MAJOR ARTICLE

Predictors of Kidney Tubular Dysfunction in HIV-Infected Patients Treated with Tenofovir: A Pharmacogenetic Study

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Background. Tenofovir is one of the most widely used antiretroviral drugs. Tenofovir undergoes renal clearance by a combination of glomerular filtration and active tubular secretion. Although rare, the mechanism by which tenofovir causes renal damage is not well characterized. We have explored the association between kidney tubular dysfunction (KTD) and polymorphisms in genes encoding drug transporters.

Methods. All consecutive, human immunodeficiency virus (HIV)–infected patients receiving tenofovir-containing antiretroviral regimens who were seen at a single institution during the first trimester of 2008 were enrolled in the study. KTD was defined by the presence of at least 2 of the following abnormalities: nondiabetic glucosuria, urine phosphate wasting, hyperaminoaciduria, β2-microglobulinuria, and increased fractional excretion of uric acid. Twelve single-nucleotide polymorphisms in the *ABCC2, ABCC4, SCL22A6, SLC22A11,* and *ABCB1* genes were analyzed using TaqMan 5′-nuclease assays.

Results. A total of 115 HIV-infected patients were examined, of whom 19 (16.5%) had KTD. The percentage of patients with KTD was higher among those with genotype CC at position −24 of *ABCC2* than among those with genotypes CT and TT (24% [16 of 68 patients] vs. 6% [3 of 47 patients]; $P = .020$). In a multivariate analysis, older age (odds ratio [OR], 1.1; 95% confidence interval [CI], 1.0–1.2; $P = .024$), lower body weight (OR, 0.9; 95% CI, 0.8–0.9; $P = .048$), and genotype CC at *ABCC2* position −24 (OR, 5; 95% CI, 1.2–21; $P = .027$) were independently associated with KTD.

Conclusions. Approximately 17% of HIV-infected patients treated with tenofovir had KTD. Homozygosity for the C allele at position −24 of the *ABCC2* gene was strongly associated with KTD in this population. This polymorphism may help to identify patients at greater risk for developing tenofovir-associated tubulopathy, and close monitoring of renal function is warranted for these patients.

Tenofovir is a nucleotide reverse-transcriptase inhibitor widely used for the treatment of human immunodeficiency virus (HIV) infection. Tenofovir undergoes renal clearance by a combination of glomerular filtration and active tubular secretion. Although large prospective trials have shown that tenofovir is relatively safe for the kidney, with a very low rate of renal insufficiency [1], cases of tubular dysfunction, including development of Fanconi syndrome, have been reported [2–8], and concern exists about the long-term use of tenofovir.

The mechanism by which tenofovir may cause renal damage is not well understood, although interference with transporter proteins in the renal tubule may play a role. Tenofovir entry into the epithelial cells of the kidney tubule through the basolateral membrane involves organic anion transporters (OATs), mainly OAT1 and, to lesser extent, OAT3 [9, 10]. These influx transporters are encoded by the solute carrier genes *SLC22A6* and *SLC22A8,* respectively. Known substrates for OAT1 include cyclic adenosine monophosphate, cyclic guanosine monophosphate, antiviral agents (acyclovir, cidofovir, and zidovudine), antibiotics, and diuretics [11]. OAT1 and OAT3 are expressed in the basolateral membrane, whereas other members of the OAT family, such as OAT4 (encoded by *SLC22A11*), are expressed

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in the luminal membrane [12]. A polymorphism in this gene (rs11231809) has previously been shown to interfere with excretion of the diuretic torsemide [13] and could play a role in the renal clearance of other compounds.

Once tenofovir enters tubular cells, its secretion is an active process that depends on efflux by transporters on the luminal membrane. Although tenofovir uptake from blood into the proximal tubule has been studied, the efflux transport through the apical surface is less well characterized. Proteins implicated in tenofovir efflux at the luminal surface include multidrug-resistance protein 2 (MRP2) [14, 15] and MRP4 [16, 17]. These proteins are encoded by the adenosine triphosphate–binding cassette (ABC) genes *ABCC2* and *ABCC4,* respectively. Both MRP2 and MRP4 are energy-dependent pumps that efflux their substrates into the glomerular filtrate [12]. For many antiviral drugs, the efflux at the luminal membrane is rate limiting, occasionally resulting in intracellular accumulation. Therefore, drugs such as cidofovir and adefovir may produce concentration-dependent renal toxicity [18]. Because tenofovir has structural similarity to these compounds (all are nucleotide analogues), its accumulation within tubular epithelial cells may interfere with renal function. Moreover, transporter expression may modulate the extent of tubular damage. The objective of this study was to explore the association between polymorphisms in *ABCC2, ABCC4, ABCB1, SLC22A6,* and *SLC22A11* and the development of tubular dysfunction in HIV-infected patients treated with tenofovir.

PATIENTS AND METHODS

Study population. All HIV-infected patients receiving tenofovir-containing therapy who were seen at a single clinic in Madrid during the first trimester of 2008 were invited to participate in this cross-sectional study of markers of tubulopathy in 24-h urine samples. The study protocol was approved by the hospital ethics committee. Proximal tubular renal dysfunction was determined on the basis of the following abnormalities: nondiabetic glucosuria (urine glucose level, >300 mg daily), total excretion of phosphorus (urine phosphorus × urine volume) >1200 mg daily, fractional tubular resorption of phosphorus (1 − [(urine phosphorus × plasma creatinine)/(plasma phosphorus × urine creatinine)]) <0.82, hyperaminoaciduria (any amino acid in urine, with the exception of hystidine, glycine, and serine), β2-microglobulinuria (β2-microglobulin level, >1 mg daily), and fractional excretion of uric acid ([(urine uric acid × plasma creatinine)/(urine creatinine × plasma uric acid)] × 100) >15%. Kidney tubular dysfunction (KTD) was defined by the presence of at least 2 of these ab-

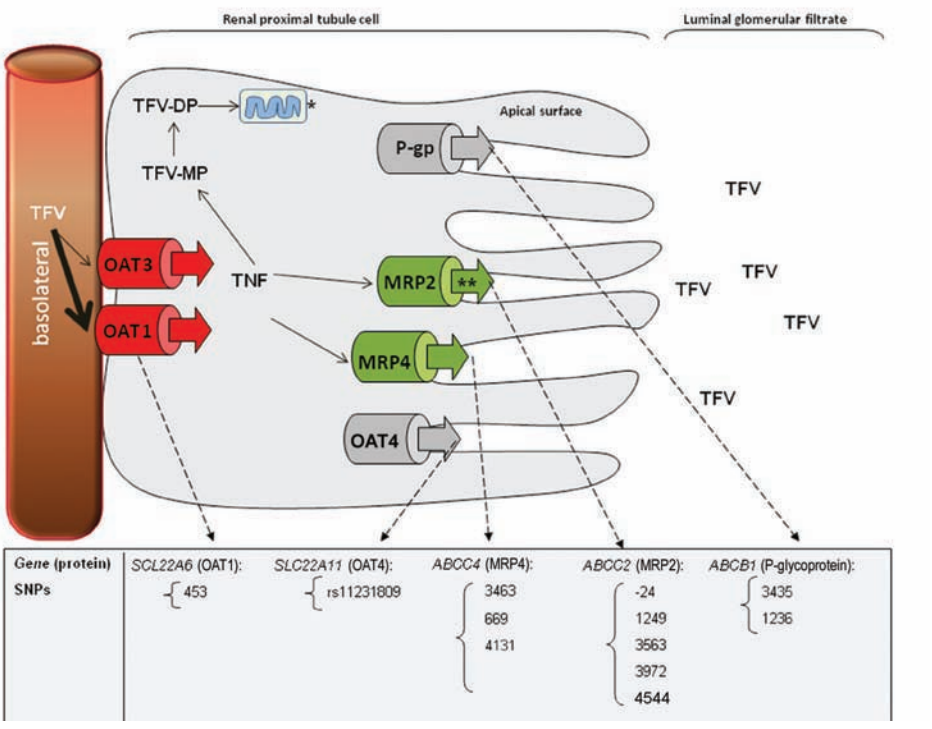

Figure 1. Protein transporters involved in tenofovir (TFV) elimination at basolateral and luminal surface of the proximal renal tubule. MRP2, multidrug-resistance protein 2; MRP4, multidrug-resistance protein 4; OAT1, organic anion transporter 1; OAT3, organic anion transporter 3; OAT4, organic anion transporter 4; P-gp, P-glycoprotein; SNP, single-nucleotide polymorphism; TFV-DP, tenofovir diphosphate; TFV-MP, tenofovir monophosphate. *Mitochondrial toxicity could be one of the mechanisms by which tenofovir causes tubular damage. **The role of MRP2 in tenofovir transport is not clear.

Table 1. Characteristics of the study population.

Characteristic	HIV-infected patients with KTD (n = 19)	HIV-infected patients with normal tubular function (n = 96)	P
Age, years	45 (40–51)	42 (36–46)	.066
Male sex	15 (79)	83 (86)	.478
Body weight, kg	65 (54–71)	68 (60–76)	.068
Ethnicity			
White	17 (89)	86 (90)	.785
Black	0 (0)	2 (2)	
Other	2 (11)	8 (8)	
Hepatitis C virus coinfection	6 (32)	33 (34)	.794
Treatment with protease inhibitors	14 (74)	43 (45)	.110
Duration of tenofovir treatment, months	42 (27–49)	33 (11–47)	.196
Diabetes	7 (37)	19 (20)	.103
Blood parameters, mg/dL			
Glucose	102 (94–107)	98 (92–104)	.286
Creatinine	0.9 (0.8–1.1)	0.9 (0.8–1.0)	.551
Phosphorus	3.8 (3.1–4.1)	3.4 (3.1–3.8)	.156
Uric acid	5.3 (4.3–6.2)	5.5 (4.8–6.5)	.526
Urea	34 (22–42)	33 (25–37)	.973
Urine parameters[a]			
Nondiabetic glucosuria	1 (5)	0 (0)	.165
Hyperaminoaciduria	6 (32)	9 (9)	**.016**
Phosphate wasting	15 (79)	32 (33)	**<.001**
β2-microglobulinuria	14 (74)	5 (5)	**<.001**
Uric acid excretion altered	7 (37)	5 (5)	**<.001**

NOTE. Data are median (interquartile range) for quantitative variables and no. (%) of patients for qualitative variables. KTD, kidney tubular dysfunction. Statistically significant P values ($P < .05$) are shown in boldface. HIV, human immunodeficiency virus.

[a] Urine parameters are considered to be qualitative variables on the basis of the following abnormalities: urine glucose level >300 mg daily in nondiabetic patients, hyperaminoaciduria (i.e., any amino acid in urine, with the exception of hystidine, glycine, and serine), either total excretion of phosphorus >1200 mg daily or fractional tubular resorption of phosphorus <0.82, β2-microglobulin level >1 mg daily, and fractional excretion of uric acid >15%.

normalities, with at least 1 being a Fanconi syndrome criterion (glucosuria in nondiabetic patients, hyperaminoaciduria, or hyperphosphaturia).

Genetic polymorphisms. Single-nucleotide polymorphisms (SNPs) in genes encoding tubular transporters were selected on the basis of functional significance and/or reported minor-allele frequencies >5%. The 12 SNPs selected were as follows: (i) *ABCC2* −24C→T, which has been associated with higher excretion of tenofovir [19]; (ii) *ABCC2* 1249G→A (exon 10), 3563T→A (exon 25), and 3972C→T (exon 28), which along with −24C→T, define a haplotype that predisposes to tenofovir-associated nephrotoxicity [20]; (iii) *ABCC2* 4544G→A (exon 32), which has been reported to be underrepresented in patients with tenofovir-associated renal toxicity [20]; (iv) *ABCC4* 3463A→G, which has been associated with decreased renal tenofovir clearance [19], *ABCC4* 4131T→G, found at high frequency in Caucasians, and *ABCC4* 669C→T, which has been found at higher frequency among patients with tenofovir-associated renal toxicity [20]; (v) *SCL22A6* 453G→A, which has been shown to influence protein expression [21]; (vi) *SLC22A11* rs11231809, which has been associated with abnormal torsemide clearance; and (vii) *ABCB1* 3435C→T and 1236C→T, which have been associated with altered P-glycoprotein expression. P-glycoprotein is capable of transporting tenofovir disoproxil fumarate, the prodrug of tenofovir, so the altered expression of P-glycoprotein in enterocytes could influence tenofovir exposure [22–24]. Figure 1 summarizes the transporter proteins, genes, and polymorphisms analyzed in the study.

Pharmacogenetic analyses. DNA was extracted from peripheral blood mononuclear cells by using a QIAamp DNA Mini Kit (QIAGEN). *ABCB1* 3435C→T and 1236C→T were identified by direct sequencing, and all other genotyping was performed by allelic discrimination using TaqMan 5′-nuclease assays with standard protocols (TaqMan SNP Genotyping As-

Table 2. Genotypes and allelic frequencies at *ABCC2, ABCC4, ABCB1, SLC22A6,* and *SLC22A11* in human immunodeficiency virus–infected patients with and without kidney tubular dysfunction (KTD).

Gene (protein), position, SNP identification, and genotype or allele	Patients with KTD (n = 19)	Patients with normal tubular function (n = 96)	P
ABCC2 (MRP2)			
−24 C→T, rs717620			
C/C	16 (84)	52 (54)	
C/T	2 (11)	37 (39)	**.045**
T/T	1 (5)	7 (7)	
C	34 (89)	141 (73)	
T	4 (11)	51 (27)	**.002**
1249 G→A, rs2273697			
G/G	13 (68)	66 (69)	
G/A	5 (26)	26 (27)	.976
A/A	1 (5)	4 (4)	
G	31 (82)	158 (82)	
A	7 (18)	34 (18)	.981
3563 T→A, rs8187694			
T/T	17 (89)	83 (86)	
T/A	2 (11)	12 (12)	.876
A/A	0 (0)	1 (1)	
T	36 (95)	178 (93)	
A	2 (5)	14 (7)	.763
3972 C→T, rs3740066			
C/C	10 (53)	31 (32)	
C/T	6 (32)	50 (52)	.201
T/T	3 (16)	15 (16)	
C	26 (68)	112 (58)	
T	12 (32)	80 (42)	.147
4544 G→A, rs8187710			
G/G	16 (84)	74 (77)	
G/A	3 (16)	14 (15)	.892
G	35 (92)	178 (93)	
A	3 (8)	14 (7)	.865
ABCC4 (MRP4)			
669 C→T, rs899494			
C/C	16 (84)	74 (77)	
C/T	3 (16)	21 (22)	.757
T/T	0 (0)	1 (1)	
C	35 (92)	171 (89)	.637
T	3 (8)	23 (12)	
3463 A→G, rs1751034			
A/A	14 (74)	58 (60)	.318
A/G	4 (21)	36 (38)	
G/G	1 (5)	2 (2)	
A	32 (84)	152 (79)	.370
G	6 (16)	40 (21)	
4131 T→G, rs3742106			
T/T	7 (37)	40 (42)	
T/G	7 (37)	42 (44)	.452
G/G	5 (26)	14 (15)	
T	21 (55)	122 (64)	
G	17 (45)	70 (36)	.199

Table 2. ***(Continued.)***

Gene (protein), position, SNP identification, and genotype or allele	Patients with KTD (n = 19)	Patients with normal tubular function (n = 96)	P
ABCB1[a] (P-gp)			
3435 C→T, rs1045642			
C/C	6 (35)	29 (32)	
C/T	11 (65)	45 (49)	.145
T/T	0 (0)	18 (20)	
C	23 (68)	103 (56)	.212
T	11 (32)	81 (44)	
1236 C→T, rs1128503			
C/C	6 (38)	31 (33)	.569
C/T	7 (44)	42 (45)	
T/T	3 (19)	21 (22)	
C	19 (59)	104 (55)	.679
T	13 (41)	84 (45)	
SLC22A6 (OAT1)			
453[b]			
G/G	13 (68)	80 (83)	
G/A	6 (32)	15 (16)	.241
A/A	0 (0)	1 (1)	
G	32 (84)	175 (91)	
A	6 (16)	17 (9)	.142
SLC22A11 (OAT4)			
rs11231809			
T/T	6 (32)	43 (45)	.509
T/A	8 (42)	36 (38)	
A/A	5 (26)	17 (18)	
T	20 (53)	122 (64)	.583
A	18 (47)	70 (36)	

NOTE. Data are no. (%) of patients for genotypes and no. (%) of alleles (i.e., twice the number of patients) for alleles. Statistically significant P values ($P < .05$) are shown in boldface. MRP2, multidrug-resistance protein 2; MRP4, multidrug-resistance protein 4; OAT1, organic anion transporter 1; OAT4, organic anion transporter 4; P-gp, P-glycoprotein; SNP, single-nucleotide polymorphism.

[a] Genotypes at *ABCB1* 3435 and *ABCB1* 1236 could not be obtained for 2 and 3 patients, respectively, in the group of patients with KTD and for 4 and 2 patients, respectively, in the group of patients with normal tubular function.

[b] Position relative to AJ249369.

says; Applied Biosystems). All primer and probe sequences are available on request.

Statistical analyses. Descriptive results of continuous variables were expressed as medians and interquartile ranges. Continuous variables were compared using a parametric test (Student's t test) or nonparametric test (Wilcoxon test), as required. For the comparison of proportions, the χ^2 test was used, with Yates or Fisher's corrections applied when needed. Bivariate and multivariate logistic regression analyses were performed for the identification of factors associated with kidney tubular toxicity. Parameters with P values <.2 in the bivariate analysis were entered into a stepwise multivariate analysis. All statistics were conducted using SPSS, version 11.0 (SPSS), and differences with P values <.05 were considered to be statistically significant. *ABCC2* and *ABCC4* haplotypes for individual samples were constructed using PHASE, version 2.1 (University of Washington, Seattle) [25, 26].

RESULTS

Study population. A total of 124 HIV-infected patients receiving stable antiretroviral regimens containing tenofovir were originally identified. Informed consent and DNA samples for genotypic analyses were obtainable from 115 of these patients. A total of 19 (16.5%) of the 115 patients fulfilled criteria for KTD. The main characteristics of the study population are given in table 1. The median follow-up time was 35 months (interquartile range, 11–47 months). There were no statistically sig-

Table 3. Distribution of *ABCC2* and *ABCC4* haplotypes among human immunodeficiency virus–infected patients with and without kidney tubular dysfunction (KTD).

Haplotypes	Patients with KTD (n = 19)	Patients with normal tubular function (n = 95)[a]	P	OR (95% CI)
ABCC2[b]				
CGAC	2 (5)	14 (7)	.698	0.7 (0.10–2.86)
CGTC	19 (50)	64 (34)	.063	1.96 (0.96–4)
CGTT	6 (16)	28 (15)	.844	1.08 (0.38–2.78)
CATC	5 (13)	32 (17)	.602	0.75 (0.24–1.96)
CATT	2 (5)	2 (1)	.146	5.26 (0.52–50)
TGTT	4 (11)	49 (26)	**.037**	0.34 (0.10–0.94)
TGTC	0 (0)	1 (1)		…
ABCC4[c]				
CAG	17 (45)	64 (34)	.203	1.59 (0.77–3.24)
CAT	13 (34)	76 (40)	.515	0.78 (0.34–1.70)
CGT	5 (13)	25 (13)		…
TGT	1 (3)	11 (6)	.480	0.44 (0.02–2.70)
CGG	0 (0)	2 (1)		…
TAG	0 (0)	3 (2)		…
TAT	2 (5)	8 (4)	.741	1.26 (0.17–5.73)
TGG	0 (0)	1 (1)		…

NOTE. Data are no. (%) of haplotypes (i.e., twice the number of patients), unless otherwise indicated. Statistically significant P values ($P < .05$) are shown in boldface.

[a] Haplotypes at *ABCC2* and *ABCC4* were not available for 1 patient with normal tubular function.
[b] *ABCC2* haplotypes were constructed on the basis of positions −24, 1249, 3563, and 3972.
[c] *ABCC4* haplotypes were constructed on the basis of positions 669, 3463, and 4161.

nificant differences in age, sex, body weight, race, hepatitis C virus coinfection, use of protease inhibitors, and the duration of tenofovir therapy (in months) between patients with and patients without KTD. Similarly, baseline biochemical parameters, such are urea, glucose, creatinine, phosphorus, and uric acid levels, did not differ significantly between the 2 groups.

Association of* ABCC2, ABCC4, SLC22A6, SLC22A11, *and* ABCB1 *with KTD. The distribution of genotypes at the *ABCC2, ABCC4, SLC22A6, ABCB1,* and *SLC22A11* genes is given in table 2. All polymorphisms were in Hardy-Weinberg equilibrium. The single SNP analysis showed a higher percentage of patients with KTD among C homozygotes at position −24 of *ABCC2,* compared with the patients with other genotypes (24% [16 of 68 patients] vs. 6% [3 of 47 patients]; $P = .020$). No other statistically significant differences were observed.

The analysis of the association of a single renal parameter with the different SNPs showed the relevance of 5 SNPs. *ABCC2* −24 genotype CC was associated with phosphorus wasting and with β2-microglobulin excretion; *ABCC2* 1249 genotype GA/AA was associated with the altered excretion of amino acids; *ABCC2* 3972 genotype CC was associated with β2-microglobulin excretion; *ABCC4* 669 genotype CC was associated with phosphorus wasting; and *ABCC4* 4131 genotype TG/GG and *OAT4* rs11231809 genotype TT were associated with uric acid excretion altered.

Association of haplotypes at* ABCC2 *and* ABCC4 *with KTD. The distribution of *ABCC2* and *ABCC4* haplotypes is given in table 3. The TGTT haplotype of *ABCC2* was less prevalent among patients with KTD than among the remaining patients (11% vs. 26%; $P = .037$). No statistically significant differences were found for *ABCC4* haplotypes.

Predictors of KTD in patients treated with tenofovir. Regression analysis was used to determine the predictors of KTD in the study population. In the multivariate analysis that included sex, age, body weight, hepatitis C virus coinfection, number of months of treatment with tenofovir, plasma HIV RNA level, use of protease inhibitors, concomitant exposure to nephrotoxic drugs, diabetes, hypertension, creatinine clearance, and relevant genotypes, the following parameters were independently associated with KTD: older age (OR, 1.1; 95% CI, 1.0–1.2; $P = .024$), lower body weight (OR, 0.9; 95% CI, 0.8–0.9; $P = .048$), and homozygosity for the C allele at position −24 of *ABCC2* (OR, 5; 95% CI, 1.2–21; $P = .027$) (table 4).

DISCUSSION

Prospective clinical trials have consistently reported a relatively low rate of renal insufficiency in patients exposed to tenofovir

Table 4. Predictors of kidney tubular dysfunction in human immunodeficiency virus (HIV)–infected patients treated with tenofovir.

	Bivariate analysis		Multivariate analysis	
Factor	OR (95% CI)	*P*	OR (95% CI)	*P*
Female sex	1.70 (0.5–5.9)	.403		
Age (per year)	1.06 (1–1.1)	**.047**	1.09 (1.02–1.18)	.024
Body weight (per kg)	0.95 (0.9–1)	**.052**	0.93 (0.88–0.99)	.048
Hepatitis C virus coinfection	1.30 (0.4–3.7)	.627	…	
Months receiving tenofovir treatment	1.03 (1–1.1)	**.039**	…	
Plasma HIV RNA level (per log copies/mL)	1.6 (0–6.7)	.334	…	
CD4 cell count (per cells/mL)	1 (0.9–1)	.795	…	
Use of nephrotoxic drugs	2.50 (0.7–8.2)	**.130**	…	
Use of protease inhibitors	2.85 (0.8–9.4)	**.087**	…	
Arterial hypertension	1.17 (0.3–3.7)	.785	…	
Diabetes	2.36 (0.8–6.8)	**.111**	…	
Creatinine clearance (per mL/min)	0.98 (0.9–1)	**.124**	…	
Genetic polymorphisms[a]				
Genotype CC at *ABCC2* −24 (vs. CT and TT)	4.51 (1.2–16.50)	**.023**	5.04 (1.20–21)	.027
ABCC2 1249A allele	1.01 (0.35–2.9)	.977	…	
ABCC2 3563A allele	0.75 (0.15–3.63)	.722	…	
ABCC2 3972T allele	0.43 (0.16 to 1.16)	**.096**	…	
ABCC2 4544A allele	1.09 (0.28–4.26)	.892	…	
ABCC4 669T allele	0.63 (0.2–2.4)	.494	…	
ABCC4 3463G allele	0.28 (0.2–1.6)	.545	…	
ABCC4 4131G allele	1.22 (0.4–3.4)	.696	…	
SLC22A6 453A allele	2.3 (0.8–6.9)	**.138**	…	
SLC22A11 rs11231809 A allele	0.60 (0.2–1.9)	.387	…	
ABCB1 3435T allele	0.806 (0.29–2.6)	.873	…	
ABCB1 1236T allele	0.856 (0.3–2.7)	.796	…	

NOTE. Factors with $P < .2$ in the bivariate analysis (shown in boldface) were entered into the multivariate analysis. For the multivariate analysis, data for statistically significant factors ($P < .05$) are shown.

[a] For the alleles, the factor is the presence of at least 1 of the designated allele.

[1, 27]. However, the renal safety profile of tenofovir is still being debated, and reports of Fanconi syndrome are emerging in the literature, often involving HIV-infected patients with prior underlying renal abnormalities and/or concomitant exposure to nephrotoxic agents [2–8]. We report the results of an investigation of the pharmacogenetic determinants of KTD in HIV-infected patients receiving stable tenofovir therapy. The most important finding was the association between KTD and genotype CC at position −24 of *ABCC2*.

The mechanism by which MRP2 influences the risk of KTD in these patients is not well understood. Three hypotheses may be considered. First, tenofovir could be excreted less efficiently by tubular cells in *ABCC2* −24C homozygotes, as suggested elsewhere [19]. Increased intracellular concentrations of tenofovir within epithelial tubular cells could be deleterious. However, tenofovir metabolites have not been found to be increased in peripheral blood mononuclear cells of carriers of genotype CC at *ABCC2* [28], although this may be accounted for by differences in the nucleotide pool equilibrium and metabolism between blood cells and epithelial tubular cells. Second, MRP2 may transport an as-yet-unidentified endogenous chemical or protein that influences tenofovir toxicity to the kidney, and this genotype is associated with alteration in this factor. This explanation is supported by the observation that, although tenofovir appears to affect hepatobiliary elimination in rats [14], it is not a substrate for human MRP2 in vitro [9, 16]. It should also be noted that in vitro studies with hugely exaggerated expression systems may overestimate the contribution of a transporter in vivo but that false-negative results are very unlikely when appropriate controls are used. Finally, the *ABCC2* −24C allele may be in linkage disequilibrium with other SNPs in genes coding for as-yet-unidentified proteins that influence tubular function.

The CATC haplotype of *ABCC2*, defined by SNPs at positions −24, 1249, 3563, and 3972, has already been associated with a higher risk of tenofovir-associated KTD [20]. However, we did not find an association with the same haplotype in our study. It must be noted, however, that these studies are not

directly comparable, because of differences in study design and, most importantly, in the criteria used to define renal toxicity. Izzedine et al. [20] defined renal toxicity on the basis of metabolic acidosis, urine potassium loss, hypophosphoremia, low uric acid levels, and aminoaciduria within 1 month after initiation of tenofovir therapy. It is possible that longer exposure could increase the proportion of patients with tubular abnormalities. In contrast, our cross-sectional study examined HIV-infected patients with a median duration of exposure to tenofovir of 34 months. Because patients who developed early renal toxicity would have been excluded, our results may have underestimated the real incidence of KTD associated with tenofovir therapy. This issue also underscores the importance of a consensus on an accurately defined phenotype for clinical diagnosis of KTD and the need for pharmacogenetic association studies in this area.

Kidney tubular damage is often multifactorial, and tenofovir-associated KTD is unlikely to be an exception. In addition to the impact of genetic polymorphisms, other factors could influence the risk for developing KTD. Indeed, age and body weight were statistically significant risk factors for KTD in our study. Interestingly, both factors tend to result in a reduced tenofovir clearance, and pharmacokinetic studies show that tenofovir elimination is dependent on the ratio of body weight to serum creatinine level [29]. With lower body weight, the ratio diminishes, and tenofovir exposure increases. Following this rationale, studies examining a potential association between tenofovir plasma concentrations and KTD are warranted.

Several limitations of our study must be acknowledged. First, only a limited number of polymorphisms in candidate transporters were examined in a limited number of patients, and other unexplored genotypes may have a greater impact on susceptibility to KTD. Second, other transporters that were not considered could be involved in the elimination of tenofovir throughout the renal tubule. Finally, the cross-sectional design of our study involving patients receiving long-term tenofovir therapy could have missed individuals with more serious renal toxicity manifested at early time points. Prospective studies, with follow-up for sufficient duration and involving patients initiating treatment with tenofovir, are warranted to confirm our findings.

In summary, these results suggest the existence of a genetic predisposition to developing tenofovir-associated renal toxicity, primarily recognizable as tubular dysfunction. Other factors, such as older age and lower body weight, may also contribute. Collectively, a high accumulation of tenofovir within tubular epithelial cells may account for an increased risk of tubular damage in patients treated with tenofovir. This concentration-dependent effect is important because tenofovir may be used for treatment of conditions other than HIV infection (e.g., chronic hepatitis B virus infection), in which dose reduction might be an option. Moreover, if tubular damage persists for long periods in the absence of renal insufficiency, laboratory and clinical manifestations other than those associated with kidney failure may develop. This is the case in premature osteoporosis due to bone mineral loss. If these preliminary data are confirmed in prospective studies, then close monitoring of tubular function is warranted for patients receiving treatment with tenofovir, particularly for *ABCC2* −24C homozygotes.

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