

INTRODUCTION

- Tenofovir (TFV) use has been associated with renal tubular damage and, less frequently, with Fanconi's syndrome, acute renal failure and eGFR decline over time;[1]
- Several associated factors have been identified:
  - ✧ pre-existing CKD, low BMI, older age and associated diabetes mellitus, didanosine use;[2,3]
  - ✧ single nucleotide polymorphisms in genes encoding for transporters such as *ABCC2* (MRP2) [rs717620, rs56220353] and *ABCC10* (MRP7) [rs9349256].[4-6]
  - ✧ High TFV plasma concentrations[7-10];
  - ✧ Boosted PIs use (associated with higher TFV exposure trough intestinal p-glycoprotein and MRP4 inhibition);[11-13]
- The suggested mechanism is TFV reduced urinary excretion, followed by "entrapment" in proximal tubular cells with consequent mitochondrial toxicity; nevertheless no study has measured TFV urinary output

AIM OF THE STUDY

To study the demographic, therapeutic and genetic determinants of tenofovir urinary concentrations in HIV-positive patients with normal renal function.

MATERIAL & METHODS:

Prospective, cross-sectional, observational study

Main inclusion criteria:

- Adult HIV-positive patients
- estimated creatinine clearance ≥60 mL/min (Cokroft-Gault equation)
- on tenofovir-containing regimens for ≥6 months
- intake of tenofovir 12±2 hours before sampling

Paired plasma and urinary concentration were measured by a validated HPLC/MS-MS method;

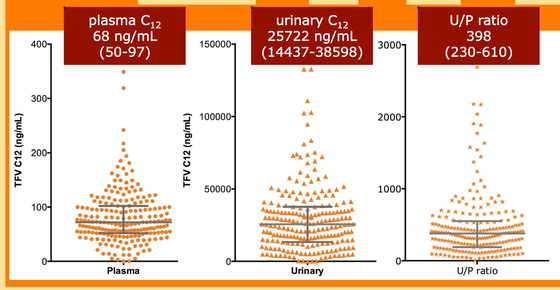
Single Nucleotide Polymorphisms by real-time PCR

- *ABCB1* (P-glycoprotein): rs1045642 (3435C>T)
- *ABCC2* (MRP2): rs717620 (-24 G>A) and rs2273697 (1249 G>A)
- *ABCC4* (MRP4): rs1059751 (*879 T>C) and rs1751034 (3348 T>C)
- *ABCC10* (MRP7): rs2125739 (2759 C>T) and rs9349256 (1791+526 G>A)
- *SLC22A6* (OAT1): rs4149170 (453 G>A)
- *SLC28A2* (CNT2): rs11854484 (124 C>T)
- *SLC29A1* (hENT1): rs760370 (1260-201 A>G)
- *AK1* (AK1): rs1109374 (145+19262 T>C)

Data are expressed as medians (interquartile ranges).

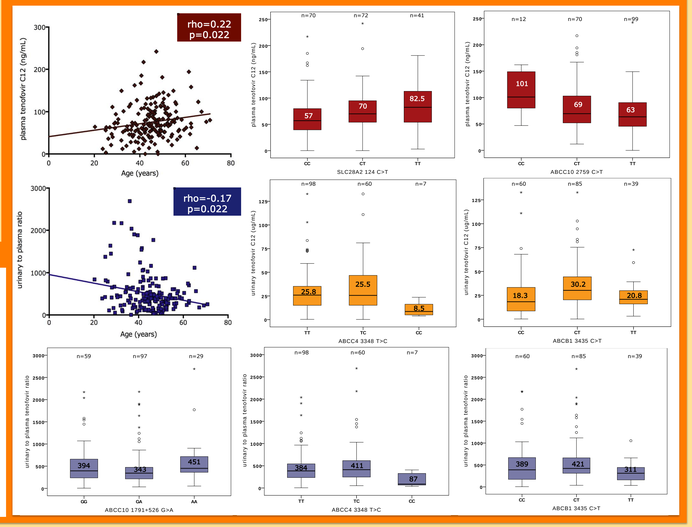
RESULTS (n=194)

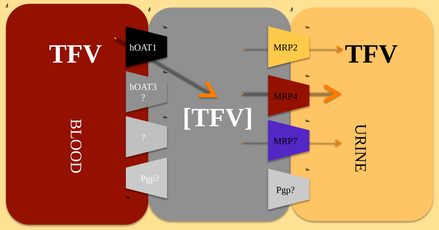
gender (male: n, %)	136	70.1%	HCV-positive n (%)				46	23.7%
ethnicity (Caucasian: n, %)	163	84%	HIV RNA <50 cps/mL n (%)				178	91.8%
age years (med, IQR)	45.3	39- 51	TDF duration months (med, IQR)				51.3	26.2-81.3
CD4 n/uL (med, IQR)	552	435-702	plasma CR mg/dL (med, IQR)				0.96	0.84-1.08
BMI Kg/m^2 (med, IQR)	23.7	21.8-26.1	estimated CR CL ml/min (med, IQR)				91.7	80-109.2
Third drugs PI	80	41.2%	ATV	6.2%	ATV/r	18.5%	LPV/r	6.2%
Third drugs NNRTI	99	51%	DRV/r	9.8%	EFV	40.7%	NVP	10.3%
Third drugs RAL	12	6.2%	ETV	0.5%	fAPV/r	0.5%	MVC	0.5%



Urinary to plasma ratio	Univariate	Multivariate linear logistic regression		
	p	p	B	95 CI
Age	0.022	0.001	-11.6	-18.45 -4.92
Gender	0.357	0.551	-	-
BMI	0.895	0.166	-	-
Ethnicity (Caucasian)	0.566	0.091	-	-
duration of TFV intake	0.541	0.873	-	-
PI use	0.128	0.304	-	-
ABCB1 3435 C>T	0.024	0.095	-	-
ABCC2 -24 G>A	0.793	-	-	-
ABCC2 1249 G>A	0.256	-	-	-
ABCC4 *879 T>C	0.578	-	-	-
ABCC4 3348 T>C	0.019	0.004	3.2	1.02-5.40
ABCC10 1791+526 G>A	0.044	0.320	-	-
ABCC10 2759 C>T	0.139	-	-	-
SLC22A6 453 G>A	0.106	-	-	-
SLC28A2 124 C>T	0.111	-	-	-
SLC29A1 1260-201 A>G	0.745	-	-	-
AK1 145+19262 T>C	0.869	-	-	-

At multivariate linear regression analysis age (p=0.001) and *ABCC4* 3348 single nucleotide polymorphisms (p=0.004) were independent predictors of TFV urinary output.





CONCLUSIONS

✧ We succeeded in measuring urinary TFV concentration collected 12 hours after drug intake: this may be a new pharmacokinetic marker to monitor tenofovir urinary output;

✧ Age and single nucleotide polymorphism in the *ABCC4* gene are independent predictors of TFV urinary excretion: this finding confirms the relationship between genetic factors and tenofovir exposure in the suggested mechanism underlying proximal tubular dysfunction (i.e. TFV "entrapment" in proximal tubular cells) (figure above);

✧ Urinary TFV output (as urinary C12/ plasma C12) is an independent predictor of proximal tubular dysfunction (measured as retinol binding protein to creatinine ratio) (*Calcagno et al, submitted*) and patients with uncommon genotype in MRP4 encoding gene (*ABCC4* 3348 CC) have higher uRBP/uCr ratios (*data not shown*);

✧ Given the interplay between genetic and pharmacokinetic factors in determining renal tubular damage, treatment tailoring in TFV-treated patients deserves further clinical evaluation.

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DISCLOSURES

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