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Pharmacogenetic Determinants of Kidney-associated Urinary and Serum Abnormalities in Antiretroviral-treated HIV-positive Patients

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Abstract

Tenofovir disoproxyl fumarate (TDF) has been associated with renal tubular abnormalities, phosphaturia and proteinuria (retinol binding protein, RBP, loss): vitamin D (VD) and PTH affect these markers.

Aim was to understand if some single nucleotide polymorphisms (SNPs) were predictors of renal abnormalities in an Italian cohort of HIV-affected patients.DNA was analyzed through real-time PCR, urinary RBP corrected by creatinine (uRBP/Cr).

The majority of patients received TDF. Abnormal uRBP/Cr was more frequent in TDF recipients: eGFR<90 mL/min and TDF were predictors in the whole cohort, whereas eGFR<90 mL/min, TDF concentrations and *CYP24A1*-3999TT in TDF-treated patients. Phosphate levels were higher low VD level patients: age<50 years, *CYP27B1*+2838CC genotype and non European ancestry were predictors. PTH levels were *border-line* higher in TDF patients: non-European ancestry, females, TDF, VD levels<30 ng/mL and *SLC28A2*-124*CT/TT* and *ABCC2*-24CC were predictors.
For the first time, SNPs were associated with PTH, phosphate, calcium and tubular dysfunction in HIV-infected patients.

Keywords: tenofovir; vitamin D; SNP; renal toxicity; HIV; cART.

1. Introduction

Combination antiretroviral therapy (cART) is the combination of three modern antiretroviral drugs and it has greatly reduced morbidity and mortality in HIV-positive patients. Despite this groundbreaking success, persons living with HIV have a significantly reduced health-adjusted life expectancy with premature and serious comorbidities, linked to lifestyle, HIV-related factors and drug-associated toxicities [1].

Tenofovir disoproxyl fumarate (TDF) is a prodrug of tenofovir (TFV), an acyclic nucleotide reverse transcriptase inhibitor active against HIV and HBV [2-4].

TDF is widely used because of its high potency, tolerability and once-daily dosing: TFV has long serum and intracellular half-lives of 17 h and 10–50 h, respectively [5]. It is excreted by the kidney through glomerular filtration and active tubular secretion and dose reduction is required in patients with significant renal impairment. TDF is actively transported in proximal tubular cells by OAT1 (encoded by *SLC22A6* gene); MRP-2 (encoded by *ABCC2* gene) and MRP-4 (encoded by *ABCC4* gene) are responsible for TFV secretion into tubular lumen and in urines [6]. Some drug-drug interactions of clinical significance were reported, such as didanosine ritonavir-boosted and unboosted atazanavir [2, 7].

TDF has been rarely associated with renal impairment and Fanconi's syndrome. More commonly, low bone mineral density and proximal tubular renal toxicity have been reported. The proposed mechanism lies in drug mitochondrial toxicity, since TFV is a mitochondrial DNA polymerase inhibitor. *In vitro* and animal studies suggested that drug accumulation, due to its potential intracellular entrapment, could induce mitochondrial DNA polymerase depletion in proximal tubular cells [8]. Since the source of energy derives from mitochondria, tubular dysfunction is observed, with loss of low weight proteins (β2- microglobulin/ retinol binding protein, RBP), amino acids, glucose, uric acid, bicarbonate and phosphate in urines. Among tubular abnormalities, phosphaturia and proteinuria are the most frequently observed:

reduced tubular phosphate reabsorption is present in 30-50% of TDF treated patients, but other factors, such as vitamin D (VD) and parathyroid hormone (PTH) levels, could affect these markers [9].

Every factor increasing TFV plasma concentration (low weight, female gender, concomitant protease inhibitor intake) or inhibiting its renal excretion (concomitant protease inhibitor or diclofenac administration) could lead to increased toxicity risk [10-13].

In fact, every year of TDF exposure is associated with increased proteinuria, chronic disease and rapid decline in kidney function [14]. Other factors involved in TDF-associated renal toxicity are several: chronic kidney disease was predicted by older age, intravenous drug use, HCV concomitant infection, female gender, lower nadir CD4+ cells, lower baseline eGFR, proximal tubular dysfunction, hypertension, diabetes and cardiovascular diseases [15-17].

In this context, single nucleotide polymorphisms (SNPs) in different genes have been related to TFV-associated tubular toxicity [18 6]. Several evidences pointed out genetic variants affecting TFV-associated kidney toxicity: for example, *ABCC2* 1249 G>A in French population, *ABCC2-24* G>A in Japanese and Spanish ones [19, 20]. The CATC *ABCC2* haplotype was assessed, but conflicting results were found: one study identified association, but another did not confirm it; results were opposite probably due to the different used renal toxicity definition [19, 21]. Furthermore, other works investigated *ABCC2, ABCC4* and *ABCC10* gene SNP role in influencing plasma or intracellular concentrations [20, 22-24].

Salvaggio *et al.* showed increased prevalence of GG genotype of *ABCC4* 3348 SNP in patients treated with TDF and showing KTD; but this was demonstrated only in the univariate analysis, but not confirmed in the multivariate one [25].

In addition, SNPs were able to affect TFV exposure: in fact, related to its association with lopinavir and ritonavir, *ABCC4* 3348 G allele carriers had TFV renal clearance values 15% lower than wild type and AUC 32% higher than wild type; furthermore, *ABCC2* -24 T allele

patients excreted 19% more TFV than wild type ones [26]. In another study from the same group, clinical and genetic determinants of intracellular TFV-DP have been studied: *ABCC4* 3348 G variant carriers have 35% higher intracellular TFV-DP concentrations [21]. Another recent research reported a 30% increase in TFV plasma concentrations in Thai patients carrying *ABCC4* rs3742106 TG/GG genotype (after adjusting for weight, eGFR and the concomitant RTV use) [27]. Moreover, *ABCC4* rs899494 C>T SNP has been related to TFV renal damage, but other studies did not confirm this association [28].

Taken together, these results showed that genetic variants have been directly associated with TDF renal dysfunction and plasma exposure, but no data are available in literature concerning the association among SNPs and phosphate, calcium, PTH and RBP values; for these reasons, the aim of this study was to investigate the role of SNPs, related to transporters, nuclear factors and VD pathway, in affecting abnormal phosphate, PTH, calcium and RBP in an Italian cohort of HIV-positive treated patients.

2. Patients and methods

Patients treated with antiretroviral drugs (TDF or other drugs for at least six months), were enrolled at the "Amedeo di Savoia" hospital (Turin, Italy) after obtaining Ethics Committee approval (Comitato Etico Interaziendale di Orbassano, Orbassano, Italy: number 63/15/12); all patients signed a written informed consent before being enrolled. TDF was administered at the dose of 245 mg qd with evening intake.

Inclusion criteria were adult age and confirmed HIV positivity. Exclusion criteria were diabetes, untreated hypertension, known renal malformations, recurrent nephrolithiasis and the concomitant use of nephrotoxic drugs (such as gancyclovir or cyclosporine). Values of phosphate, calcium, 25-hydroxyvitamin D (25-VD) and PTH were evaluated at baseline.

Whole blood was withdrawn in EDTA tubes, genomic DNA was isolated from blood samples (MagnaPure Compact, Roche, Monza, Italy) and genotypes were assessed through a real-time polymerase chain reaction allelic discrimination system (LightCycler 480, Roche, Monza, Italy). Investigated gene SNPs were: *ABCB1* (encoding P-glycoprotein) 3435 C>T (rs1045642), *ABCC2* (encoding multidrug resistance protein 2) 1249 G>A (rs2273697), *ABCC2*-24 G>A (rs717620), *ABCC4* (encoding multidrug resistance protein 4) *879 T>C (rs1059751), *ABCC4* 3348 T>C (rs1751034), *ABCC10* (encoding multidrug resistance protein 7) 1875+256 G>A (rs9349256), *SLC28A2* (concentrative nucleoside transporter 2) 124 C>T (rs11854484), *SLC22A6* (encoding organic anion transporter 1) 453 G>A (rs4149170), *HNF4α* (encoding hepatocyte nuclear factor alpha) 975 C>G (rs1884613), *CYP27B1* (encoding cytochrome 27B1 enzyme responsible for VD active metabolite 1,25-dyhydroxyvitamin D (1,25-VD) production) +2838 C>T (rs4646536), *VDR* Cdx2 A>G (rs11568820), *CYP24A1* (encoding cytochrome 27B1 enzyme responsible for VD inactive metabolite 24,25-dyhydroxyvitamin D (24,25-VD) production) 3999 T>C (rs2248359).

These genes have been chosen since TFV is transported by ABCB1, ABCC2, ABCC4, ABCC10 and OAT1 transporters [20, 27, 29, 30]. Furthermore, SNPs in *SLC28A2* gene were analyzed, since TFV is a nucleoside analogue and this is a concentrative nucleoside transporter. *HNF4α* gene SNPs were evaluated since this is a transcription factor, which is able to affect the expression of some transporters genes, such as ABC ones [31]. Finally, *CYP27B1*, *CYP24A1*, and *VDR* genes were selected due to they are VD enzyme activator, inactivator and receptor. All these SNPs have been *a priori* selected according to their allele frequency and distribution in our Caucasian analyzed population.

Urinary RBP (uRBP) was measured on spot urines using enzyme immunoassay kit (Arbor Assays, Ann Arbor, Michigan, USA) with a limit of detection of 4.09 ng/ml. uRBP was corrected for spot urinary creatinine (Cr, measured through automated Jaffe` methods on Roche Diagnostics clinical biochemistry analysers; Roche Diagnostics, Basel, Switzerland). uRBP/Cr normality ranges were provided by the kit manufacturer: below 130 ug/g for patients aged > 50 years and 172 ug/g for patients aged < 50 years.

All variables were tested for normality through the Shapiro-Wilk test. Normal variables were described as average and standard deviation, non-normal ones as median values and interquartile range (IQR) and categorical ones as numbers and percentages. Allele frequencies were tested for Hardy-Weinberg equilibrium. Kruskal-Wallis and Mann-Whitney tests were used for differences in continuous variables between genetic groups, considering statistical significance with a two-sided p-value < 0.05. Stepwise multivariate linear (for phosphate, PTH and calcium levels) and logistic (for abnormal uRBP/Cr) regression analysis were performed including variables with a *p*-value below 0.2 at univariate analysis. Considered variables were: gender, ethnicity, age, BMI, Cr, eGFR, hepatitis B or C co-infections, treatments, TFV plasma and urine concentrations and SNPs, but only the significant ones were reported in results. Bonferroni correction has been performed, since an adjustment made

to *p* values is needed when several dependent or independent statistical tests are being performed simultaneously on a single data set.

Tests were performed with IBM SPSS Statistics 24.0 for Windows (Chicago, Illinois, USA).

3. Results

Baseline characteristics for the 444 included patients are reported in Table 1; 377 (84.9%) were receiving TDF. Variant genotype frequencies (%) were calculated and no linkage disequilibrium is present (table S1); the Hardy-Weinberg conditions were met for all SNPs, with exception of *SLC28A2 124.*

Abnormal uRBP/Cr

Abnormal uRBP/Cr levels were different between TDF and no TDF-containing regimens (p<0.001): 38.9% (N=145) of TDF treated patients and 1.3% (N=5) of TDF-untreated individuals showed abnormal uRBP/Cr levels.

In TDF treated patients, *ABCB1* 3435 TT (p=0.023) and *ABCC10* 1875+256 *GA/AA* (p=0.031) variants were related to abnormal uRBP/Cr (figure 1). However when applying the Bonferroni correction, no factors remained in the final model (p-value set at <0.005).

In logistic regression analysis, eGFR <90 mL/min, TFV plasma/urine ratio and *CYP359 TT* genotype were considered predictive factors of abnormal uRBP/Cr levels for TDF treated patients (table 2), whereas only eGFR <90 mL/min and TDF treatment in all the patients (table 3). Particularly, for TDF treated patients: n=95 (52.7%) abnormal uRBP/Cr patients with eGFR < 90 mL/min *vs.* n=80 (36.5%) for eGFR > 90 mL/min ones, p=0.010; whereas for TDF not-treated patients: n=3 (11.1%) abnormal uRBP/Cr patients with eGFR < 90 mL/min *vs.* n=2 (10%) for eGFR > 90 mL/min, p=0.927.

Phosphate levels

No significant statistical difference of phosphate concentrations was found between patients treated with TDF and those treated with different compounds (p=0.199).

An inverse correlation between VD levels and phosphate concentrations was here observed (p=0.046, R= -0.96). *ABCC10* 1875+256 (p=0.007, 3.1 (IQR 2.8; 3.5) mg/dL for GG genotype *vs.* 3 (2.6; 3.4) mg/dL for GA/AA genotypes), *SLC28A2* 124 (p<0.001, 3.2 (IQR 2.9; 3.6) mg/dL for CC genotype *vs.* 2.9 (2.6; 3.3) mg/dL for CT/TT genotypes) and *VDR* Cdx2 (p=0.005, 3.3 (IQR 2.8; 3.9) mg/dL for AA genotype *vs.* 3 (2.7; 3.4) mg/dL for AG/GG genotypes,)) SNPs were associated with phosphate levels (table 4).

Predictive value of variables was finally evaluated through linear regression (table 5): Bonferroni correction has been performed and only ethnicity and *CYP27B1*+2838 remained in the model (adjusted p-value<0.003; figure 2). Finally, age < 50 years, *CYP27B1*+2838 CC genotype and non European ancestry were predictors of higher concentrations.

Parathyroid hormon levels

PTH levels were *border-line* higher in patients treated with TDF (52.25 pg/mL (37.58-48.20) *vs.* 48.65 pg/mL (34.03-58.70), p=0.050).

An inverse correlation between VD levels and PTH concentrations was observed (p<0.001, R=-0.240). *ABCC2*-24 variant associated with PTH concentrations (p=0.028, 66.6 (54.8; 103.9) pg/mL for AA genotype *vs.* 51.6 (36.9; 67.5) pg/mL for AG/GG genotypes, table 4). At linear regression analysis (table 6), after Bonferroni correction, baseline VD levels < 30 ng/mL and *SLC28A2* 124 CT/TT were associated with PTH levels and in the final model non-European ancestry, female gender, treatment with TDF, baseline vitamin D levels < 30 ng/mL and *SLC28A2* 124 *CT/TT* and *ABCC2*-24 CC SNPs were independent predictors of PTH (figure 3).

Calcium levels

A border-line statistical difference in calcium concentrations was observed between individuals treated with TDF and not treated ones (9.49 (9.20-9.80 vs. 9.51 (9.40-8.90), p=0.052).

ABCC10 1875+256 (p=0.007, 9.5 (9.2; 9.8) mg/dL for GG genotype *vs.* 9.4 (9.2; 9.7) mg/dL for GA/AA genotypes) and *ABCC2*-24 (p=0.029, 9.5 (9.2; 9.8) mg/dL for GG genotype *vs.* 9.6 (9.3; 9.8) mg/dL for GA/AA genotypes) gene polymorphisms were associated with calcium concentrations (table 4).

Predictive value of variables was finally evaluated through linear regression: no factors were retained.

4. Discussion

Tubular dysfunction is a complex interplay of TDF use and different risk factors, which lead to a range of prevalence of this pathology between 7 and 75%, depending on the considered factors: no clear definition of tubular dysfunction is established, as a different number of tubular biomarkers was evaluated in different studies [9].

In this study, the role of some known factors in predicting renal and metabolic outcomes were confirmed: age and ethnicity (serum phosphate levels and PTH), VD levels and TDF use (PTH) and TDF exposure (uRBP/Cr). For the first time, *ABCC2*-24 and *SLC28A2* gene polymorphisms were associated with PTH levels in HIV-infected patients. Finally, SNPs in genes associated to VD metabolic pathways were found to be associated with tubular dysfunction as defined by abnormally high uRBP/Cr (*CYP24A1* 3999) and low serum phosphate concentrations (*CYP27B1*+2838).

In a previous study, TFV urinary clearance (as measured by urinary to plasma 12-h concentration ratio) was associated with SNPs in *ABCC10* gene, along with protease inhibitors co-administration [32]. Furthermore, TFV plasma concentration was related to creatinine clearance, protease inhibitors co-administration and variant in *SLC28A2* gene. It is noteworthy that lower intracellular TFV diphosphate concentrations were found in patients harboring *SLC28A2* variants (*rs1060896* and *124*) [33, 34].

Subsequently, since in literature, substrate studies for CNT28A2 (encoded by *SCL28A2* gene) and TFV are lacking, our group investigated if TDF was CNT2 substrate and observed that it is not [35].

The relationship between TDF use and VD levels is fully elucidated. VD may be relevant because of its role in maintaining calcium and phosphate homeostasis through intestinal uptake and bone re-absorption. A large proportion of patients present VD deficiency [36].

A study reported an association between the highest quintile of TFV plasma concentrations with higher VD binding protein, lower free 1,25-VD, higher 25-VD, and higher serum calcium. In the same work, higher TFV plasma levels were related to higher VD binding protein and lower free 1,25-VD, suggesting functional VD deficiency explaining TDF-associated increased PTH [37].

TDF-associated bone changes may be caused by the drug effect on PTH, since its secretion increases soon after starting therapy [38-40]: VD deficiency could exacerbate this increase, but TDF-related higher PTH levels are found even in sufficient VD quantity people [39, 41, 42]. VD supplementation could reduce TDF-associated PTH increase, but does not ameliorate phosphate loss [42, 43].

1,25-VD is increased by TDF administration in opposition with the expected trend of VD deficiency, but consistent with higher PTH secretion [37]. Another study showed that among suboptimal VD status patients (< 30 ng/mL), PTH levels > than 87 pg/mL were commons in TDF users compared to nonusers (p=0.018), whereas among TDF users, PTH was elevated in suboptimal VD patients (p=0.045); finally, the regression analysis suggested that PTH was directly predicted by TDF use and inversely by 25-VD levels [41]. Rosenvinge *et al.* investigated patients and treatment characteristics related to VD deficiency: they found 25 associated to TDF-linked hyperparathyroidism and also with higher estimated glomerular filtration rate [39].

TDF is known to cause proximal tubular disease in kidney, where VD is converted to the active form. Thus it could be possible a relationship between TDF, VD and kidney, but also in this case, data seem to be converse.

Particularly, TDF leads to impaired renal function, hyperphosphaturia, hypophosphatemia, hypertension and increased renal vascular resistance due to downregulation of the sodium-

phosphorus cotransporter and upregulation of angiotensin II and AT1 receptor. TDF also increases oxidative stress, as evidenced by higher Thiobarbituric Acid Reactive Substances (TBARS) and lower total glutathione (GSH) levels, and induces dyslipidemia. Association of TDF and VD deficiency aggravated renovascular effects and TDF-induced nephrotoxicity, due to changes in the redox state and involvement of RAAS [44]: in fact this hormone has been related with renal and cardiovascular diseases , due of its effects on oxidative stress, lipid metabolism and renin-angiotensin-aldosterone system (RAAS). *Tarcin et al.* demonstrated that VD deficient individuals presented higher plasma levels of TBARS [45]. Besides the effects of VD deficiency on oxidative stress, studies have demonstrated that HIV-infected individuals exhibit a deficiency of GSH aggravating the redox state [46].

Furthermore, several studies have shown that VD is a negative endocrine regulator of RAAS and its concentration has been inversely associated with the prevalence of metabolic syndrome [44].

Scherzer *et al.* showed that TDF use was associated with proteinuria and chronic kidney disease. Furthermore, VD deficiency itself has been related to increased prevalence of proteinuria in adults [14].

Moreover, some investigations indicated no association between VD deficiency and NNRTI use; whereas others are in contrast [39, 47]: in fact, a study of 2014 aimed to investigate the effects of VD deficiency on TDF-induced nephrotoxicity. Wistar rats were divided into four groups: control, receiving a standard diet for 60 days; VD deficiency, receiving a VD-free diet for 60 days; TDF, receiving a standard diet for 60 days with the addition of TDF (50 mg/kg food) for the last 30 days; and VD deficiency+TDF receiving a VD-free diet for 60 days + TDF for the last 30 days. These data showed that VD deficient rats presented hypophosphatemia, hypocalcemia and higher PTH levels. These alterations were expected

since the lack of VD reduces calcium intestinal absorption, leading to a lower calcium levels of calcium and PTH higher production by the parathyroid gland. PTH, in turn, acts on bone tissue in order to attenuate the decrease in serum calcium and the increase in phosphorus excretion. It is well known that VD and calcium are potent PTH regulators, suggesting hypocalcemia and hypovitaminosis D combined with a slight decrease in renal function, as observed in VD deficient animals, may be responsible for PTH increase. The hyperphosphaturic effect of TDF may explain PTH lower levels in VD deficient+TDF when compared with VD deficient group [44]. Low VD levels also induce podocyte loss and the development of glomerulosclerosis through direct cellular effects, compromising the integrity of the glomerular filtration membrane [48].

This study demonstrates variants in genes associated to VD pathway remained in the final regression model for phosphate and abnormal uRBP/Cr. No relationship has been suggested concerning these variables and VD levels at baseline. Probably, this could be due to that CYP27B1 and CYP24A1 are enzymes involved in VD activation and inactivation, respectively, and only 25-VD precursor and not 1,25-VD and 24,25-VD ones was quantified [49].

In logistic regression analyses, eGFR < 90 mL/min was suggested as a predictor of abnormal uRBP/Cr. Furthermore, TDF treatment is a predictor of abnormal uRBP/Cr in all the patients and its plasma/urine ratio concentrations in TDF treated ones; finally, *CYP24A1* 3999 TT is a predictor of toxic RBP levels in TDF administered individuals.

These data have to be confirmed, also considering the advent of the new TFV formulation (TAF), which enhanced delivery to lymphatic tissues, resulting in about 5-fold higher TFV-DP concentrations in PBMCs and ~90% lower circulating TFV compared to TDF [50].

RTV and COBI increase TDF AUC (area under the curve) by 25-37%: for this reason, TAF dose is lowered from 25 to 10 mg daily, whereas TDF dose is maintained to 300 mg. Hill *et al.*performed a meta-analysis, based on 11 trials (8111 patients), comparing TDF and TAF treatments: in the absence of concomitant boosting drugs no significant advantage of TAF over TDF was observed [51]. Nevertheless switch studies suggested an improvement in tubular damage markers in patients switching to TAF even when co.administered with integrase strand trasnfer inhibitors. The clinical advantage of this change as well as the potential use of TDF in patients without risk factors (including genetic variants) need to be assessed longitudinally.

Another consideration could be cost implications: in 2015, Walensky *et al.* evaluated the economic impact of TAF over TDF on societies and found an annual premium of $1000 over the TDF average wholesale price [52]. Consequently, the budget impact could affect the administered drug choice, since now new national and international guidelines recommend to start therapy in all HIV-infected patients: this is particularly important for developing countries. Additionally some counties even in rich-resource countries favor generic TDF on TAF in the absence of concomitant clinical and pharmacological risk factors [53].

In this study, several limitations were acknowledged, such as the single cohort, the limited number of controls and multiple comparisons requiring a larger sample size; furthermore data on bone mineral density were not available.

In conclusion, this data could be useful to select patients who really could be treated with TDF: particularly, it could be possible that in patients treated with TDF/FTC and integrase strand transfer inhibitors, showing favourable genetic characteristics, TDF could be used without specific renal adverse events.

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6. Conflict of Interest

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CHARACTERISTICS		TENOFOVIR	OTHER TREATMENTS
n		377	67
Age (year), median [IQR]		47 [39.6-53.2]	51 [44.9-57.4]
Male gender, n (%)		273 (72.4)	50 (76.9)
BMI (Kg/m^2), median [IQR]		24.1 [22.2-25.6]	23.5 [22.1-25.9]
Ancestry, n (%)	European	331 (87.8)	60 (89.6)
	American	13 (3.4)	2 (2.9)
	African	30 (8.0)	5 (7.5)
	Asian	3 (0.8)	-
Chronic hepatitis C, n (%)		87 (23.1)	12 (9.4)
Chronic hepatitis B, n (%)		22 (5.9)	3 (4.8)
Cirrhotics, n (%)		21 (5.6)	2 (3.2)
CD4+, median [IQR]		544 [408-681.5]	635 [491.3-863.8]
Nadir, median [IQR]		221 [136-341]	209 [98-258]
Viral load < 50 copies mL, n (%)		351 (93.1)	60 (92.3)
Concomitant treatment	Protease inhibitors or elvitegravir, n (%)	135 (35.8)	31 (48.4)
	Integrase inhibitors (no elvitegravir), n (%)	18 (4.8)	2 (3.1)
	Non nucleoside inhibitors, n (%)	221 (58.6)	31 (48.4)
Vitamin D, n (%) <10 ng/mL 10-30 ng/mL >30 ng/mL		 43 (11.3) 197 (52) 133 (35.1)	 3 (4.8) 39 (62.9) 19 (30.6)
Blood creatinine, median [IQR]		1.0 (0.9-1.1)	1.1 [0.9-1.2]
Estimated glomerular filtration CG rate at baseline (mL/min), median [IQR]		93.4 [81.2-111.2]	84.8 [70.9-92.7]
Estimated glomerular filtration rate MDRD at baseline (mL/min), median [IQR]		84.2 [75.7-94.8]	79.6 [69.3-86.5]
Calcium levels, median [IQR]		9.5 [9.2-9.8]	9.5 [9.4-9.8]
Phosphate levels, median [IQR]		2.9 [2.6-3.3]	3 [2.6-3.4]
Parathormone levels, median [IQR]		52 [36.7-68.5]	49.1 [33.9-63.6]
Tenofovir plasma levels (ng/mL), [IQR]		67 [49-94.5]	-
Urine creatinine, median [IQR]		128.4 [97.6-179.2]	103.2 [70.7-120.7]
Abnormal urinary retinol binding protein levels, n (%)		145 (38.9)	5 (1.3)
Tenofovir urine levels (ng/mL), [IQR]		23490 [13953-33809]	-

Table 1. Baseline characteristics of the studied population

	uRBP/Cr (N=377)			
	UNIVARIATE		MULTIVARIATE	
	***p* VALUE**	**OR (95% IC)**	***p* VALUE**	**OR (95% IC)**
Integrase inihibitors (no elvitegravir)	0.141	0.421 (0.133; 1.333)		
Chronic hepatitis C	0.082	0.454 (0.945; 2.627)		
eGFR < 90 mL/min	0.011	1.937 (1.165; 3.221)	0.016	1.924 (1.132; 3.272)
Ethnicity (Caucasian)	0.047	2.105 (1.011; 4.387)		
Tenofovir plasma/urine ratio	0.040	4E+23 (13.458; 1.29E+46)	0.020	1E+34 (257227; 1.3E+63)
ABCB1 3435 TT	0.028	1.742 (1.063; 2.855)		
CYP24A1 3999 TT	0.163	0.685 (0.403; 1.165)	0.049	1.847 (0.904; 3.773)
ABCC10 1875+256 GA/AA	0.151	1.401 (0.885; 2.218)		
VDR Cdx2 AG/GG	0.103	1.754 (0.893; 3.446)		

Table 2. Significant values in the univariate (<0.2) and multivariate (<0.05) logistic regression analysis for uRBP/Cr levels prediction in TDF treated patients.

	uRBP/Cr (N=487)			
	UNIVARIATE		MULTIVARIATE	
	***p* VALUE**	**OR (95% IC)**	***p* VALUE**	**OR (95% IC)**
Ethnicity (Caucasian)	0.030	2.196 (1.080; 4.463)		
BMI > 30	0.153	0.474 (0.170; 1.320)		
eGFR < 90 mL/min	0.017	0.569 (0.358; 0.906)	0.014	0.536 (0.326-0.883)
Chronic hepatitis C	0.027	1.726 (1.066; 2.796)		
Integrase inihibitors (no elvitegravir)	0.113	0.403 (0.131; 1.238)		
NN	0.145	1.353 (0.901; 2.031)		
TDF treatment	0.004	3.427 (1.480; 7.937)	0.001	7.605 (2.209; 26.180)
ABCB1 3435 TT	0.039	1.633 (1.025; 2.601)		
CYP27B1 +2838 TT	0.192	0.758 (0.500; 1.149)		
VDR Cdx2 AG/GG	0.073	1.825 (0.946; 3.523)		
CYP24A1 3999 TT	0.169	0.700 (0.422; 1.163)		
ABCC10 1875+256 GA/AA	0.045	1.570 (1.009; 2.443)		

Table 3. Significant values in the univariate (<0.2) and multivariate (<0.05) logistic regression analysis for uRBP/Cr levels prediction in patients treated with different drugs.

POLYMORPHISM	GENOTYPE	*p*-value	PHOSPHATE LEVELS (mg/dL, N=441)	p-value2	PARATHORMON LEVELS (pg/mL, N=420)	p-value3	CALCIUM LEVELS (mg/dL, N=442)
ABCC10 1875+256	GG	0.007	3.1 (IQR 2.8; 3.5)			0.007	9.5 (IQR 9.2; 9.8)
	GA/AA		3 (IQR 2.6; 3.4)				9.4 (IQR 9.2; 9.7)
SLC28A2 124	CC	<0.001	3.2 (IQR 2.9; 3.6)				
	CT/TT		2.9 (IQR 2.6; 3.3)				
VDR Cdx2	AA	0.005	3.3 (IQR 2.8; 3.9)				
	AG/GG		3 (IQR 2.7; 3.4)				
ABCC2-24	GG					0.029	9.5 (IQR 9.2; 9.8)
	GA/AA						9.6 (IQR 9.3; 9.8)
	AA			0.028	66.6 (IQR 54.8; 103.9)		
	GG/GA				51.6 (IQR 36.9; 67.5)		

Table 4. Gene SNPs influence on phosphate, parathormon and calcium levels

	Phosphate			
	UNIVARIATE		MULTIVARIATE	
	***p* VALUE**	**OR (95% IC)**	***p* VALUE**	**OR (95% IC)**
BMI at baseline				
Age < 50 years	0.033	0.130 (0.010; 0.250)	0.032	0.147 (0.013; 0.282)
Sex (female)	< 0.001	0.377 (0.250; 0.505)		
Ethnicity (non Caucasian)	< 0.001	0.437 (0.261; 0.613)	0.002	0.317 (0.120; 0.514)
Baseline blood creatinine < 1.2 mg/dL	0.144	0.154 (-0.053; 0.360)		
Chronic hepatitis B	0.114	-0.201 (-0.449;0.048)		
Protease inhibitor and elvitegravir treatment	0.006	0.170 (0.050; 0.291)		
Treatment without non nucleoside inhibitors	0.001	0.195 (0.077; 0313)		
Tenofovir urine/plasma ratio > 384	0.087	0.115 (-0.017; 0.248)		
ABCC2-24 GA/AA	0.099	-0.106 (-0.231; 0.020)		
SLC28A2 124 CT/TT	< 0.001	-0.237 (-0.358; -0.115)		
ABCC10 1875+256 GA/AA	0.001	-0.452 (-0.707; -0.196)		
SLC22A6 453 AA	0.147	0.325 (-0.115; 0.764)		
ABCC4 751*879 TC/CC	0.160	-0.092 (-0.221; 0.037)		
ABCC4 3348 TC/CC	0.106	0.100 (-0.021; 0.221)		
CYP27B1 +2838 CC	0.001	0.452 (0.196; 0.707)	0.001	0.456 (0.190; 0.721)

Table 5. Significant values in the univariate (<0.2) and multivariate (<0.05) linear regression analysis for the prediction of phosphate levels.

	PTH			
	UNIVARIATE		MULTIVARIATE	
	***p* VALUE**	**OR (95% IC)**	***p* VALUE**	**OR (95% IC)**
Sex (female)	0.026	7.119 (0.864; 13.375)	0.045	6.429 (0.150; 12.709)
Ethnicity (non caucasian)	0.015	10.709 (2.100; 19.318)	0.004	13.390 (4.232; 22.547)
Baseline vitamin D levels < 30 ng/mL	< 0.001	11.725 (6.037; 17.412)	< 0.001	12.522 (7.004; 18.039)
Treatment with tenofovir	0.072	7.127 (-0.652; 14.907)	0.045	7.732 (0.166; 15.298)
ABCC2-24 AA	0.015	23.524 (4.608; 42.439)	0.007	24.989 (6.792; 43.186)
SLC28A2 124 CT/TT	0.052	5.742 (-0.053; 11.538)	0.005	8.515 (2.528; 14.503)
ABCC10 1875+256 GA/AA	0.100	-4.838 (-10.603; 0.926)		
VDR Cdx2 AG/GG	0.166	-5.805 (-14.027; 2.418)		

Table 6. Significant values in the univariate (<0.2) and multivariate (<0.05) linear regression analysis for the prediction of PTH levels

SNP	% HOMOZIGOUS WILD TYPE	% HETEROZYGOUS	% HOMOZYGOUS MUTANT
ABCB1 3435 C>T	*32.2 CC*	*44.4 CT*	*23.4 TT*
ABCC2 1249 G>A	*62.8 GG*	*31.8 GA*	*5.4 AA*
ABCC2-24 G>A	*68.2 GG*	*29.5 GA*	*2.3 AA*
ABCC4*879 T>C	*29.3 TT*	*54.7 TC*	*16.0 CC*
ABCC4 3348 T>C	*62.8 TT*	*31.8 TC*	*5.4 CC*
ABCC10 1875+256 G>A	*34.9 GG*	*49.1 GA*	*16.0 AA*
SLC28A2 124 C>T	*34.5 CC*	*39.6 CT*	*25.9 TT*
SLC22A6 453 G>A	*75.5 GG*	*22.7 GA*	*1.8 AA*
HNF4α 975 C>G	*63.5 CC*	*28.2 CG*	*8.3 GG*
CYP27B1 +2838 C>T	*5.4 CC*	*63.9 CT*	*57.7 TT*
VDR Cdx2 A>G	*13.0 AA*	*32.7 AG*	*54.3 GG*
CYP24A1 3999 T>C	*21.6 TT*	*52.3 TC*	*26.1 CC*

Table S1. Variant allele frequencies

FIGURE 1

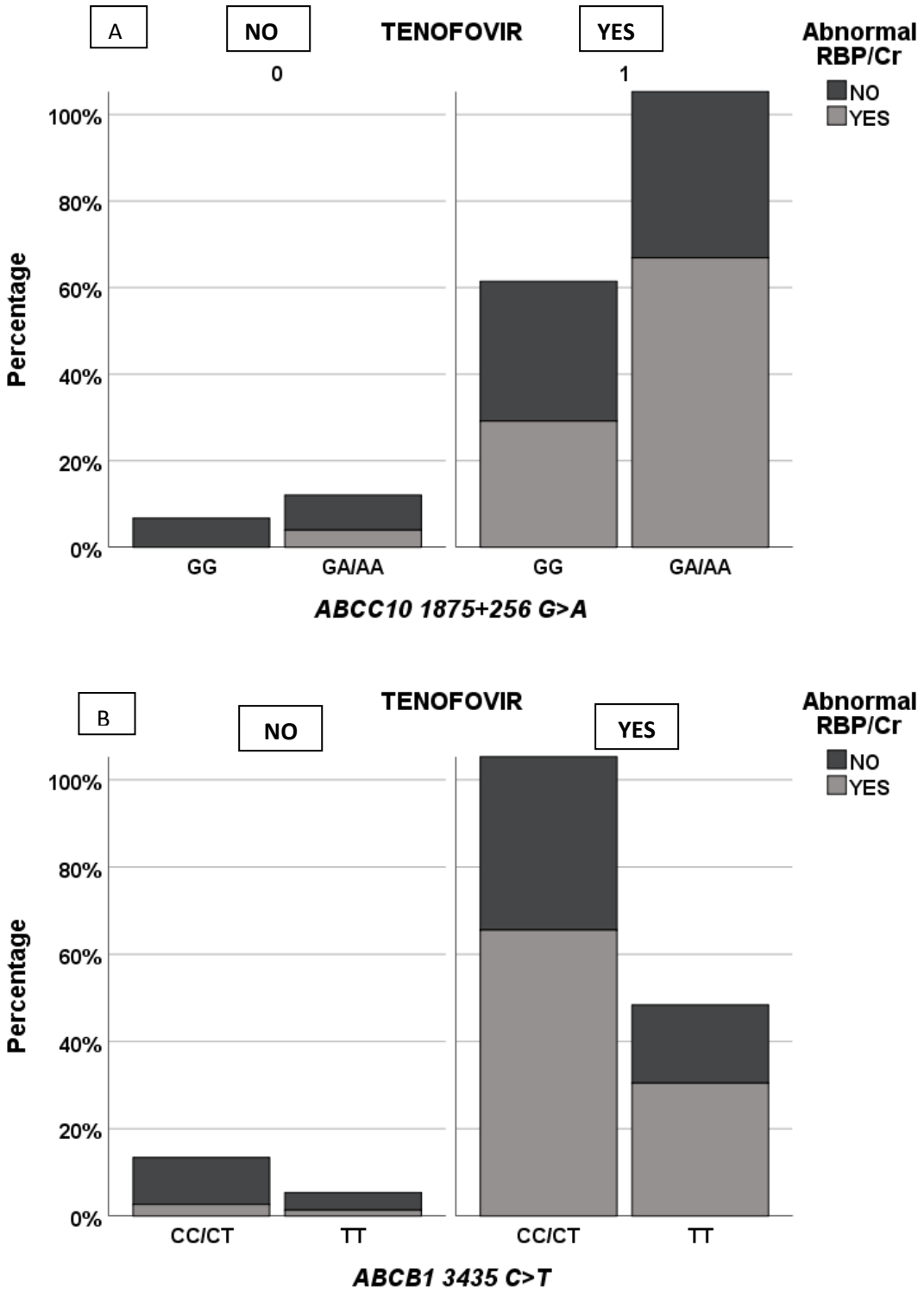

FIGURE 1: *ABCC10 1875+256 G>A* (A) and *ABCB1 3435 C>T* (B) gene SNPs and abnormal RBP/Cr association in tenofovir and not-treated patients

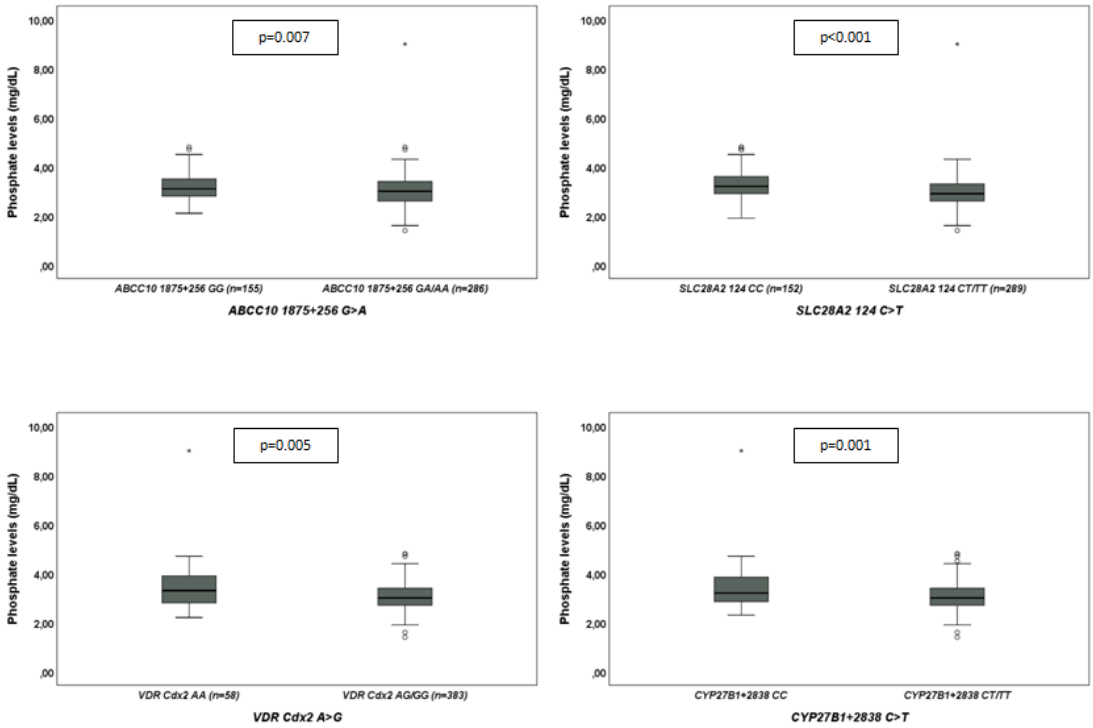

FIGURE 2: Gene SNPs influence on phosphate concentrations

FIGURE 3

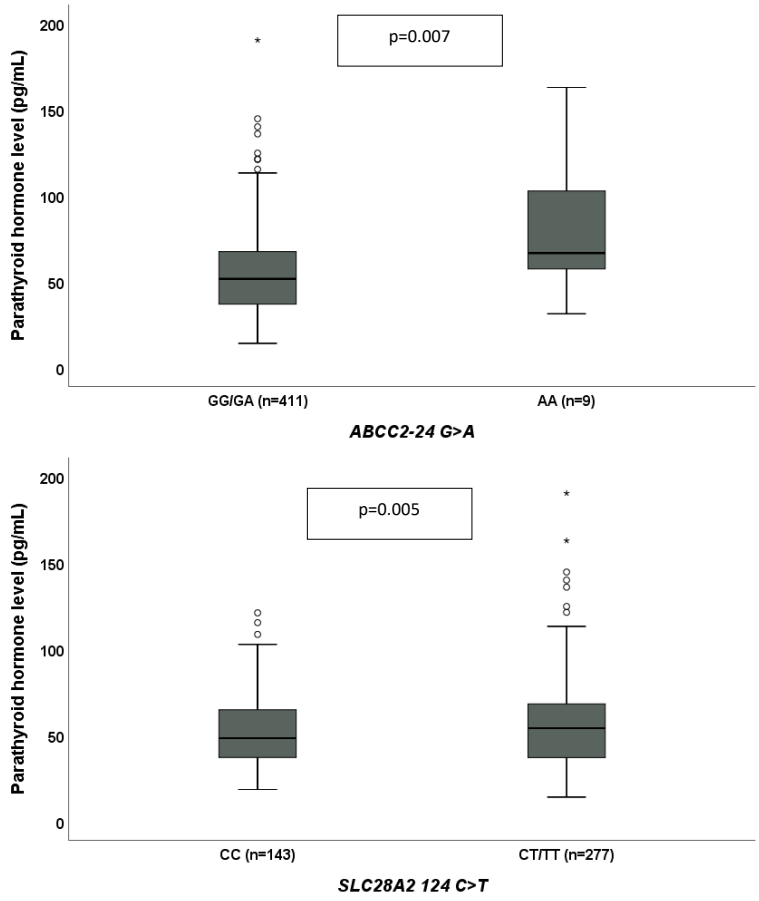

FIGURE 3: *ABCC2*-24 G>A and *SLC28A2* 124 C>T gene variant association with parathormone concentrations.