Single Nucleotide Polymorphisms in *ABCC2* Associate With Tenofovir-Induced Kidney Tubular Dysfunction in Japanese Patients With HIV-1 Infection: A Pharmacogenetic Study

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Background. Tenofovir is a widely used antiretroviral drug although it can cause kidney tubular dysfunction (KTD). The aim of this study was to determine the association between polymorphisms in genes encoding drug transporters and KTD in Japanese patients treated with tenofovir.

Methods. The association between tenofovir-induced KTD and 14 single nucleotide polymorphisms (SNPs) in the *ABCC2*, *ABCC4*, *ABCC10*, *SCL22A6*, and *ABCB1* genes was investigated in 190 Japanese patients. KTD was diagnosed by the presence of at least 3 abnormalities in the following parameters: fractional tubular resorption of phosphate, fractional excretion of uric acid, urinary β2-microglobulin, urinary α1-microglobulin, and urinary N-acetyl-β-D-glucosaminidase. Genotyping was performed by allelic discrimination using TaqMan 5′-nuclease assays with standard protocols. Associations between genotypes and KTD were tested by univariate and multivariate logistic regression analyses.

Results. KTD was diagnosed in 19 of the 190 (10%) patients. Univariate and multivariate analyses showed a significant association between KTD and genotype CC at position −24 CC (adjusted odds ratio [OR], 20.08; 95% confidence interval [CI], 1.711–235.7; $P = .017$) and genotype AA at position 1249 (adjusted OR, 16.21; 95% CI, 1.630–161.1; $P = .017$) of *ABCC2*. Multivariate analysis showed higher adjusted OR for patients with both homozygotes (adjusted OR, 38.44; 95% CI, 2.051–720.4; $P = .015$). *ABCC2* haplotype −24T and 1249G was a protective haplotype for KTD (OR, 0.098; 95% CI, .002–.603; $P = .003$

Conclusions. This is the first study of our knowledge to identify the association between SNPs in *ABCC2* and tenofovir-induced KTD in an Asian population. Close monitoring of renal function is warranted in tenofovir-treated patients with these SNPs.

Tenofovir disoproxil fumarate (TDF), a prodrug of tenofovir, is a nucleotide reverse transcriptase inhibitor widely used for the treatment of human immunodeficiency virus type 1 (HIV-1) infection and hepatitis B infection [1–4]. Tenofovir is excreted by a combination of glomerular filtration and active tubular secretion. Although the nephrotoxicity of tenofovir is regarded mild and tolerable [5–7], several cases of tenofovir-induced nephrogenic diabetes insipidus, Fanconi syndrome, and acute renal failure have been reported, and prognosis of renal function with long-term tenofovir use remains unknown [8–10].

The mechanism of tenofovir-induced kidney damage is not fully understood. However, mitochondrial damage in the proximal renal tubular cells was observed in patients with prominent tenofovir-induced kidney tubular dysfunction (KTD) [11, 12].

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Because the characteristics and severity of tenofovir-induced KTD vary widely among individuals, the role of host genetics has drawn a particular attention. Single nucleotide polymorphisms (SNPs) in transporter proteins of renal tubular cells have been investigated to elucidate their roles in tenofovir-induced KTD [13–15].

Tenofovir enters kidney tubular cells through the basolateral membrane and is transported mainly by organic anion transporter (OAT) 1 and, to a lesser extent, OAT 3, encoded by genes *SLC22A6* and *SLC22A8*, respectively [16]. Tenofovir is excreted into the urine at the apical membrane by 2 transporters on the luminal membrane; multidrug resistance protein (MRP) 4 and MRP 2, encoded by the adenosine triphosphate–binding cassette (ABC) genes *ABCC4* and *ABCC2,* respectively [17, 18]. Although the role of MRP4 in transporting tenofovir has been well established, that of MRP 2 remains controversial [19, 20]. Recently, MRP 7, encoded by *ABCC10* gene, was also reported to take part in the excretion of tenofovir [21]. P-glycoprotein is a membrane protein expressed on the cells of renal proximal tubule, intestine, and hepatocytes. Encoded by *ABCB1* gene, P-glycoprotein transports TDF, the prodrug of tenofovir. SNPs on *ABCB1* might alter the expression of P-glycoprotein and thus affect exposure of tenofovir [22–24].

Previous studies reported inconsistent findings on the association of the SNPs of the transporter protein on tenofovir-induced KTD [13–15]. Several pathological processes could induce KTD, such as active infection, inflammation, diabetic nephropathy, concurrent use of nephrotoxic drugs, and preexisting renal impairment, and thus it is difficult to evaluate KTD induced exclusively by tenofovir [25]. Moreover, drug interaction with other antiretrovirals, especially ritonavir-boosted protease inhibitors, modifies tenofovir clearance and thus the severity of tenofovir-induced KTD [26, 27]. Previous studies examined patients treated with various antiretroviral combinations, which might also contribute to the inconsistent findings. Thus, the effect of SNPs on tenofovir-induced KTD remains to be clarified and isolated from other abovementioned conventional risk factors for KTD [15, 28]. Of note, the population investigated in previous studies on the role of SNPs in tenofovir-induced KTD was mostly whites, and patients of other genetic background have hardly been examined.

Based on the above background, the present study was designed to elucidate the association between polymorphisms in genes encoding drug transporters in renal tubular cells and tenofovir-induced KTD, in a setting designed to exclude other predisposing or intervening factors: the inclusion of Japanese patients with HIV infection on the same antiretroviral combination with suppressed HIV-1 viral load, and free of preexisting renal impairment, major comorbidities, and active infections.

METHODS

Ethics Statement

This study was approved by the Human Genetics Research Ethics Committee of the National Center for Global Health and Medicine, Tokyo, Japan. Each patient included in this study provided a written informed consent for genetic testing and publication of clinical data for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study Design

We performed a single-center cohort study to cross-sectionally elucidate the association between SNPs in genes encoding renal tubular transporters in Japanese patients with HIV infection and tenofovir-induced KTD.

Study Subjects

The study included consecutive Japanese patients with HIV infection, aged >17 years, with HIV-1 viral load <200 copies/mL, and on at least 4-week treatment with once-daily ritonavir (100 mg)–boosted darunavir (800 mg) plus fixed dose tenofovir (300 mg)/emtricitabine (200 mg), seen at our clinic between 1 October 2011 and 31 March 2012. The exclusion criteria were (1) active infection, (2) malignancy, (3) diabetes mellitus, defined by the use of anti-diabetic agents or fasting plasma glucose >126 mg/dL or plasma glucose >200 mg/dL on two different days, (4) alanine aminotransferase 2.5 times more than the upper limit of normal, (5) estimated glomerular filtration rate (eGFR) calculated by Cockcroft-Gault equation of <50 mL/minutes [creatinine clearance = [(140 − age) × weight (kg)]/(serum creatinine × 72)(×0.85 for females)] [29], and (6) patients without consent to the study.

Measurements

Blood and spot urine samples were collected either on the day of enrollment or on the next visit, together with body weight measurement. The blood samples were used to measure serum creatinine, serum uric acid, serum phosphate, CD4 count, and C-reactive protein, whereas urine samples were used to measure phosphate, uric acid, creatinine, β2-microglobulin (β2M), α1-microglobulin (α1M), and N-acetyl-β-D-glucosaminidase (NAG). The values of β2M, α1M, and NAG measured in the urine samples were expressed relative to urinary creatinine of 1 g/L (/g Cr).

Urinary concentrations of β2M and α1M were measured with latex aggregation assay kits (β2M: BMG-Latex X1 “Seiken”; Denka Seiken Co, Niigata, Japan; α1M: Eiken α1M-III; Eiken Chemical Co, Tokyo, Japan), and those of NAG by colorimetric assay of enzyme activity with 6-methyl-2-pyridyl-N-acetyl-1-thio-β-D-glucosaminide as substrate (Nittobo Medical Co, Tokyo).

Definition of Renal Proximal Tubular Dysfunction

KTD was defined as the presence of at least 3 abnormalities in the following 5 parameters: fractional tubular resorption of phosphate {1 − [(urine phosphate × serum creatinine)/(urine creatinine × serum phosphate)]} × 100 of <82%, fractional excretion of uric acid {[(urine uric acid × serum creatinine)/(urine creatinine × serum uric acid)] × 100)} of >15%, β2-microglobulinuria (β2M > 1000 μg/g Cr), α1-microglobulinuria (α1M > 16.6 mg/g Cr), and high-NAG level in urine (NAG > 5.93 U/g Cr). The above cutoff levels were selected on the basis of data reported previously by various investigators [15, 30, 31].

The potential risk factors for KTD were determined according to previous studies and collected together with the basic demographics from the medical records [6, 27, 32, 33]. They included age, sex, body weight, and presence or absence of other medical conditions (concurrent use of nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, and nonsteroidal antiinflammatory agents, coinfection with hepatitis B, defined by positive hepatitis B surface antigen, coinfection with hepatitis C, defined by positive HCV viral load, hypertension, defined by current treatment with antihypertensive agents or 2 successive measurements of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg at the clinic, dyslipidemia, defined by current treatment with lipid-lowering agents or 2 successive measurements of either low-density lipoprotein cholesterol >140 mg/dL, high-density lipoprotein cholesterol <40 mg/dL, total cholesterol >240 mg/dL, triglyceride >500 mg/dL. At our clinic, blood pressure and body weight are measured every visit. We used the data on or closest to and preceding the day of blood/urine sample collection by no more than 180 days.

Genetic Polymorphisms

SNPs in genes encoding tubular transporters were selected on the basis of their functional significance, findings of previously published reports, and/or reported minor-allele frequencies >5% in the Japanese [13–15, 21, 28]. The allele frequency data for the Japanese were obtained from the Japanese Single Nucleotide Polymorphisms (JSNP) database [34]. The 14 SNPs selected were (1) *ABCC2* (encodes MRP2) −24C → T (in the promoter; rs717620); 1249G → A (Val417Ile; rs2273697); 2366C → T (Ser789Phe; rs56220353); 2934G → A (Ser978Ser; rs3740070), (2) *ABCC4* (encodes MRP4) 559G → T (Gly187Trp; rs11568658); 912G → T (Lys304Asn; rs2274407); 2269G → A (Glu757Lys; rs3765534); 3348A → G (Lys1116Lys; rs1751034); 4135T → G [in the 3′ untranslated region (UTR); (rs3742106)]; 4976T → C (3′ UTR; rs1059751), (3) *ABCC10* (encodes MRP10) 526G → A (intron; rs9349256); 2759T → C (Ile920Thr; rs2125739), (4) *SLC22A6* (encodes OAT1) 180C → T (Asn60Asn; rs11568630), and (5) *ABCB1* (encodes P-glycoprotein) 2677T → A/G (A:Ser893Thr, G:Ser893Ala; rs2032582).

Pharmacogenetic Analyses

Genomic DNA was extracted from peripheral-blood leukocytes using the protocol described in the sheet enclosed with the QIAamp DNA MiniKit (Qiagen, Valencia, California). All genotyping was performed by allelic discrimination using TaqMan 5′-nuclease assays with standard protocols (TaqMan SNP Genotyping Assays; Applied Biosystems, Foster City, California). The primer and probe sequences are available on request.

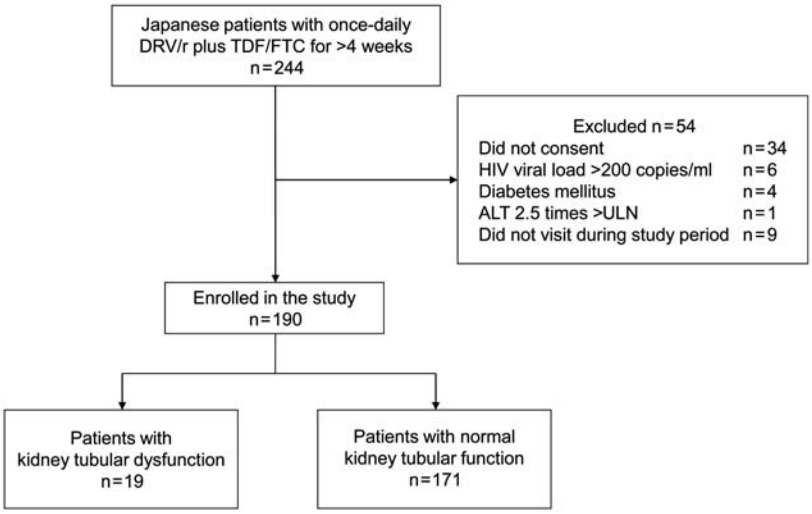

Figure 1. Patient enrollment. Abbreviations: ALT, alanine transaminase; DRV/r, ritonavir-boosted darunavir; HIV, human immunodeficiency virus; TDF/FTC, tenofovir/emtricitabine; ULN, upper limit of normal.

Statistical Analysis

Baseline characteristics were compared between patients with KTD and without tubular dysfunction by the Student *t* test for continuous variables and by either the χ^2 test or Fisher exact test for categorical variables. Statistical comparisons for genotype frequencies between 2 groups were made by use of 2×3 table Fisher exact test (2×6 table for rs2032582). Associations between genotypes and KTD were tested by univariate and multivariate logistic regression analyses. The impact of other variables was estimated with univariate analysis, and those with $P < .20$ were incorporated into multivariate analysis, in addition to the basic demographics such as age and sex. Statistical significance was defined at 2-sided *P* value < .05. We used odds ratios (ORs) and 95% confidence intervals (95% CIs) to estimate the impact of each variable on KTD. The Haploview software was used to test Hardy-Weinberg equilibrium and *ABCC2* and *ABCC4* haplotype analysis. All other statistical analyses were performed with the Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, Illinois).

RESULTS

A total of 190 patients who provided blood and urine samples and satisfied the inclusion and exclusion criteria were enrolled in the study (Figure 1). KTD was diagnosed in 19 of the 190 patients (10%). The baseline characteristics and laboratory data for patients with and without KTD are listed in Table 1. Patients with KTD were older ($P < .001$), had smaller body weight ($P = .006$) and lower eGFR ($P = .003$), and were more likely to be hypertensive than patients with normal tubular function ($P = .088$). The median duration of tenofovir therapy was 71.5 weeks (interquartile range [IQR]: 36.8–109.2 weeks) for the entire study population, which was not different between the 2 groups ($P = .888$).

Table 1. Characteristics of Patients With and Without Kidney Tubular Dysfunction

	Patients With KTD (n = 19)	Patients With Normal Tubular Function (n = 171)	*P* Value
Variables for kidney tubular markers			
Urinary β2M (μg/g Cr)[a]	3066 (2247–10068)	209.2 (114.2–536.2)	<.001
Urinary α1M (mg/g Cr)[a]	26.5 (19.8–37.4)	7.95 (5.02–11.9)	<.001
Urinary NAG (U/g Cr)[a]	9 (6.2–14.3)	3.74 (2.84–4.95)	<.001
Fractional tubular resorption of phosphate[a]	83.9 (81.7–92)	91.9 (88.8–94.4)	<.001
Fractional excretion of uric acid[a]	9.7 (8.1–12.4)	6.4 (5.0–9.0)	<.001
Contribution of each parameter to KTD			
Urinary β2M > 1000 μg/g Cr, No. (%)	19 (100)	21 (12.3)	<.001
Urinary α1M > 16.6 mg/g Cr, No. (%)	18 (94.7)	17 (9.9)	<.001
Urinary NAG >5.93 U/g Cr, No. (%)	17 (89.5)	23 (13.5)	<.001
Fractional tubular resorption of phosphate <82%, No. (%)	5 (26.3)	2 (1.2)	<.001
Fractional excretion of uric acid >15%, No. (%)	2 (10.5)	4 (2.3)	.112
Characteristics			
Sex (male), No. (%)	18 (94.7)	166 (97.1)	.473
Age[a]	60 (41–62)	38 (32–42)	<.001
Route of transmission (homosexual contact), No. (%)	16 (84.2)	153 (89.5)	.528
Weight (kg)[a]	56 (53.5–66.5)	67.2 (58.1–75)	.006
Estimated glomerular filtration rate (mL/minutes/1.73 m^2)[a]	75.5 (62.8–93.5)	87.7 (77.5–98)	.003
Serum creatinine (mg/dL)[a]	0.85 (0.68–0.96)	0.80 (0.73–0.88)	.168
CD4 cell count (μL)[a]	380 (194–501)	379 (275–533)	.261
Serum phosphate (mg/dL)[a]	3.4 (2.7–3.7)	3.2 (2.9–3.6)	.815
Serum uric acid (mg/dL)[a]	4.7 (4.2–5.7)	5.6 (4.8–6.4)	.080
Nephrotoxic drug, No. (%)	2 (10.5)	12 (7.0)	.420
Hepatitis C, No. (%)	0 (0)	3 (1.8)	.728
Hepatitis B, No. (%)	2 (10.5)	24 (14)	.501
Dyslipidemia, No. (%)	4 (21.1)	54 (31.6)	.253
Hypertension, No. (%)	8 (42.1)	42 (24.6)	.088
C-reactive protein (mg/dL)[a]	0.07 (0.03–0.28)	0.07 (0.03–0.16)	.277
Duration of treatment with TDF (weeks)[a]	60.3 (17.7–115.4)	73.3 (37.7–109.1)	.888

Abbreviations: KTD, kidney tubular dysfunction; NAG, N-acetyl-β-D-glucosaminidase; TDF, tenofovir disoproxil fumarate.

[a] Median (interquartile range).

Table 2. Genotype Frequencies at *ABCC2*, *ABCC4*, *ABCC10*, *SLC22A6*, and *ABCB1* in Patients With and Without Kidney Tubular Dysfunction

Genotype	Amino Acid	Patients With KTD (n = 19)	Patients With Normal Tubular Function (n = 171)	*P* Value[a]
ABCC2 (MRP2)				
−24 C → T, rs717620				
C/C		18 (94.7)	108 (63.2)	
C/T		1 (5.3)	52 (30.4)	.018
T/T		0 (0)	11 (6.4)	
1249 G → A, rs2273697	Val417Ile			
G/G		11 (57.9)	133 (77.8)	
A/G		5 (26.3)	34 (19.9)	.017
A/A		3 (15.8)	4 (2.3)	
2366 C → T, rs56220353	Ser789Phe			
C/C		19 (100)	167 (97.7)	
C/T		0 (0)	3 (1.8)	1.000
T/T		0 (0)	1 (0.6)	
2934 G → A, rs3740070	Ser978Ser			
G/G		18 (94.7)	159 (93.0)	
G/A		1 (5.3)	11 (6.4)	1.000
A/A		0 (0)	1 (0.6)	
ABCC4 (MRP4)				
559 G → T, rs11568658	Gly187Trp			
G/G		13 (68.4)	133 (77.8)	
G/T		4 (21.1)	34 (19.9)	.126
T/T		2 (10.5)	4 (2.3)	
912G → T, rs2274407				
G/G		13 (68.4)	102 (59.6)	
T/G		6 (31.6)	52 (30.4)	.461
T/T		0 (0)	17 (9.9)	
2269 G → A, rs3765534	Glu757Lys			
G/G		15 (78.9)	129 (75.4)	
G/A		2 (10.5)	35 (20.5)	.241
A/A		2 (10.5)	7 (4.1)	
3348 A → G, rs1751034	Lys1116Lys			
A/A		13 (68.4)	98 (57.3)	
A/G		3 (15.8)	58 (33.9)	.185
G/G		3 (15.8)	15 (8.8)	
4135 T → G, rs3742106				
T/T		6 (31.6)	46 (26.9)	
T/G		7 (36.8)	79 (46.2)	.707
G/G		6 (31.6)	46 (26.9)	
4976T → C, rs1059751				
T/T		6 (31.6)	46 (26.9)	
T/C		5 (26.3)	86 (50.3)	.090
C/C		8 (42.1)	39 (22.8)	
ABCC10 (MRP7)				
526G → A, rs9349256				
G/G		4 (21.1)	32 (18.7)	
A/G		9 (47.4)	65 (38)	.569
A/A		6 (31.6)	74 (43.3)	

Table 2 continued.

Genotype	Amino Acid	Patients With KTD (n = 19)	Patients With Normal Tubular Function (n = 171)	*P* Value[a]
2759T → C, rs2125739	Ile920Thr			
T/T		15 (71.4)	131 (77.5)	
T/C		6 (28.6)	31 (18.3)	.488
C/C		0 (0)	7 (4.1)	
SLC22A6 (OAT1)				
180C → T, rs11568630				
C/C		18 (94.7)	164 (95.9)	
C/T		1 (5.3)	7 (4.1)	.577
T/T		0 (0)	0 (0)	
ABCB1 (P-glycoprotein)				
2677T → A/G, rs2032582	A:Ser893Thr G:Ser893Ala			
T/T		0 (0)	47 (27.5)	
T/A		3 (15.8)	14 (8.2)	
G/G		4 (21.1)	36 (21.1)	.002
G/T		8 (42.1)	46 (26.9)	
G/A		1 (5.3)	24 (14)	
A/A		3 (15.8)	4 (2.3)	

Abbreviation: KTD, kidney tubular dysfunction.

[a] By Fisher exact test.

Table 2 summarizes the distribution of genotypes at the *ABCC2*, *ABCC4*, *ABCC10*, *SLC22A11*, and *ABCB1* genes in the 2 groups. All polymorphisms were in Hardy-Weinberg equilibrium with a cutoff *P* value of .001. In single SNP analysis, a higher percentage of patients with KTD were found among genotype CC at position −24 and genotype AA at position 1249 of *ABCC2*, compared to patients with other genotypes (−24 CC; 14.3% [in 18 of 126 patients] vs 1.6% [in 1 of 64 patients]; $P = .004$; 1249 AA; 42.9% [in 3 of 7 patients] vs 8.7% [in 16 of 183 patients]; $P = .023$), respectively. The percentage of patients with KTD was also higher among genotype AA at position 2677 of *ABCB1*, compared to patients with other genotypes (2677 AA; 42.9% [in 3 of 7 patients] vs. 8.7% [in 16 of 183 patients]; $P = .023$). KTD was marginally associated with genotype AA at position 559 and genotype GG at position 4976 of *ABCC4* ($P = .112$, and .090, respectively).

Association of Genotypes with KTD

Univariate analysis showed a significant association between KTD and patients with genotype CC at position −24 (OR, = 10.50; 95% CI, 1.369–80.55; $P = .024$) and patients with genotype AA at position 1249 (OR, 7.828; 95% CI, 1.609–38.10; $P = .011$) of *ABCC2* (Table 3). The risk for KTD was higher in patients with both genotype CC at position −24 and genotype AA at position 1249 (OR, 31.88; 95% CI, 3.131–324.5; $P = .003$). Genotype AA at position 2677 of *ABCB1* was also significantly associated with KTD (OR, 7.828; 95% CI, 1.609–38.10; $P = .011$). Furthermore, old age (per 1 year, OR, 1.165; 95% CI, 1.100–1.233; $P < .001$), low body weight (per 1 kg decrement, OR, 1.076; 95% CI, 1.021–1.135; $P = .007$), and low eGFR (per 1 mL/minutes/1.73 m^2 decrement, OR, 1.052; 95% CI, 1.016–1.090; $P = .004$) were also associated with KTD.

Multivariate analysis identified genotype CC at position −24 and genotype AA at position 1249 of *ABCC2* as independent risks for KTD after adjustment for sex, age, weight, eGFR, and hypertension (adjusted OR, = 20.08; 95% CI, 1.711–235.7; $P = .017$; adjusted OR, 16.21; 95% CI, 1.630–161.1; $P = .017$), respectively (Table 4). Patients with both of the abovementioned two homozygotes showed higher adjusted OR in multivariate analysis (adjusted OR, 38.44; 95% CI, 2.051–720.4; $P = .015$; Table 4). On the other hand, genotype AA at position 2677 of *ABCB1* was not significantly associated with KTD in multivariate analysis adjusted for the abovementioned variables (adjusted OR, 1.686; 95%CI, .163–17.43; $P = .661$).

Association of Haplotypes at ABCC2 and ABCC4 with KTD

Haplotype construction was performed with the 4 identified SNPs with $P < .10$ in univariate analysis: *ABCC2*, −24 C → T, 1249 G → A; *ABCC4*, 559 G → T, 4976T → C (Table 4). Haplotypes with frequency of >1% were analyzed. *ABCC2* haplotype CA was significantly associated with TDF-induced KTD (OR, 2.910; 95% CI, 1.295–6.221; $P = .011$), whereas *ABCC2* haplotype TG was a protective haplotype (OR, 0.098; 95% CI, .002–.603; $P = .003$). *ABCC4* haplotype TT was marginally

Table 3. Univariate Analysis of Risks for Kidney Tubular Dysfunction in Patients With HIV Infection Treated With Tenofovir

Characteristic	OR	95% CI	*P* Value
Female sex	1.844	.204–16.67	.586
Age per 1 year	1.165	1.100–1.233	<.001
Weight per 1 kg decrement	1.076	1.021–1.135	.007
CD4 count per 1/μL decrement	1.002	.999–1.004	.261
Baseline eGFR per 1 mL/minutes/1.73 m^2 decrement	1.052	1.016–1.090	.004
Concurrent use of nephrotoxic drugs	1.559	.322–7.555	.581
Hepatitis B	0.721	.156–3.319	.674
C-reactive protein per 1 mg/dL	1.551	.689–3.494	.289
Hypertension	2.234	.843–5.922	.106
Dyslipidemia	0.578	.183–1.823	.349
Duration of treatment with tenofovir disoproxil fumarate (weeks)	0.999	.992–1.007	.888
ABCC2			
−24 CC	10.50	1.369–80.55	.024
1249 AA	7.828	1.609–38.10	.011
−24 CC plus 1249 AA	31.88	3.131–324.5	.003
2934 GG	1.358	.167–11.07	.775
ABCC4			
559 TT	4.912	.837–28.81	.078
912 TT	1.466	.531–4.042	.460
2269 AA	2.756	.530–14.34	.228
3348 GG	1.950	.510–7.463	.329
4135 GG	1.254	.450–3.494	.665
4976 CC	2.462	.925–6.547	.071
ABCC10			
526 GG	1.158	.360–3.725	.805
2759 TT	0.619	.220–1.738	.363
ABCB1			
2677 AA	7.828	1.609–38.10	.011

Abbreviations: CI, confidence interval; eGFR: estimated glomerular filtration rate; HIV, human immunodeficiency virus; OR, odds ratio.

[a] Due to low prevalence of minor alleles, rs56220353, rs11568630, and rs2274407 were not included in this analysis.

associated with tenofovir-induced KTD (OR, 2.497; 95% CI, .902–6.949; *P* = .077).

Table 4. Multivariate Analysis for the Risk of Tenofovir-Induced Kidney Tubular Dysfunction With Homozygotes at −24 and 1249 of *ABCC2* in Patients With HIV Infection

ABCC2	Adjusted OR	95% CI	*P* Value
Homozygote at −24 CC	20.08	1.711–235.7	.017
Homozygote at 1249 AA	16.21	1.630–161.1	.017
Homozygotes at −24 CC plus 1249 AA	38.44	2.051–720.4	.015

Each variable was adjusted for sex, age, weight, estimated glomerular filtration rate, and hypertension.

Abbreviations: CI, confidence interval; OR, odds ratio.

DISCUSSION

The present study demonstrated that genotype CC at position −24 and genotype AA at position 1249 of *ABCC2* gene are associated with tenofovir-induced KTD in Japanese patients with HIV-1 infection. The effect of SNPs was more evident in patients with both −24 CC and 1249 AA homozygotes than in those with either homozygote only. The findings of this study resolve long-term controversy over the role of genetic polymorphisms in tenofovir-induced KTD and confirm the effect of the SNPs in *ABCC2* gene in tenofovir-induced KTD.

CA haplotype (−24C, 1249A) of *ABCC2* was associated with tenofovir-induced KTD, whereas TG was a protective haplotype (Table 5). Izzedine et al [13] reported the role of CATC haplotype (−24C, 1249A, 3563T, 3972C) of *ABCC2* in KTD. However, 3563T did not play such role in this haplotype analysis, because the prevalence of 3563T is 0% in the Japanese, according to the HapMap data, and haplotype with only −24C plus 1249A still exhibited its effect on tenofovir-induced KTD (Table 5; www.hapmap.org). The reported association between tenofovir-induced KTD and 526G and 2759C of *ABCC10* described by Pushpakom et al [21] was also not reproduced in this study. Furthermore, SNPs in *ABCC4*, *SLC22A6*, and *ABCB1* investigated in the present study did not show a significant association with tenofovir-induced KTD (Table 3).

Three main aspects of our study are important. First, this is the first study to our knowledge that elucidated the effect of SNPs on tenofovir-induced KTD conducted in a country other than European countries or the United States. Our study examined Japanese patients of genetic background different from patients of previous studies, which consisted mostly of whites. While SNPs −24C and 1249A of *ABCC2* have been speculated to correlate with tenofovir-induced KTD in previous studies, the present study confirmed that these SNPs are risk factors for tenofovir-induced KTD in nonwhites.

The result that the SNPs in *ABCC2* are a risk for tenofovir-induced KTD can also be applied to patients with other genetic backgrounds who host SNPs −24C and 1249A. Notably, the impact of SNPs on tenofovir-induced KTD might be more significant in Africans and Indians than in Japanese or whites, considering that the allele frequencies of −24C and 1249A are higher in these population according to the HapMap data (−24C; Africans 96.9%, Indians 92.6%, Japanese 80.8%, whites 81.9%, 1249A; Africans 21.7%, Indians 30.7%, Japanese 8.9%, whites 23.7%; www.hapmap.org).

Second, the study was designed to evaluate the exclusive effect of SNPs on tenofovir-induced KTD by excluding

Table 5. Association Between Haplotype in *ABCC2* and *ABCC4* and Kidney Tubular Dysfunction

		Allele/Haplotype Frequency, %			
SNP Marker/Haplotype	Allele	KTD Group (n = 19)	Control Group (n = 171)	OR (95% CI)[a]	*P* Value
ABCC2					
−24 C → T	C	97.4	78.4	10.22 (1.658–419.8)	.003
1249 G → A	A	28.9	12.3	2.91 (1.345–6.296)	.011
ABCC2 haplotype	CA	28.9	12.3	2.91 (1.295–6.221)	.011
	TG	2.6	21.6	0.098 (.002–.603)	.003
ABCC4					
559 G → T	T	21.1	12.3	1.905 (.705–4.614)	.213
4976 T → C	T	48	55.3	0.746 (.375–1.470)	.399
ABCC4 haplotype					
TT	TT	17.6	7.9	2.497 (.902–6.949)	.077

Abbreviations: CI, confidence interval; KTD, kidney tubular dysfunction; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] ORs and *P* values are for comparisons of allele/haplotype frequencies between the kidney tubular dysfunction and control groups.

possible predisposing factors for KTD, for example, active infection, malignancies, diabetes mellitus, and preexisting renal impairment, which are known risks for KTD [35]. Patients who showed no HIV-1 viral suppression were also excluded. Furthermore, the enrolled patients were Japanese only, and this helped to examine a study population with comparatively similar genetic background. The study population was also on the same antiretroviral regimen (ritonavir-boosted darunavir plus tenofovir/emtricitabine), and this also helped to evaluate more precisely the effect of SNPs, because plasma concentration of tenofovir is affected by concomitant antiretrovirals and the delta change in plasma tenofovir concentration likely differs in the presence of each concomitant drug [26].

Third, SNPs were examined in 190 patients in this study. To our knowledge, the number of enrolled patients is the largest among the studies that have so far examined the effect of SNPs on tenofovir-induced KTD. Thus, this feature provided the study a higher statistical power than previous studies.

Why are polymorphisms in *ABCC2* a risk for tenofovir-induced KTD, even though it is controversial whether MRP2 plays a role in the excretion of tenofovir via the luminal membrane? [18, 20] The exact mechanism has not been determined yet, but we speculate 2 hypotheses. First, there might be unknown endogenous substances that influence tenofovir nephrotoxicity in renal tubular cells, and SNPs in *ABCC2* modulate the function or transportation of such substances [15]. Second, MRP2 may indeed take part in transporting tenofovir, because various substances including methotrexate are reported to be a substrate of MRP2, and *ABCC2* mutation alters excretion of those substances [36, 37]. Further studies are warranted to elucidate the exact mechanism of these SNPs on tenofovir-induced KTD. Furthermore, the impact of these SNPs on KTD with long-term TDF use needs to be evaluated in prospective studies.

Several limitations need to be acknowledged. First, not all polymorphisms in genes of the targeted transporter proteins were examined. Thus, we might have missed other important SNPs on the function of tenofovir transportation. There might be other unknown transporter proteins for tenofovir excretion in the kidney that contribute to susceptibility to tenofovir-induced KTD as well. Second, the diagnostic criteria for TDF-induced KTD are not uniformly established in the field and are different in the published studies. The criteria applied in this study are not entirely similar to the ones used in previous studies that examined the role of SNPs in tenofovir-induced KTD. However, by excluding other predisposing factors for KTD and enrolling a large number of patients, this study succeeded in providing a clear-cut association between SNPs and tenofovir-induced KTD.

In conclusion, the present study demonstrated that SNPs in *ABCC2* associate with tenofovir-induced KTD in Japanese patients, in a setting that excluded other predisposing factors. Assessment of renal tubular function is more cumbersome and costly to monitor than serum creatinine. However, monitoring tubular function is clinically important, because undetected long-term tubular dysfunction might lead to premature osteopenia due to phosphate wasting and accelerated progression of renal dysfunction. Close monitoring of tubular function is warranted in patients with *ABCC2* −24C and 1249A under TDF treatment.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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