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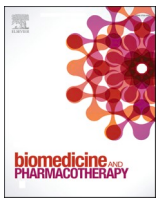

Pharmacogenetics of tenofovir drug transporters in the context of HBV: Is there an impact?

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ABSTRACT

Background: Current treatments for chronic hepatitis B management include orally administered nucleos(t)ide analogues, such as tenofovir (TDF), which is an acyclic adenine nucleotide analogue used both in HBV and human immune deficiency virus (HIV). The course of HBV infection is mainly dependent on viral factors, such as HBV genotypes, immunological features and host genetic variables, but a few data are available in the context of HBV, in particular for polymorphisms of genes encoding proteins involved in drug metabolism and elimination. Consequently, the aim of this study was to evaluate the potential impact of genetic variants on TDF plasma and urine concentrations in patients with HBV, considering the role of HBV genotypes.

Methods: A retrospective cohort study at the Infectious Disease Unit of Amedeo di Savoia Hospital, Torino, Italy, was performed. Pharmacokinetic analyses were performed through liquidi chromatography, whereas pharmacogenetic analyses through real-time PCR.

Findings: Sixty - eight patients were analyzed: *ABCC4* 4976 C>T genetic variant showed an impact on urine TDF drug concentrations ($p = 0.014$). In addition, *SLC22A6* 453 AA was retained in the final regression multivariate model considering factors predicting plasma concentrations, while *ABCC4 4976 TC/CC* was the only predictor of urine concentrations in the univariate model.

Interpretation: In conclusion, this is the first study showing a potential impact of genetic variants on TDF plasma and urine concentrations in the HBV context, but further studies in different and larger cohorts of patients are required.

Research in context:

Evidence before this study:

Different studies evaluated the role of polymorphisms in genes encoding enzymes and transporters on the clinical outcome and toxicity of tenofovir, but particularly in the context of HIV. Instead, a few data are available in the context of tenofovir pharmacogenetics in HBV.

Added value of this study:

This study investigates for the first time the role of some genetic variants in affecting tenofovir exposures both in plasma and urine, suggesting potential genetic predicting factors of sub-therapeutic or toxic levels.

Implications of all the available evidence:

This study highlights potential genetic factors which could be evaluated before starting therapy, in order to identify which patients could have reduced drug concentrations, associated with possible treatment failure, or high drug levels, possibly related to toxicity.

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1. Introduction

Chronic hepatitis B virus (HBV) infection affects approximately 296 million people worldwide, with 1.5 million new infections each year, becoming a major public health problem [1].

In 2019, hepatitis B (HB) caused 820 000 deaths, mostly due to cirrhosis and hepatocellular carcinoma [1].

Chronic HB (CHB) affected-individuals serve as the reservoir for acute infection in susceptible subjects. The prevalence of this source is influenced by ethnicity and geography. Indeed, 70–90 % of the population shows past or current HBV infection serologic markers in the areas of the world with the highest HBV prevalence (e. g. China, Korean and sub-Saharan Africa) [2].

The clinical evidence of acute hepatitis B (AHB) varies according to the age at onset of infection: it is commonly asymptomatic and progresses to chronicity in newborns and young children, while in adults it remains symptomatic, with a fulminant progress in less than 1 % [3–5].

A review considering data between 1965 and 2013 from 161 countries estimated the worldwide prevalence of HB antigen to be 3.61 % [6].

Current treatments for CHB management include orally administered nucleos(t)ide analogues (NAs), and subcutaneous or intramuscolar peginterferon (PEG - IFN). Globally, NAs are the most administered drugs: the first-line therapy should be composed by an oral antiviral such as tenofovir alafenamide (TAF), tenofovir disoproxil fumarate (TDF) or entecavir (ETV) leading to optima virological response, while PEG - IFN has lower efficacy and tolerability.[7].

TDF is an acyclic adenine nucleotide analogue used both in HBV and human immune deficiency virus (HIV) with higher viral suppression, tolerability and histologic improvement. It is a prodrug, and it is quickly absorbed metabolized to tenofovir, which acts by inhibiting the HIV-1 reverse transcriptase enzyme. After oral administration, tenofovir is distributed mainly in the kidney, liver and gut. Basically, it is metabolized in the liver and eliminated through kidney. Once in plasma, tenofovir disposition followed two compartment kinetics and its clearance is 44.7 L/h (40.2;49.5) for a 70 Kg individual [8,9]. Its plasma half life is about 17 h, the maximum concentration is 0.33 ± 0.12 μg/mL and the area under the curve is 3.32 ± 1.37 μg*h/ mL after administering TDF 300 mg dosage [10]. Tenofovir volume of distribution at steady-state is 1.3 ± 0.6 L/Kg and 1.2 ± 0.4 L/Kg, for intravenous administration of 1 mg/Kg and 3 mg/Kg, respectively.

The course of HBV infection is mainly dependent on viral factors, such as HBV genotypes, immunological features, and host genetic variables [11]. In particular, polymorphisms of genes encoding proteins involved in drug metabolism and elimination have been reported to have an impact on TDF concentration in HIV - affected patients, but a few data are available in the context of HBV treatment. Consequently, the aim of this study was to evaluate the potential impact of single nucleotide polymorphisms (SNPs) of genes encoding enzymes and transporters on TDF plasma and urine concentrations in patients with HBV, considering the role of HBV genotypes.

2. Materials and methods

2.1. Characteristics of enrolled patients

A retrospective cohort study at the Infectious Disease Unit of Amedeo di Savoia Hospital, Torino, Italy, ASL Città di Torino, was performed. 68 patients HBeAg positive and negative naïve or experienced treated with TDF were enrolled from March 2015 and June 2019. HIV - coinfected patients were excluded from the study. The study was approved by our local ethics committee (number of protocol 002360, date: January 15th, year: 2015) and it was conducted in agreement with the Helsinki Declaration. All patients involved in the study providing written informed consent for the enrolment.

The following baseline data were collected: age, sex, BMI, geographic origin, HBV genotype (A, B, C, D, E), level of education, probable route of transmission according to medical history (unknown, sexual, IDU, vertical, familiar, iatrogenic) and information about other medications and co - morbidities.

2.2. HBV test

Serum HBV DNA levels were quantified by real-time PCR (COBAS AmpliPrep / COBAS TaqMan HBV Test 2.0, Roche Molecular Systems, NJ, USA). HBV genotyping was performed using INNO-LiPA (Innogenetics, Belgium). HBsAg, HBeAg, and anti - HBe were detected using an Elecsys assay (Roche Diagnostics, Italy), but quantification of the S antigen was instead carried out using an ARCHITECT analyser (Abbott Diagnostics, Ireland) with a range of 0.05–250.0 IU / mL; values of qHBsAg > 250.0 IU / mL were subsequently diluted and retested.

2.3. Pharmacokinetic analysis

Pharmacokinetic analysis was conducted at the through concentration (Ctrough, samples and urine collected before drug administration) at the last visit. Plasma samples were obtained from whole blood after centrifugation at 4 °C, 3000 r.p.m. for 10 min and stored at − 20 °C for pharmacokinetic analysis.

Urines were collected between 08:30 and 10:30 a.m.: patients were instructed to urinate at home and then to wait for sample withdrawal. Urine samples were directly frozen at − 20 °C.

Plasma and urinary concentrations were determined by previous fully validated UHPLC – MS / MS method [12,13].

2.4. Genetic analysis

Genomic DNA extraction was obtained using QIAamp whole blood mini kit (Qiagen, Valencia, CA, USA) according to the instructions of the manufacturer. Genotyping was conducted by real time-based allelic discrimination (BIORAD, Milan, Italy). The following SNP were analysed:

ABCB1 3435 C > T (rs1045642), *ABCC2* - 24 G > A (rs717620), *ABCC2* 1249 G > A (rs2273697), *ABCC4* 4976 T > C (rs1059751), *ABCC4* 3463 T > C (rs1751034), *ABCC10* 1791 + 526 G > A (rs9349256), *SLC22A6* 453 G > A (rs4149170), *SLC28A2* 124 C > T (rs11854484).

2.5. Statistical analysis

IBM SPSS Statistics software 28.0 for Windows (Chicago, Illinois, USA) was used for statistical analysis. Continuous variables are reported as the median with the interquartile range [IQR] from the 25th to the 75th percentile, while categorical variables are reported as frequencies and percentages.

All variables were compared using the Shapiro - Wilk test. Categorical variables were compared using the Mann-Whitney and Kruskal - Wallis tests. Continuous variables were evaluated using Spearman's correlation. Associations were assessed using the $\chi 2$ test. Univariate and multivariate analyses for plasma and urinary TDF levels were performed using a linear regression model. Multivariate analysis was adjusted for the following variables: age, gender, BMI, baseline qHBsAg, HBV DNA baseline, HBV genotype, estimated gomerular filtration rate (eGFR), liver stifness, presence of HBV resistance, and treatment experience.

3. Results

Sixty - eight samples obtained from the recruited patients were analyzed. Patients characteristics regarding gender, age, Body Mass

Index (BMI), geographical origin, HBV infection and treatment were reported in Table 1.

The role of genetic variants encoding enzymes and transporters involved in drug metabolism and elimination was investigated: *ABCC4* 4976 C > T genetic polymorphism had an impact on urine TDF drug concentrations ($p = 0.014$), as reported in Fig. 1.

Demographic, pharmacological, genetic and biochemical factors able to predict TDF plasma and urine concentrations were evaluated in linear regression analyses, as reported in Tables 3 and 4, respectively: *SLC22A6* 453 AA was retained in the final regression multivariate model considering plasma concentrations, while *ABCC4 4976 TC / CC* was the only predictor of urine concentrations in the univariate model.

4. Discussion

Anti-HB drugs include a low genetic barrier of resistance, such as lamivudine, telbivudine, and adefovir and drugs with a high genetic barrier, such as ETV, TDF or TAF [14]. Despite its high genetic barrier and increased probability of efficacy, TDF is associated with major side effects, as kidney failure, hypophosphatemia, osteoporosis with consequent bone fractures [15,16]. Different studies suggested to monitor routinely eGFR and phosphatemia in TDF-treated patients in order to early identify possible impairment. In the last few years, TAF was introduced as a better alternative to TDF for HBV treatment, particularly in older patients, individuals with bone or kidney disease and in HIV co-infected [17]. The therapeutic drug monitoring (TDM) of TDF is generally performed in the clinical management of people living with HIV. The role of plasma and urinary exposure of TDF was associated with renal damage and treatment failure in HIV affected patients [18], but a few no data are available in the context of CHB patients treated with TDF. Consequently, a study evaluated the role of TDF plasma and urinary levels in affecting the clinical outcome in CHB patients [19]. In details, eGFR was 68 mL/min in naïve patients, while in those pre-treated with adefovir dipivoxil was 55.5 mL/min ($p < 0.001$). HBV E genotype was associated with lower TDF levels ($\beta = -0.829$, $p < 0.001$). In particular, this genotype was related to a reduction in HBsAg during treatment with ETV, predicting a longer time to HBsAg loss, compared to A and D genotypes [20]. Consequently, the aim of one of our study was to quantify qHBsAg decline in HBeAg-negative CHB HBV genotype E patients treated with TDF or ETV. Sixty -five West African patients were recruited: 61.5 % was treated with ETV and 38.5 with TDF. Serological and virological response was significantly lower in patients treated with ETV compared to TDF, after 5 years of treatment.

TDF is not a substrate of cytochrome P450 enzymes and its elimination in the proximal renal tubule is managed mostly by membrane transporters. Consequently, clinical pharmacogenomics needs to focus on drug transporters-related genes [21]. For example, the study by Decloedt *et al.* showed that *ABCB1* rs1989830 and *ABCC5* rs11921035

Table 1
Baseline characteristics of study population.

Characteristics of enrolled patients (N = 68)	N (%) or median [IQR]
Therapy Duration (years)	8.7 [7–11]
Age (years)	49.50 [34.25–62.25]
Gender (M)	51 [75]
BMI	24 [22.65–26.38]
Naive	32 (47.1)
Experienced	36 (52.9)
qHBsAg (Log IU / mL)	3.27 [2.61–3.99]
HBV-DNA (Log IU / mL)	2.72 [1.3–5.44]
Basal liver stiffness (KPa)	7 [6–10.43]
Presence of liver cirrhosis	14 (20.6)
ALT (IU / mL)	43.5 [22–81.75]
AST (IU / mL)	33 [22.50–81.75]
eGFR (mL / min)	80.15 [69.25–88.87]
Plasmatic TDM (ng / mL)	45 [34–57.5]
Urinary TDM (ng / mL)	17490 [12307.5–24858]
Urinary TDF/ Plasma TDF ratio	393.66 [254.44–622.99]
Geographical origin N (%)	
Italy	32 (47.1)
Europe, other than Italy	9 (13.3)
Africa	14 (20.6)
China	12 (17.6)
South America	1 (1.5)
HBV genotypes N (%)	
A	8 (11.8)
B	3 (4.4)
C	9 (13.2)
D	37 (54.4)
E	11 (16.2)
Employment status N (%)	
Unemployed	23 (33.8)
Workers	45 (66.2)
Educational level N (%)	
None	8 (36.4)
Junior high school	10 (45.5)
High school	2 (9.1)
University	2 (9.1)
Route of trasmission N (%)	
Sexual	4 (5.9)
Intravenous drug use	6 (8.8)
Perinatal	1 (1.5)
Familiar	17 (25.5)
Health-care associated	7 (10.3)
Unknown	33 (48.5)
HBeAg N (%)	
Positive	24 (35.3)
Negative	44 (67.7)
HDV coinfection N (%)	
IgG positive	4 (5.9)
IgG negative	64 (94.1)

Distribution of allelic frequencies of analyzed genetic variants were reported in Table 2.

Table 2
Allelic frequencies of analyzed genetic variants.

	Homozigous Wild type (%)	Heterozygous (%)	Homozygous Mutant (%)
ABCC2 - 24 G > A	66.7 (GG)	24.6 (GA)	8.7 (AA)
SLC22A6 453 G > A	62.5 (GG)	32.3 (GA)	5.2 (AA)
ABCC2 1249 G > A	57.4 (GG)	39.5 (GA)	3.1 (AA)
ABCC10 1791+526 G > A	29.0 (GG)	49.5 (GA)	21.5 (AA)
ABCB1 3435 C > T	25.6 (CC)	43.6 (CT)	30.8 (TT)
ABCC4 4976 T > C	30.0 (TT)	49.5 (TC)	20.5 (CC)
ABCC4 3463 T > C	60.6 (TT)	27.5 (TC)	11.9 (CC)

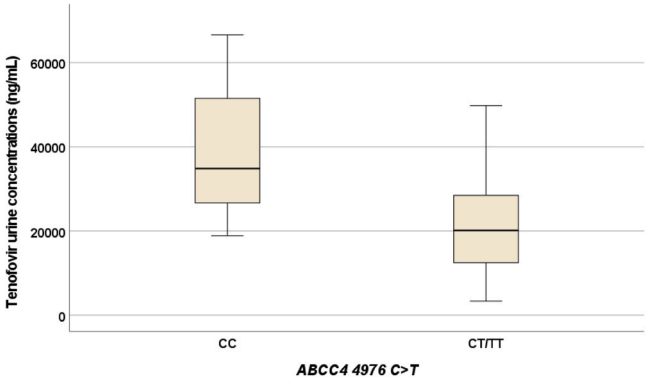

Fig. 1. *ABCC4* 4976 C > T genetic variant impact on urine tenofovir drug concentrations ($p = 0.014$).

Table 3
Logistic regression analysis: factors able to predict tenofovir plasma levels. Bold represents statistically significant values.

	Tenofovir plasma concentrations			
	UNIVARIATE		MULTIVARIATE	
	p VALUE	OR (95 % IC)	*p* VALUE	OR(95 % IC)
Gender (male)	0.487	3.986 (- 7.389; 15.361)		
Age ≥ 60 years	0.257	-6.254 (- 17.164; 4.656)		
Caucasians	0.755	0.461 (- 2.473;3.394)		
Genotype E	0.235	7.980 (- 5.301; 21.262)		
BMI > 25	0.541	3.082 (- 6.935; 13.098)		
Naïve	0.384	-4.318 (- 14.166; 5.530)		
Metavir	0.600	0.987 (- 2.749; 4.724)		
Cirrhosis	**0.043**	**13.985 (0.456; 27.514)**	0.926	0.777 (-16.043;17.598)
ALT baseline	0.960	0.003 (- 0.098; 0.103)		
AST baseline	0.504	-0.010 (- 0.039; 0.019)		
Creatinine baseline	0.250	-8.966 (- 24.449; 6.516)		
eGFR baseline	0.638	0.081 (- 0.261;0.423)		
Stiffness baseline	**0.025**	**0.880 (0.115; 1.645)**	0.088	0.850 (-0.134;1.833)
***ABCC2* 24 GA / AA**	0.787	2.404 (- 15.570; 20.378)		
***SLC22A6* 453 AA**	**0.002**	**67.391 (27.194; 107.589)**	**<0.001**	**69.689 (30.970;108.408)**
ABCC2 1249 AA	0.517	-14.873 (- 60.933;31.987)		
ABCC10 526 AA	0.472	-6.468 (- 24.629;11.592)		
ABCC4 4976 TC / CC	0.527	-5.118 (- 21.380;11.144)		
ABCC4 3463 TC / CC	0.371	7.449 (- 9.271;24.170)		
ABCB1 3435 TC / CC	0.052	- 15.576 (- 31.266;0.114)		

(ABC, encoding ATP-binding cassette, BMI= Body Mass Index, ALT= alanine transaminase, AST= aspartate aminotransferase, eGFR= Estimated Glomerular Filtration Rate).

Table 4
Logistic regression analysis: factors able to predict tenofovir urine levels. Bold represents statistically significant values (ABC, encoding ATP-binding cassette, BMI= Body Mass Index, ALT= alanine transaminase, AST= aspartate aminotransferase, eGFR= Estimated Glomerular Filtration Rate).

	Tenofovir urine concentrations	
	UNIVARIATE	
	p VALUE	OR(95 % IC)
Gender (male)	0.754	1188.667 (- 6353.514; 8730.847)
Age ≥ 60 years	0.486	2545.751 (- 4711.115; 9802.617)
Caucasians	0.383	-848.267 (- 2777.690; 1081.156)
Genotype E	0.197	-5722.517 (- 14485.997; 3040.964)
BMI > 25	0.879	-507.175 (- 7146.845; 6132.495)
Naïve	0.436	2559.198 (- 3950.482; 9076.878)
Metavir	0.862	216.266 (- 2258.672; 2691.204)
Cirrhosis	0.060	8604.007 (- 378.691; 17586.705)
ALT baseline	0.853	6.162 (- 60.101; 72.425)
AST baseline	0.983	-0.214 (- 19.658; 19.230)
Creatinine baseline	0.401	-3890.764 (- 13121.427; 5339.899)
EGFR baseline	0.178	152.150 (- 71.152; 375.453)
Stiffness baseline	0.359	241.827 (- 280.361; 764.016)
***ABCC2* 24 GA / AA**	0.956	-341.033 (- 12890.440; 12208.374)
***SLC22A6* 453 AA**	0.902	2041.371 (- 31372.355; 35455.098)
ABCC2 1249 AA	0.697	- 6305.784 (- 38843.851; 26232.283)
ABCC10 526 AA	0.695	- 2493.233 (- 15268.616; 10282.149)
ABCC4 4976 TC / CC	**< 0.001**	**-18197.232 (- 27924.870; −8469.595)**
ABCC4 3463 CC	0.326	- 5820.727 (- 17701.688; 6060.234)
ABCB1 3435 TC / CC	0.931	- 500.459 (- 12136.126; 1135.209)

were associated with tenofovir cerebrospinal fluid to plasma ratio and emtricitabine, respectively [22], whereas, Rungtivasuwan showed that tenofovir clearance in AG/GG patients for *ABCC4* 3463 is increased of 11 % compared to AA genotype patient [23]. In details, TDF transport in proximal tubular cells is regulated by OAT1, encoded by *SLC22A6* gene, while its secretion into tubular lumen and in urines is managed by MRP - 2 and MRP - 4, which are encoded by *ABCC2* and *ABCC4* genes, respectively [24,25].

Frequent side-effects related to TDF treatment were: phosphaturia, proteinuria and reduced kidney function [25]. Furthermore, factors such as older age, HCV concomitant infection, drug use, female gender, low baseline eGFR, proximal tubular dysfunction, hypertension, cardiovascular diseases, and diabetes were also predictors of chronic kidney disease [26,27]. Furthermore, TDF renal toxicity is influenced by plasma exposure and genetic polymorphisms in genes encoding for transporters.

In fact, in this context, different SNPs have been associated with TDF tubular toxicity [24,28]: for example, *ABCC2* 1249 G > A in French population, *ABCC2* - 24 G > A in Japanese and Spanish populations [29,30]. *ABCC2, ABCC4* and *ABCC10* genes SNP role in influencing plasma or intracellular concentrations were investigated [30–33]. Particularly, the role of SNPs in predicting renal abnormalities in a cohort of HIV-affected patients was performed and, for the first time, SNPs were associated with parathyroid hormone (PTH), phosphate, calcium and tubular dysfunction in people living with HIV [34]. In details, abnormal urinary retinol binding protein (uRBP)/ creatinine (Cr) ratio resulted more frequent in TDF treated patients: eGFR < 90 mL / min and TDF use were predictors in all the population, eGFR < 90 mL/min, TDF concentrations and *CYP24A1* - 3999 TT in patients treated with TDF.

TDF treated patients showed border-line higher PTH levels and, considering the whole population, female gender, non-European ancestry, TDF use, VD levels lower than 30 ng/mL and *SLC28A2* −124 CT / TT and *ABCC2* - 24 CC were predictors of PTH levels in the final regression model [34]. Finally, at the multivariate analysis investigating the factors predicting the urinary to plasma TDF ratio, *ABCC10* GA / AA genotypes and protease inhibitor treatment resulted predictors.

As suggested before, TDF plasma exposure seems to be affected by *SLC28A2* gene (encoding the concentrative nucleoside transporter 2, CNT2): in particular, *SLC28A2* 124 CT / TT genotype was associated with increased tenofovir plasma exposure [35], but another study by our group showed TDF and TAF are not CNT2 substrates [36].

Another study showed *ABCC4* rs899494 and rs1059751 genetic variants were related to eGFR and urinary B2 microglobulin/creatinine, respectively, although with a different trend compared to previous studies. Furthermore, changes in eGFR resulted affected by *COL27A1* SNP [37].

In this article we found that *ABCC4* 4976 TC / CC genotype group is the only factor retained in the univariate linear regression analysis for urine tenofovir concentrations. Concerning this genetic variant, a study showed *ABCC4* 4976 C allele was not associated with the severity of nephrotoxicity in people living with HIV treated with TDF [34]: in fact, Bonferroni correction showed no significant association between this variant and levels of uRBP / Cr, phosphate, PTH or calcium. This seems to be in accordance with what highlighted in this article, with CC genotype patients showing higher TDF urine concentrations. Another article suggested that patients carrying C allele of *ABCC4* 4976 had beta-2-microglobinuria, wherease *ABCC2* 24 C and *ABCC2* 1429 A alleles, which were previously overexpressed in HIV patients taking TDF and showing kidney tubular dysfunction [38], were not present in the beta-2-microglobinuria group [32]. In this article, we found that *SLC22A6* 453 AA is the only predictive factor of plasma concentrations

in the linear regression analysis: it is the first time this genetic variant was associated with tenofovir plasma concentrations in HBV patients. In the literature, this SNP was associated with anti-HIV protease inhibitors concentrations: genotype GG carriers had significantly lower ($p = 0.047$) cerebrospinal fluid (CSF) / plasma ratio compared to GA / AA genotype [39]. In addition, the A allele was not related to nephrotoxicity in TDF - treated patients, although in this study we suggest higher TDF concentrations in AA carriers in the regression analyses [40].

Other articles showed the impact of polymorphisms on other genes (not transporters): for example, a study by Cindi *et al.* reported that IFNL4 rs12979860 and LINC01684 rs9305223 and rs142693425 were associated with tenofovir clearance [41].

It is important to highlight that not all the studies suggest an impact of genetics: in fact, another study by our group evaluated the impact of demographic, pharmacokinetic, and pharmacogenetic factors in affecting TDF discontinuation for renal outcomes in HIV-affected patients. In this study, 304 patients were recruited: after a median time of 28.3 months, 27 patients discontinued TDF for renal toxicity. A higher probability of TDF discontinuation was associated with male gender, older age, no Caucasian ethnicity, absence of intravenous drug abuse, protease inhibitors drugs, indinavir use, HCV-positivity, reduced CD4 cell count, detectable HIV-RNA, lower eCrCl, spot-urine proteinuria) and higher TDF exposure, but no genetic polymorphism had an impact [18].

In conclusion, this is the first study showing a possible role of genetics in TDF plasma and urine exposures in HBV infected patients, showing a possible impact of *SLC22A6* 453 SNP on tenofovir plasma exposure and *ABCC4* 4976 on urine concentrations. Main limits were the small simple size and and the timing of TDF evaluation. Consequently, further studies in larger and different cohorts of patients are required to confirm the potential impact of pharmacogenetics in the context of HBV. In particular, it could be useful to evaluated if these SNPs could have an impact in terms of clinical outcome or toxicity.

CRediT authorship contribution statement

Jessica Cusato: Writing – original draft, Project administration, Investigation, Conceptualization. **Alessandra Manca**: Writing – original draft, Project administration, Investigation, Conceptualization. **Alice Palermiti**: Software. **Jacopo Mula**: Software. **Miriam Antonucci**: Methodology. **Francesco Chiara**: Visualization, Conceptualization. **Amedeo De Nicolò**: Writing – review & editing, Supervision. **Tommaso Lupia**: Data curation. **Giacomo Stroffolini**: Data curation. **Lucio Boglione**: Writing – review & editing, Supervision. **Antonio D'Avolio**: Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Sharing Statement

Data are available within the text. Patient data are available on request due to privacy and ethical restrictions.

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