

**Table 2. Comparison of Clinical Characteristics of Patients With and Without Invasive Amebiasis**

	Amebic Colitis (n = 11)	Extraintestinal IA <sup>a</sup> (n = 7)	Non-IA (n = 1189)	PValue IA vs Non-IA
Age (years), average (SD)	35.9 (12.3)	38.2 (11.0)	37.5 (10.8)	.81
Japanese nationality, no. (%)	10 (90.9)	6 (85.7)	1068 (89.8)	.71
Male sex, no. (%)	11 (100)	7 (100)	1119 (94.1)	.62
MSM, no. (%)	11 (100)	6 (85.7)	929 (78.1)	.15
TPHA test-positive, no. (%)	5 (45.5)	2 (28.6)	451/1175 (38.4)	.91
HBV exposure, <sup>a</sup> no. (%)	6 (54.5)	5 (71.4)	630/1178 (53.5)	.15
HCV Ab-positive, no. (%)	0/11 (0)	0/7 (0)	42/1172 (3.6)	1.00
Anti- <i>E. histolytica</i> at baseline, median (IQR)	×100 (<×100–×800)	×400 (×100–×400)	<×100 (<×100–<×100)	<.001
Anti- <i>E. histolytica</i> at the onset of IA, median (IQR)	×800 (×200–×800)	×400 (×100–×800)	...	
Follow-up period, median months (IQR)	7.8 (3.3–25.1)	10.5 (4.9–17.9)	25.5 (7.0–47.3)	

Data were compared using  $\chi^2$  test, Student *t* test, or Mann–Whitney *U* test for qualitative or quantitative variables, respectively.

Abbreviations: Ab, antibody; Anti-*E. histolytica*, anti *Entamoeba histolytica* antibody; HBV, hepatitis B virus; HCV, hepatitis C virus; IA, invasive amebiasis; IQR, interquartile range; MSM, men who have sex with men; SD, standard deviation; TPHA, *Treponema pallidum* hemagglutination.<sup>a</sup>Extraintestinal cases include one case of appendicitis and 6 cases of liver abscess.

(confirmed by identification of erythrophagocytic trophozoites in surgically removed specimen), amebic liver abscess in 6, and amebic colitis in 11 (confirmed by identification of erythrophagocytic trophozoites in stool samples). The median anti-*E. histolytica* titer at baseline was significantly higher among patients who developed invasive amebiasis than that among those who did not, but the other clinical and laboratory parameters were not different between the 2 groups (Table 2). Although no significant differences in the frequency of invasive amebiasis were evident in patients with ×100 ( $P = .77$ ) and ×200 ( $P = .18$ ) anti-*E. histolytica* titers at baseline, compared with negative anti-*E. histolytica* patients (<×100), the frequency was higher in patients with ×400 ( $P < .001$ ), ×800 ( $P = .025$ ), and  $\geq$ ×1600

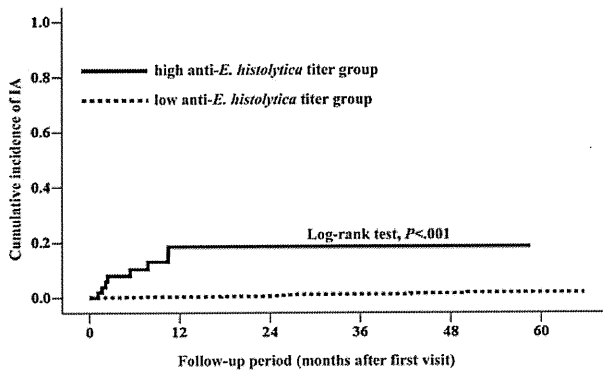
( $P < .001$ ) anti-*E. histolytica* titers at baseline, compared with negative anti-*E. histolytica* patients. Univariate and multivariate analyses also showed that future development of invasive amebiasis correlated only with high titer of anti-*E. histolytica* antibody at baseline ( $\geq$ ×400: Univariate, HR: 20.985, 95% confidence interval [CI], 8.085–54.467; multivariate, HR: 22.079, 95% CI, 7.964–61.215) (Table 3). Furthermore, the risk of development of invasive amebiasis was significantly higher in the high anti-*E. histolytica* titer group (patients with anti-*E. histolytica* titer  $\geq$ ×400 at baseline) than in the low anti-*E. histolytica* titer group (patients with anti-*E. histolytica* titer  $\leq$ ×200 at baseline; log-rank test:  $\chi^2 = 80.203$ ,  $P < .001$ , Kaplan–Meier estimate, Figure 2). Moreover, most patients of the high anti-*E. histolytica*

**Table 3. Risk Analysis for Development of Invasive Amebiasis by Cox Proportional Hazard Regression Model**

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	PValue	HR (95% CI)	PValue
older age (by 1 y)	0.989 (.947–1.033)	.624		
Japanese nationality	1.334 (.305–5.840)	.702		
Male sex	21.884 (.002–241297.39)	.516		
MSM	4.318 (.573–32.518)	.156	4.048 (.488–33.584)	.195
TPHA test-positive	0.901 (.348–2.335)	.831		
HBV exposure-positive	2.183 (.778–6.124)	.138	1.839 (.644–5.249)	.255
HCV Ab-positive	0.047 (.000–2697.344)	.584		
Anti- <i>E. histolytica</i> titer $\geq$ ×400	20.985 (8.085–54.467)	<.001	22.079 (7.964–61.215)	<.001

The Cox proportional-hazards regression analysis was used to estimate the impact of anti-*E. histolytica* titer at baseline on the incidence of invasive amebiasis. The impact of basic clinical characteristics, such as sexuality and serology status of other STIs, was estimated with univariate Cox proportional hazards regression. Multivariate Cox hazards regression analysis using variables identified in univariate analysis with *P* values of < .20. In all analyses, statistical significance was defined as *P* value of < .05.

Abbreviations: Ab, antibody; Anti-*E. histolytica*, anti *Entamoeba histolytica* antibody; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; IA, invasive amebiasis; MSM, men who have sex with men; TPHA, *Treponema pallidum* hemagglutination.



**Figure 2.** Incidence of invasive amebiasis in low and high anti-*E. histolytica* titer groups. Differences in the time from first visit to the diagnosis of invasive amebiasis (IA) between the low anti-*E. histolytica* titer group ( $\leq \times 200$  at baseline) and high anti-*E. histolytica* titer group ( $\geq \times 400$  at baseline) were analyzed by Kaplan-Meier method. Log-rank test was used to determine the statistical significance. Abbreviations: Anti-*E. histolytica*, anti-*Entamoeba histolytica* antibody; IA, invasive amebiasis.

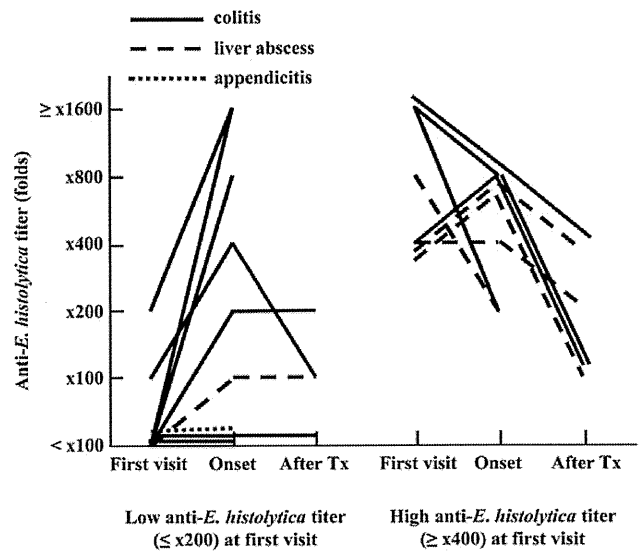
titer group developed invasive amebiasis during the first year of follow-up, whereas those of the low anti-*E. histolytica* titer group developed this complication more lately and new cases of invasive amebiasis were diagnosed throughout the follow-up period.

#### Transitional Changes in Anti-*E. histolytica* Titer Among Patients Who Developed Amebiasis

The median anti-*E. histolytica* titer was significantly higher at the onset of invasive amebiasis than that at first visit in patients with low baseline anti-*E. histolytica* titer ( $\leq \times 200$ ;  $P = .028$ , Wilcoxon signed-rank test) (Figure 3). In contrast, the median anti-*E. histolytica* titers at these 2 time points were not different in patients with high baseline anti-*E. histolytica* titer ( $\geq \times 400$ ;  $P = .18$ , Wilcoxon signed-rank test). Serum samples taken after nitroimidazole treatment (median time from the commencement of treatment 289 days [range 174–841]) were available in 10 patients. Anti-*E. histolytica* titers were lower after the treatment in 7 of the 10 patients, compared with the baseline values. To define the natural decay of anti-*E. histolytica*, we measured serum anti-*E. histolytica* titers at 9 months after study enrollment in 37 patients with high anti-*E. histolytica* titer at baseline but did not develop invasive amebiasis during the study period. The titers were lower, or similar to the baseline in 19 and 15 patients, respectively, whereas the remaining 3 patients showed 2-fold increase in the titer.

## DISCUSSION

In the present study, the seroprevalence of anti-*E. histolytica* antibody among HIV-1-infected patients was 21.3%, which was



**Figure 3.** Anti-*E. histolytica* titer before and after diagnosis of invasive amebiasis. Anti-*E. histolytica* titer at the onset of IA was compared to that at baseline (first visit to the clinic) by Wilcoxon signed-rank test. Anti-*E. histolytica* titers after treatment were measured at 219 days [range: 174–252] and 367 days [272–841] after the completion of treatment of patients with low and high anti-*E. histolytica* titer at first visit, respectively. Abbreviations: Anti-*E. histolytica*, anti-*Entamoeba histolytica* antibody; IA, invasive amebiasis.

much higher than those reported in other developed countries where amebiasis is considered as an STI [3, 9, 23, 24]. In addition, our results showed that sexually active MSM tend to be seropositive for *E. histolytica* infection, in agreement with previous studies from our group [27, 28].

The pathogenesis of amebiasis, such as incubation period after cyst ingestion and the mechanism of spontaneous remission, remains unclear. Although previous study showed anti-*E. histolytica*-positive children were more susceptible to *E. histolytica* infection than their seronegative counterparts [31], the clinical significance of anti-*E. histolytica* seropositivity and its titer in asymptomatic individuals had not been fully assessed. We measured serum anti-*E. histolytica* immunoglobulin M (IgM) levels in 18 patients at the onset of invasive amebiasis [32], but the level was detectable only in 3 patients with amebic colitis and 1 patient with liver abscess. The present study demonstrated that patients with high anti-*E. histolytica* titer ( $\geq \times 400$ ) at first visit developed invasive amebiasis much more frequently than those with low anti-*E. histolytica* titer ( $\leq \times 200$ ). The cumulative risk for invasive amebiasis among patients with high anti-*E. histolytica* titer at baseline rapidly increased during the first one year of follow-up but plateaued thereafter, suggesting that exacerbation of subclinical amebiasis occurs frequently within one year in these patients. On the other hand, the cumulative risk for invasive amebiasis among patients with low anti-*E. histolytica* titer at baseline increased more slowly and

developed at the same pace throughout the follow-up period, suggesting that the invasive amebiasis in these patients represented new infection rather than exacerbation of subclinical infection. The median anti-*E. histolytica* titer at the onset of invasive amebiasis in patients of high anti-*E. histolytica* titer group was not higher than that at first visit, whereas the titer increased at the onset compared with that at baseline in low anti-*E. histolytica* titer group. In addition, uni- and multivariate analyses identified high titer of anti-*E. histolytica* antibody at baseline as the only significant risk factor for future development of invasive amebiasis; seropositivity to other STIs was not a significant factor. These results add support to the aforementioned hypothesis regarding the difference in the pathology of invasive amebiasis between the high and low anti-*E. histolytica* groups. In this study, 15 asymptomatic but anti-*E. histolytica*-positive patients were treated with metronidazole at first visit (excluded from the follow-up analysis study), and none of them developed invasive amebiasis (median follow-up period, 11.7 months), suggesting the potential effectiveness of preemptive therapy for asymptomatic individuals with high anti-*E. histolytica* titer.

In conclusion, our results showed a relatively high prevalence of amebiasis in HIV-1-infected individuals in Japan, and that subclinical amebiasis is common among these individuals. The results emphasize the difficulty of disease control in not only individual patients with amebiasis but also in epidemiological control of this condition due to the long duration of subclinical infection of *E. histolytica*. Anti-*E. histolytica* testing for high-risk individuals could be helpful in early diagnosis of subclinical amebiasis, and early treatment of patients with such infection could prevent the development of invasive amebiasis and the transmission to others in the same community. Further studies to clarify the pathogenesis of invasive amebiasis are warranted.

## Notes

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# Low Raltegravir Concentration in Cerebrospinal Fluid in Patients With ABCG2 Genetic Variants

Kiyoto Tsuchiya, PhD,\* Tsunefusa Hayashida, PhD,\* Akinobu Hamada, PhD,†‡ Shingo Kato, PhD,§ Shinichi Oka, MD, PhD,\*|| and Hiroyuki Gatanaga, MD, PhD\*||

**Abstract:** Adenosine triphosphate-binding cassette transporter G2 (ABCG2) is expressed on the cerebrospinal fluid (CSF) side of choroid plexus epithelial cells, which form the blood-CSF barrier. Raltegravir was recently identified as a substrate of ABCG2. In the present study, we analyzed the relationship between single-nucleotide polymorphisms of ABCB1 and ABCG2 genes and raltegravir concentrations in 31 plasma and 14 CSF samples of HIV-infected patients treated with raltegravir-containing regimens. The mean CSF raltegravir concentration was significantly lower in CA (25.5 ng/mL) and AA (<10 ng/mL) genotypes at position 421 in ABCG2 gene compared with CC (103.6 ng/mL) genotype holders ( $P = 0.016$ ).

**Key Words:** antiretroviral therapy, raltegravir, cerebrospinal fluid concentrations, blood-cerebrospinal fluid barrier, adenosine triphosphate-binding cassette transporter G2

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## INTRODUCTION

Anatomical sanctuary sites in HIV-infected patients, where local drug exposure is lower than systemic compartment, are currently under intense investigation because they are suspected of hindering viral elimination by antiretroviral therapy (ART) and acting as sites for the selection of drug-resistant viruses during combination treatment. Especially the

brain, the largest sanctuary site, in which residual viruses may cause chronic encephalitis and neurocognitive disorders, is one of the hottest foci of current HIV researches. Raltegravir, one of the preferred integrase inhibitors in the current ART guidelines, is highly effective in penetrating the central nervous system,<sup>1</sup> although a high interpatient variability has also been reported.<sup>2,3</sup>

Anatomically, the blood-cerebrospinal fluid (CSF) barrier makes tight junction and consists of choroid plexus epithelial cells in the cerebral ventricle. The adenosine triphosphate-binding cassette transporter B1 (ABCB1), also known as P-glycoprotein or multidrug resistance protein 1, and the adenosine triphosphate-binding cassette transporter G2 (ABCG2), also known as breast cancer resistance protein, are expressed on the CSF side of choroid plexus epithelial cells, and both are involved in the active transport of drugs.<sup>4,5</sup> Moreover, ABCB1 and ABCG2 are also expressed in the intestines and contribute to the absorption of the drugs. Recently, raltegravir was found to be a substrate of both ABCB1 and ABCG2.<sup>6</sup> In the present study, we analyzed the relations between raltegravir plasma and CSF concentrations and single-nucleotide polymorphisms (SNPs) of ABCB1 and ABCG2 genomes.

## MATERIALS AND METHODS

HIV-1-infected patients treated with raltegravir-containing regimens (raltegravir 400 mg twice daily with 2 nucleotide/nucleoside reverse transcriptase inhibitors and/or protease inhibitors) were recruited at the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan. Blood samples were withdrawn into heparinized tubes 12 hours after raltegravir dosing (trough level), and the plasma was separated and stored at  $-80^{\circ}\text{C}$ . Stocked residues of CSF samples taken 3–4 hours after raltegravir dosing for clinical purposes were also subjected to analysis. The Ethics Committee for Human Genome Studies at the National Center for Global Health and Medicine approved this study (NCGM-A-000122-02) and allowed us the use of only residues of samples that were originally obtained for clinical purposes. Each patient provided a written informed consent.

Plasma and CSF raltegravir concentrations were measured by the reverse-phase high-performance liquid chromatography (HPLC) method. Briefly, 200  $\mu\text{L}$  of plasma or CSF and 400  $\mu\text{L}$  of ethyl acetate were vortexed in a tube for 10 seconds and centrifuged. The organic phase was transferred to a new tube and evaporated to dryness. Subsequently, the

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From the \*AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan; †Department of Clinical Pharmacology, Group for Translational Research Support Core, National Cancer Center Research Institute, Tokyo, Japan; ‡Department of Medical Oncology and Translational Research, Kumamoto University, Kumamoto, Japan; §Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo, Japan; and ||Center for AIDS Research, Kumamoto University, Kumamoto, Japan.

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Correspondence to: Hiroyuki Gatanaga, MD, PhD, AIDS Clinical Center, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan (e-mail: higatana@acc.ncgm.go.jp).

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residue was reconstituted in 250 µL of mobile phase, and 50 µL was injected into HPLC. Chromatography was performed, using Chromaster HPLC system (Hitachi, Tokyo, Japan) with RF-10A fluorescence detector (Shimadzu, Kyoto, Japan). Inertsil ODS-3 column (150 × 4.6 mm, 5-µm particle size; GL Sciences, Tokyo, Japan) was used as the analytical column. The flow rate was maintained at 1.5 mL per minute with fluorescence detection at 307 nm (excitation) and 415 nm (emission). The mobile phase consisted of acetonitrile/ethanol/phosphoric acid/water (20.8:20.8:0.1:58.3, vol/vol). Raltegravir calibration standards ranged from 10 to 2500 ng/mL. The accuracy of the analysis at 3 concentration levels ranged from -8.4% to +4.9%. Intraassay and interassay precisions were <4.8% and <7.6%, respectively. This assay was validated for both plasma and CSF raltegravir concentrations.

Genomic DNA was isolated from peripheral blood mononuclear cell, using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Genotyping of allelic variants of ABCB1 1236 C>T (rs1128503), 2677 G>T/A (rs2032582), 3435 C>T (rs1045642), 4036 A>G (rs3842), and ABCG2 421 C>A (rs2231142) was carried out using the TaqMan Drug Metabolism Assays by the ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA), according to the protocol provided by the manufacturer.

Differences between the groups were analyzed for statistical significance using the Kruskal-Wallis test. *P* values <0.05 denoted the presence of statistically significant difference. Analysis was performed using the SPSS Statistics software version 21 (IBM, Armonk, NY).

**RESULTS**

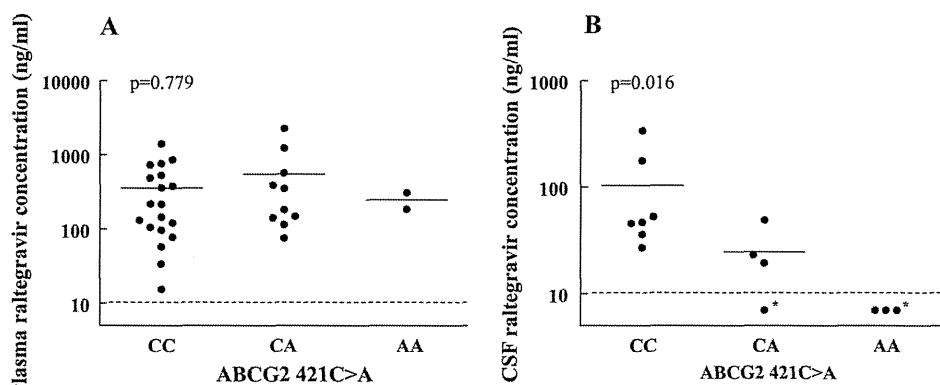
Plasma samples were collected from 31 patients, and stocked CSF samples from another group of 14 patients were used for the measurement of raltegravir concentrations.

All 45 patients (Japanese = 44, Myanmarian = 1) were subjected to SNP analysis of ABCB1 and ABCG2 genomes (Table 1). At position 1236 of ABCB1 gene, CC, CT, and TT genotypes were identified in 7, 21, and 17 patients, respectively. At position 2677, GG, GT, TT, GA, TA, and AA genotypes were identified in 8, 14, 11, 7, 4, and 1 patients, respectively. At position 3435, CC, CT, and TT genotypes were identified in 14, 17, and 14 patients, respectively. At position 4036, AA, AG, and GG genotypes were identified in 25, 18, and 2 patients, respectively. None of the genotypes of these SNPs in ABCB1 genome showed significant correlation with raltegravir concentration in plasma or CSF. At position 421 of ABCG2 gene, CC, CA, and AA genotypes were identified in 26, 14, and 5 patients, respectively. There was no significant correlation between the genotype at position 421 and trough concentration of raltegravir in plasma (Fig. 1A). However, in all 3 AA genotype holders, CSF raltegravir concentration was less than the lower limit of quantification (10 ng/mL) (Fig. 1B). Furthermore, in one of 4 CA genotype holders, CSF raltegravir concentration was below the detection limit, although it was higher than 25 ng/mL in any of the 7 CC genotype holders. The CA and AA genotype holders had significantly lower raltegravir concentrations in the CSF than the CC

**TABLE 1.** Genotype Frequencies of ABCB1 and ABCG2 Polymorphisms and Raltegravir Concentrations

		n	Raltegravir Concentration (ng/mL)*	<i>P</i>
Plasma (n = 31)				
ABCB1				
1236 C>T	rs1128503			
CC		3	480.1 ± 347.7	0.485
CT		16	489.5 ± 602.4	
TT		12	289.9 ± 324.5	
2677 G>T/A	rs2032582			
GG		5	254.6 ± 161.2	0.254
GT		12	569.9 ± 716.6	
TT		6	197.1 ± 70.5	
GA		4	648.9 ± 208.4	
TA		4	215.1 ± 219.4	
3435 C>T	rs1045642			
CC		9	358.2 ± 253.9	0.680
CT		13	533.5 ± 659.0	
TT		9	287.8 ± 362.0	
4036 A>G	rs3842			
AA		17	408.5 ± 535.7	0.594
AG		13	402.5 ± 457.7	
GG		1	572.8	
ABCG2				
421 C>A	rs2231142			
CC		19	355.5 ± 366.8	0.779
CA		10	550.1 ± 699.0	
AA		2	247.8 ± 88.7	
CSF (n = 14)				
ABCB1				
1236 C>T	rs1128503			
CC		4	140.8 ± 151.5	0.330
CT		5	35.6 ± 19.5	
TT		5	23.2 ± 14.7	
2677 G>T/A	rs2032582			
GG		3	31.7 ± 20.0	0.137
GT		2	36.1 ± 23.6	
TT		5	23.2 ± 14.7	
GA		3	188.0 ± 146.5	
AA		1	<10	
3435 C>T	rs1045642			
CC		5	122.5 ± 137.5	0.325
CT		4	30.4 ± 18.6	
TT		5	24.6 ± 17.5	
4036 A>G	rs3842			
AA		8	41.5 ± 56.6	0.061
AG		5	103.0 ± 132.5	
GG		1	<10	
ABCG2				
421 C>A	rs2231142			
CC		7	103.6 ± 116.0	0.016
CA		4	25.5 ± 16.8	
AA		3	<10	

\*Data are mean ± SD for concentrations ≥10 ng/mL.



**FIGURE 1.** ABCG2 421C>A genotype and raltegravir concentration in plasma at trough level (A) and in CSF (B). Horizontal straight line indicates mean value; dashed line indicates the lower limit of quantification (10 ng/mL). \*<10 ng/mL of raltegravir concentration.

genotype holders ( $P = 0.016$ ), when the concentration below the lower limit of quantification was considered 10 ng/mL.

## DISCUSSION

ABCG2 is diffusely expressed, whereas ABCB1 is weakly expressed on the CSF side of choroid plexus epithelial cells,<sup>7,8</sup> suggesting that the contribution of ABCB1 may be minor and that ABCG2 expression level in the choroid plexus is more likely to influence raltegravir concentration in the CSF than ABCB1. Previous studies indicated that genetic polymorphism of ABCG2 altered the protein expression level in plasmid transfection experiments.<sup>9,10</sup> Especially, C to A nucleotide substitution at position 421 significantly reduced the expression. The low expression induced by this nucleotide substitution may impair raltegravir transport from capillary blood to CSF, resulting in low raltegravir concentrations in CSF in holders of the CA/AA genotype at position 421. However, this SNP did not alter plasma raltegravir concentration significantly. Transporters other than ABCG2 may also exist in the intestines and further enhance raltegravir absorption. The presence of any antiretroviral at a concentration lower than that required for viral suppression could select drug-resistant HIV variants. In fact, we reported previously one patient with CSF raltegravir-resistant HIV variant, although the variant was not detected in the plasma.<sup>11</sup> The present study indicate that the genotype of this patient was AA at position 421 and that raltegravir concentration was below the lower limit of quantification in the CSF of this patient. Special attention should be paid to the raltegravir-containing ART of individuals with the CA/AA genotype at position 421 with active viral replication in the CNS, such as patients with HIV encephalitis.

Our study has certain limitations. Raltegravir concentrations were measured in plasma at trough level in 31 patients, and it was measured in stocked CSF samples of another group of 14 patients. First, we could not investigate the correlation between plasma and CSF concentrations because no paired plasma and CSF samples from the same subjects were available. Second, the time of CSF sampling in relation to raltegravir dosing varied among 3–4 hours. However, the population pharmacokinetic modeling of raltegravir concentration in the CSF showed a stable time course regardless of the dosing time.<sup>2,12</sup> Therefore, it is unlikely that the

sampling time had a large impact on CSF concentration of the CA/AA genotype at position 421 in ABCG2 gene. Further analysis of the correlation between ABCG2 genotype and raltegravir CSF concentration is warranted.

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# Long-term exposure to tenofovir continuously decrease renal function in HIV-1-infected patients with low body weight: results from 10 years of follow-up

Takeshi Nishijima<sup>a,b</sup>, Yohei Kawasaki<sup>c</sup>, Noriko Tanaka<sup>c</sup>,  
Daisuke Mizushima<sup>a,b</sup>, Takahiro Aoki<sup>a</sup>, Koji Watanabe<sup>a</sup>, Ei Kinai<sup>a</sup>,  
Haruhito Honda<sup>a</sup>, Hirohisa Yazaki<sup>a</sup>, Junko Tanuma<sup>a</sup>,  
Kunihisa Tsukada<sup>a</sup>, Katsuji Teruya<sup>a</sup>, Yoshimi Kikuchi<sup>a</sup>,  
Hiroyuki Gatanaga<sup>a,b</sup> and Shinichi Oka<sup>a,b</sup>

**Objectives:** To investigate the effect of long-term tenofovir disoproxil fumarate (TDF) use on renal function, especially in patients with low body weight who are vulnerable to TDF nephrotoxicity.

**Design:** A single-center, observational study in Tokyo, Japan.

**Methods:** We performed a 10 years cohort study of 792 HIV-1-infected patients. The effect of long-term TDF use on estimated glomerular filtration rate (eGFR) was investigated on treatment-naïve patients who started TDF-containing antiretroviral therapy ( $n = 422$ ) and those who started abacavir-containing antiretroviral therapy as control ( $n = 370$ ). Three renal endpoints were examined by the logistic regression model: decrement in eGFR of higher than 10 ml/min per 1.73 m<sup>2</sup> relative to the baseline, more than 25% decrement in eGFR, and eGFR lower than 60 ml/min per 1.73 m<sup>2</sup> at least 3 months apart. The loss in eGFR was estimated using linear mixed models for repeated measures.

**Results:** The median weight at baseline was 63 kg. TDF use increased the risk of all three renal outcomes compared with the control group: higher than 10 ml/min per 1.73 m<sup>2</sup> decrement in eGFR [adjusted odds ratio (OR) = 2.1, 95% confidence interval (CI) 1.45–3.14,  $P < 0.001$ ], more than 25% decrement (adjusted OR = 2.1, 95% CI 1.50–2.90,  $P < 0.001$ ), and eGFR lower than 60 ml/min per 1.73 m<sup>2</sup> at least 3 months apart (adjusted OR = 3.9, 95% CI 1.62–9.36,  $P = 0.002$ ). The cumulative mean loss relative to the control after 1, 2, 3, 4, and 5 years of TDF exposure was –3.8, –3.6, –5.5, –6.6, and –10.3 ml/min per 1.73 m<sup>2</sup>, respectively, indicating that the loss in eGFR increased over time ( $P < 0.001$ ).

**Conclusion:** In this cohort of patients with low body weight, TDF exposure increased the risk of renal dysfunction. Furthermore, the loss in eGFR relative to the control increased continuously up to 5 years.

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**Keywords:** HIV-1, low body weight, renal dysfunction, tenofovir disoproxil fumarate, treatment-naïve

<sup>a</sup>AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, <sup>b</sup>Center for AIDS Research, Kumamoto University, Kumamoto, and <sup>c</sup>Biostatistics Section, Department of Clinical Research and Informatics, Clinical Science Center, National Center for Global Health and Medicine, Tokyo, Japan.

Correspondence to Hiroyuki Gatanaga, MD, PhD, AIDS Clinical Center, National Center for Global Health and Medicine, 1-21-1, Toyama, Shinjuku, Tokyo 162-0052, Japan.

Tel: +81 3 3202 7181; fax: +81 3 5273 6483; e-mail: hihatana@acc.ncgm.go.jp

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## Introduction

Tenofovir disoproxil fumarate (TDF) is one of the most widely used nucleotide reverse transcriptase inhibitors (NRTIs) for the treatment of HIV-1 infection in both resource-rich and resource-limited settings [1,2], and also for the treatment of hepatitis B infection [3,4]. Furthermore, TDF, at a fixed dose with emtricitabine, has been approved in the United States for the prevention of sexual transmission of HIV-1 in uninfected adults as preexposure prophylaxis [5,6].

TDF is known to cause renal proximal tubular dysfunction [7–10] and also reduces estimated glomerular filtration rate (eGFR) more than other NRTIs [11–13]. To date, the extent of TDF-induced renal dysfunction is regarded as mild and tolerable [14,15], and one meta-analysis recommended that TDF use should not be restricted even when regular monitoring of renal function and serum phosphate levels is impractical [16]. Furthermore, although evidence is limited, most of the TDF-induced loss in renal function is considered to occur during the first year of exposure [12,15].

However, a large proportion of studies that investigated TDF nephrotoxicity were based on an analysis of a relatively short observation period, typically a few years, and little information is available on the effect of long-term TDF use on the prognosis of renal function. This is important as HIV-1 infection requires lifelong antiretroviral therapy (ART). In this regard, although small body weight is a well established risk factor for TDF nephrotoxicity [16,17], the TDF-related renal dysfunction has hardly been evaluated in patients with small body weight, who are potentially at higher risk for larger drug exposure and, thus, more severe toxicity [17–20].

Based on the above background, the current study was designed to investigate the effects of long-term TDF use on renal function in HIV-1-infected patients with low body weight, using 10 years data from our observational cohort study.

## Methods

### Study design and patients

We performed a single-center cohort study of HIV-1-infected patients using the medical records at AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo. The effect of long-term TDF use on renal function was investigated on treatment-naïve patients who started TDF-containing ART, and those who started abacavir (ABC)-containing ART as the control. ABC was chosen as the control because this NRTI is not known to be nephrotoxic and is not renally eliminated [21] and because the Japanese guidelines for

the treatment of HIV-1 infection placed both TDF and ABC as the preferred NRTIs throughout the observational period [22]. The inclusion criteria were treatment-naïvety, Japanese, age older than 17 years, and treatment with either the recommended 300 mg/day dose of TDF or 600 mg/day dose of ABC-containing standard ART (consisting of one nonnucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI) or integrase strand transfer inhibitor (INSTI), and two NRTIs) at our clinic between 1 January 2004 and 31 December 2011. Furthermore, the following exclusion criteria were applied: start of ART at other facilities, baseline eGFR of lower than 60 ml/min per 1.73 m<sup>2</sup>, discontinuation of TDF or ABC within 90 days after initiation of ART, or start of ART with both TDF and ABC. Of the 1334 patients who started ART at our clinic during the study period, 792 patients fulfilled these criteria and constituted the study patients (see Figure, Supplemental Digital Content 1, <http://links.lww.com/QAD/A537>, which shows patient enrollment process). The study patients were followed up until 31 December 2013. Censoring occurred at discontinuation of TDF or ABC, referral to other hospitals, loss to follow-up, death, or end of the observation period. The inclusion of Japanese patients only served to examine a population with relatively small body stature, compared with whites and African Americans [17]. The selection of TDF or ABC at baseline was left to the discretion of the attending physician, because both drugs were the preferred NRTIs during the study period in the Japanese guidelines [22]. The attending physician also selected the key drug (NNRTI, PI, or INSTI). In Japan, TDF became available from April 2004 and ABC from September 1999.

The study was approved by the human research ethics committee of National Center for Global Health and Medicine. All patients included in this study provided written informed consent for their clinical and laboratory data to be used and published for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

### Measurements

eGFR was calculated using the Japanese equation based on standardized serum creatinine, sex, and age, which was developed by the Japanese Society of Nephrology (JSN):  $eGFR = 194 \times [\text{serum creatinine}]^{-1.094} \times [\text{age}]^{-0.287} \times [0.739 \text{ if woman}]$  [23]. This equation was used because the Japanese equation performs better than The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [24] for patients with small body stature, such as Japanese, especially in individuals with GFR lower than 60 ml/min per 1.73 m<sup>2</sup> [25]. The 2013 practice guidelines for patients with CKD published by JSN also recommend the use of this equation for the Japanese, rather than CKD-EPI, which was derived mostly from whites and African Americans [25,26].

The baseline eGFR was estimated for each patient from age, sex, and serum creatinine measurements made closest to and preceding the commencement of ART by no more than 90 days. Patients visited our clinic at least every 3 months for monitoring CD4<sup>+</sup> cell count, HIV-1 viral load, and eGFR as the prescription period under the Japanese healthcare system is limited to 3 months. Thus, for calculation of follow-up eGFR value, we collected serum creatinine values measured closest to every 90 day within a range of 45 days from initiation of ART.

The potential risk factors for renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical records [16,19,27,28]. They included age, sex, body weight, BMI = {body weight (kg)/[height (m)]<sup>2</sup>}, history of AIDS, route of HIV-1 transmission, baseline laboratory data (CD4<sup>+</sup> cell count, HIV viral load, and serum creatinine), and presence or absence of other medical conditions (concurrent use of ritonavir-boosted PIs (PI/r), concurrent nephrotoxic drugs such as ganciclovir and sulfamethoxazole/trimethoprim, diabetes mellitus defined by using antidiabetic agents or fasting plasma glucose higher than 126 mg/dl or plasma glucose higher than 200 mg/dl on two different days, hypertension defined by current treatment with antihypertensive agents or two successive measurements of SBP higher than 140 mmHg or DBP higher than 90 mmHg at the clinic, dyslipidemia defined by current treatment with lipid-lowering agents, coinfection with hepatitis B defined by positive hepatitis B surface antigen, coinfection with hepatitis C defined by positive HCV viral load, and current smoking). At our clinic, body weight and blood pressure were measured on every visit, whereas other variables were measured in the first visit and at least once annually. We used the data on or closest to and preceding the day of starting ART by no more than 180 days.

### Statistical analysis

The primary exposure variable was TDF use over the control (ABC) as part of the initial ART. Three renal endpoints were applied in this study; we primarily focused on decrement in eGFR of higher than 10 ml/min per 1.73 m<sup>2</sup> relative to the baseline [29], because this endpoint is considered appropriate for patients with well maintained renal function, such as the study population; more than 25% decrement in eGFR relative to the baseline [17,18]; and two consecutive measurements of eGFR lower than 60 ml/min per 1.73 m<sup>2</sup> at least 90 days apart [30]. Changes in eGFR were plotted from the baseline measurement until occurrence of each of the three renal endpoints, and the logistic regression model was used to estimate the effect of TDF use over control on the occurrence of these renal endpoints. The model was adjusted for baseline eGFR, baseline body weight, nephrotoxic drug use, PI/r use, CD4<sup>+</sup> cell count, hypertension, dyslipidemia, and diabetes mellitus, which

are established risk factors for TDF nephrotoxicity [13,16,27,28]. Baseline age was not added to the model to avoid over adjustment because the equation for eGFR calculation already includes age, and the baseline age was not associated with TDF use, indicating that age is not a confounding factor for the association between TDF use and eGFR. Furthermore, older age at baseline was shown to be a predictive variable for lower baseline eGFR (linear regression,  $P < 0.0001$ ). In this case, adding predictive covariates to the logistic regression model will have detrimental effects on precision [31].

To investigate the effect of body weight on TDF-related nephrotoxicity, we did subgroup analysis for baseline weight categories: at least 70 kg and lower than 70 kg. Then, the multivariate logistic analysis for the renal endpoint of the occurrence of higher than 10 ml/min per 1.73 m<sup>2</sup> decrement in eGFR was conducted for each subgroup.

To further investigate the effect of TDF on renal function, we estimated the decrement in eGFR in the TDF group relative to the control group by calculating the difference in eGFR loss between the TDF and control group from baseline to 5 years after initiation of ART by 90 days intervals with a linear mixed models for repeated measures. We constructed the model with a random effect for patients. This model also included fixed effects for assigned treatment, baseline eGFR, baseline body weight, nephrotoxic drug use, PI/r use, CD4<sup>+</sup> cell count, hypertension, dyslipidemia, and diabetes mellitus. Interaction terms for time by treatment were included.

As additional analyses, the statistical analyses using eGFR calculated with CKD-EPI equation adjusted with the Japanese coefficient were also performed:  $eGFR = 0.813$  (a Japanese coefficient)  $\times 141 \times \min(SCr/\kappa, 1)^\alpha \times \max(SCr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$  (if female), where SCr is serum creatinine,  $\kappa$  is 0.7 for women and 0.9 for men,  $\alpha$  is  $-0.329$  for women and  $-0.411$  for men, min indicates the minimum of SCr/ $\kappa$  or 1, and max indicates the maximum of SCr/ $\kappa$  or 1 [32].

Statistical significance was defined at two-sided  $P < 0.05$ . We used odds ratios (ORs) with 95% confidence intervals (95% CIs) as a measure of the effect of TDF use on renal endpoints. All statistical analyses were performed with SAS Software, version 9.3 (SAS Institute, Cary, North Carolina, USA).

## Results

Of the 792 study patients, 422 patients started TDF-containing ART (TDF group) whereas the remaining 370 patients formed the control group who started ABC-containing ART (see Figure, Supplemental Digital

**Table 1. Baseline characteristics of patients who started tenofovir disoproxil fumarate-containing antiretroviral therapy and controls (abacavir-containing antiretroviral therapy).**

	Study patients (n=792)	TDF (n=422)	Control (ABC) (n=370)	P
Sex (male), n (%)	769 (97)	412 (98)	357 (97)	0.40
Age <sup>a</sup>	36 (31–43)	36 (31–43)	36 (31–44)	0.23
Weight (kg) <sup>a</sup>	63 (57.8–70.4)	62.9 (57.2–69.8)	63.8 (58.0–71.4)	0.25
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	22 (20.1–24.1)	21.9 (20.1–23.8)	22.2 (20.3–24.6)	0.23
eGFR (ml/min per 1.73 m <sup>2</sup> ) <sup>a</sup>	95.7 (84–110)	96.5 (84.7–111.5)	95.4 (83.7–108.6)	0.32
Serum creatinine (mg/dl) <sup>a</sup>	0.74 (0.66–0.82)	0.73 (0.66–0.82)	0.74 (0.67–0.83)	0.27
CD4 <sup>+</sup> cell count (/μl) <sup>a</sup>	189 (78–266)	199 (85–281)	183 (73–241)	0.002
HIV RNA viral load (log <sub>10</sub> /ml) <sup>a</sup>	4.76 (4.26–5.23)	4.76 (4.26–5.23)	4.76 (4.27–5.26)	0.93
Ritonavir-boosted protease inhibitors, n (%)	673 (85)	368 (87)	305 (82)	0.073
Protease inhibitors (unboosted), n (%)	28 (4)	8 (2)	20 (5)	0.011
NNRTIs, n (%)	48 (6)	20 (5)	28 (8)	0.10
INSTIs, n (%)	45 (6)	28 (7)	17 (5)	0.22
Hypertension, n (%)	118 (15)	41 (10)	77 (21)	0.001
Dyslipidemia, n (%)	9 (1)	5 (1)	4 (1)	1.00
Diabetes mellitus, n (%)	29 (4)	9 (2)	20 (5)	0.021
Concurrent use of nephrotoxic drugs, n (%)	218 (28)	88 (21)	130 (35)	<0.001
Hepatitis B, n (%)	62 (8)	57 (14)	5 (1)	<0.001
Hepatitis C, n (%)	37 (5)	20 (5)	17 (5)	1.00
History of AIDS, n (%)	183 (23)	89 (21)	94 (25)	0.15
Homosexual contact, n (%)	689 (87)	364 (86)	325 (88)	0.94
Current smoker, n (%)	369 (47)	193 (46)	176 (48)	0.57
ART duration (years) <sup>a</sup>	3.52 (2.29–5.18)	3.19 (2.20–4.67)	4.59 (2.48–5.18)	<0.001

ABC, abacavir; ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; TDF, tenofovir disoproxil fumarate.

<sup>a</sup>Median (interquartile range).

Content 1, <http://links.lww.com/QAD/A537>, which shows patient enrollment process). Table 1 shows the characteristics of the study population at baseline. The majority of the study population was men, comparatively young, and had a small body stature [median weight, 63 kg (interquartile range [IQR] 57.8–70.4 kg), median BMI 22.0 kg/m<sup>2</sup> (IQR 20.1–24.1)]. There was no difference in baseline eGFR between the two groups ( $P=0.32$ ). More than 80% of the patients of the two groups used PI/r. Patients of the TDF group had higher CD4<sup>+</sup> cell count ( $P=0.002$ ) and were less likely to have hypertension ( $P=0.001$ ), diabetes mellitus ( $P=0.021$ ), and on concurrent nephrotoxic drugs ( $P<0.001$ ), than the control. The median duration of ART was longer in the control group [median, 1675 days, interquartile range (IQR), 904–1890 days] than in the TDF group [median, 1164 days, IQR, 802–1705 days] ( $P<0.001$ ). The total observation period was 1347.5 patient-years for the TDF group and 1379.3 patient-years for the controls.

During the observation period, an eGFR decline from baseline of higher than 10 ml/min per 1.73 m<sup>2</sup> occurred in 348 (82.5%) of the TDF group and 265 (71.6%) of the control group (TDF use over control: adjusted OR 2.1, 95% CI 1.45–3.14,  $P<0.001$ ) (Table 2). Furthermore, higher baseline eGFR, higher CD4<sup>+</sup> cell count also increased the risk of higher than 10 ml/min per 1.73 m<sup>2</sup> decrement in eGFR.

More than 25% decrement in eGFR occurred in 172 (40.8%) patients of the TDF group and 97 (26.2%) of the

control (adjusted OR = 2.1, 95% CI 1.50–2.90,  $P<0.001$ ) (Table 3), and two consecutive measurements of eGFR lower than 60 ml/min per 1.73 m<sup>2</sup> were encountered in 26 (6.2%) patients of the TDF group and in 14 (3.8%) of the control (adjusted OR = 3.9, 95% CI 1.62–9.36,  $P=0.002$ ) (Table 4).

Subgroup analysis by baseline body weight above and below 70 kg showed that among patients with body weight at least 70 kg, TDF use relative to the control marginally increased the risk of higher than 10 ml/min per 1.73 m<sup>2</sup> decrement in eGFR (adjusted OR = 1.7, 95% CI 0.83–3.29,  $P=0.15$ ), whereas among patients weighing lower than 70 kg, the effect of TDF use was more evident (adjusted OR = 2.5, 95% CI 1.55–4.00,  $P<0.001$ ) than that among the entire study population (see Table 1, Supplemental Digital Content 2, <http://links.lww.com/QAD/A537>, which shows effects of initiating TDF-containing ART over control on higher than 10 ml/min per 1.73 m<sup>2</sup> decrement in eGFR according to baseline body weight).

Figure 1 shows the results of the linear mixed models for repeated measures up to 5 years. The adjusted cumulative mean loss increased continuously over the years in both the TDF and control groups: in TDF group, from –11.8 ml/min per 1.73 m<sup>2</sup> at 1 year of TDF to –23.7 ml/min per 1.73 m<sup>2</sup> at 5 years of TDF exposure, and in the control, from –8.0 ml/min per 1.73 m<sup>2</sup> at 1 year to –13.5 ml/min per 1.73 m<sup>2</sup> at 5 year of ART exposure. The adjusted cumulative mean loss in the TDF group

**Table 2. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over control on >10 ml/min per 1.73 m<sup>2</sup> decrement in estimated glomerular filtration rate: multivariate logistic regression analysis.**

	Adjusted OR	95% CI	P
TDF use relative to the control	2.1	1.45–3.14	<0.001
Baseline eGFR per 1 ml/min per 1.73 m <sup>2</sup> increment	1.1	1.05–1.08	<0.001
Weight per 1 kg increment	1.0	0.99–1.01	0.92
Use of nephrotoxic drugs	0.8	0.50–1.25	0.31
Use of ritonavir-boosted protease inhibitors	1.3	0.78–2.16	0.32
CD4 <sup>+</sup> cell count per 1/μl increment	1.0	1.00–1.00	<0.001
Hypertension	2.1	1.17–3.64	0.013
Dyslipidemia	1.0	0.21–4.60	0.98
Diabetes mellitus	1.9	0.63–5.86	0.25

ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

relative to the control continuously increased over time: at 1 year of exposure –3.8 ml/min per 1.73 m<sup>2</sup>, at 2 years –3.6 ml/min per 1.73 m<sup>2</sup>, at 3 years –5.5 ml/min per 1.73 m<sup>2</sup>, at 4 years –6.6 ml/min per 1.73 m<sup>2</sup>, and at 5 years –10.3 ml/min per 1.73 m<sup>2</sup> (see Table 2, Supplemental Digital Content 3, <http://links.lww.com/QAD/A537>, which shows adjusted loss in eGFR in the TDF group relative to the control estimated with mixed model for repeated measures). There was significant interaction between time and TDF use ( $P < 0.001$ ), suggesting that the adjusted mean loss in eGFR relative to the control increased significantly over time.

Additional analyses of renal function calculated with CKD–EPI equation also showed that TDF use doubled the risk of higher than 10 ml/min per 1.73 m<sup>2</sup> decrement (adjusted OR = 2.1, 95% CI 1.57–2.86,  $P < 0.001$ ) and more than 25% decrement (adjusted OR = 1.8, 95% CI

**Table 3. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over control on >25% decrement in estimated glomerular filtration rate relative to baseline: multivariate logistic regression analysis.**

	Adjusted OR	95% CI	P
TDF use over control	2.1	1.50–2.90	<0.001
Baseline eGFR per 1 ml/min per 1.73 m <sup>2</sup>	1.0	1.03–1.04	<0.001
Weight per 1 kg increment	1.0	0.98–1.01	0.37
Nephrotoxic drug use	0.7	0.47–1.03	0.073
Ritonavir-boosted protease inhibitor use	0.9	0.58–1.44	0.69
CD4 <sup>+</sup> cell count per 1/μl increment	1.0	1.00–1.00	0.007
Hypertension	1.5	0.96–2.49	0.074
Dyslipidemia	0.7	0.13–3.69	0.67
Diabetes mellitus	1.8	0.77–4.30	0.17

ART, antiretroviral therapy; CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

1.12–2.99,  $P = 0.017$ ). The effect of TDF use on the renal endpoint of lower than 60 ml/min per 1.73 m<sup>2</sup> was also marginally significant (adjusted OR = 2.7, 95% CI 0.71–10.5,  $P = 0.15$ ). The adjusted cumulative mean loss increased continuously in both the TDF and control groups: in TDF group, from –6.3 ml/min per 1.73 m<sup>2</sup> at 1 year to 15.0 ml/min per 1.73 m<sup>2</sup> at 5 years of TDF exposure, and in the control, from –4.1 ml/min per 1.73 m<sup>2</sup> at 1 year to –8.3 ml/min per 1.73 m<sup>2</sup> at 5 year of ART exposure. The cumulative mean loss in the TDF group relative to the control after 1, 2, 3, 4, and 5 years of TDF exposure was –2.2, –2.3, –3.2, –4.4, and –6.7 ml/min per 1.73 m<sup>2</sup>, respectively, which indicated that the loss in eGFR relative to control increased over time ( $P < 0.001$ ).

### Discussion

In this 10 years observational cohort of treatment-naive patients with low median body weight of 63 kg, initiation of TDF-containing ART doubled the risk of higher than 10 ml/min per 1.73 m<sup>2</sup> decrement or more than 25% decrement in eGFR relative to baseline, compared with the control patients who started ABC-containing ART, and also increased four-fold the risk of deterioration of eGFR to lower than 60 ml/min per 1.73 m<sup>2</sup>. The effect of TDF on the decrement in eGFR was more evident in patients with body weight of lower than 70 kg (TDF use over control: adjusted OR = 2.5, 95% CI 1.55–4.00,  $P < 0.001$ ) compared with the entire study population (adjusted OR = 2.1, 95% CI 1.45–3.14,  $P < 0.001$ ), whereas the effect of TDF on renal dysfunction was only marginally significant among patients with body weight of at least 70 kg (adjusted OR = 1.7, 95% CI 0.83–3.29,  $P = 0.15$ ).

More importantly, eGFR of the patients who started TDF-containing ART decreased continuously during the 5-year observation compared with the controls who started ABC-containing ART. The adjusted mean loss relative to the control increased from –3.8 ml/min per 1.73 m<sup>2</sup> at 1 year of TDF exposure to –5.5 ml/min per 1.73 m<sup>2</sup> at 3 years, and to –10.3 ml/min per 1.73 m<sup>2</sup> at 5 years of TDF exposure. This decrement in eGFR associated with TDF use is alarming considering that the aging-related decrement in normal renal function is only 0.4 ml/min per year [33]. The findings of the present study warrant long-term monitoring of renal function in HIV-1-infected patients with low body weight who start TDF-containing ART.

The present study has three main strengths. First, to our knowledge, this is the first study that elucidated the effect of long-term TDF use on the prognosis of renal function among HIV-1-infected patients with low body weight. Low body weight has been identified as a risk for TDF

**Table 4. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over the control on estimated glomerular filtration rate <60 ml/min per 1.73 m<sup>2</sup>; multivariate logistic regression analysis.**

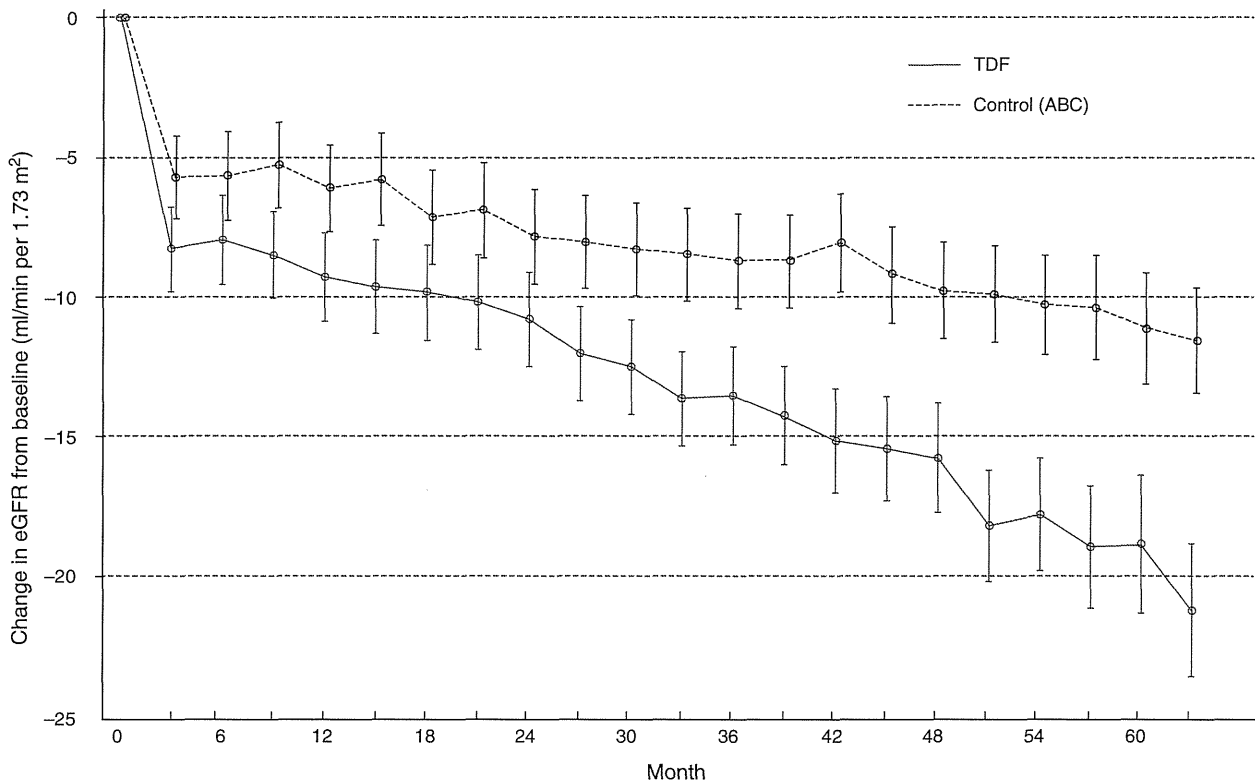
	Adjusted OR	95% CI	P
TDF use over control	3.9	1.62–9.36	0.002
Baseline eGFR per 1 ml/min per 1.73 m <sup>2</sup>	0.9	0.83–0.90	<0.001
Weight per 1 kg increment	1.0	0.93–1.00	0.069
Use of nephrotoxic drugs	0.6	0.22–1.52	0.27
Use of ritonavir-boosted protease inhibitors	1.4	0.47–3.89	0.57
CD4 <sup>+</sup> cell count per 1/μl increment	1.0	1.00–1.00	0.94
Hypertension	1.9	0.73–5.13	0.18
Dyslipidemia	2.1	0.23–18.7	0.52
Diabetes mellitus	3.7	0.85–16.2	0.083

ART, antiretroviral therapy; CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

nephrotoxicity [16,17], and it is noteworthy that many patients with HIV-1 infection are of small body stature.

Of 35.3 million estimated to be infected with HIV-1 at the end of 2012, most were from sub-Saharan Africa (25 million) and south and south-east Asia (3.9 million) [34], and studies from these regions report that HIV-1-infected patients were of low body weight (mean weight of 57.6 kg in treatment-naïve patients in Zimbabwe and Uganda [35], median 60 kg in west India [36], median 56.5 kg in Thailand [18], and mean 55 kg in Vietnam [37]). Considering that body weight of these patients are even lower than that in the present study of 63 kg, the effect of long-term TDF use on renal function might be more severe among patients in these regions.

Second, the study enrolled only treatment-naïve patients and followed their renal function up to 5 years after initiation of standard ART with one key drug and two NRTIs (including either TDF or ABC as control). This study design, together with its observational setting, allowed examination of the effect of long-term TDF use on the prognosis of renal function after the start of ART under ‘real-world’ setting, making the results of the present study more generalizable.



**Fig. 1. Adjusted mean change in estimated glomerular filtration rate from baseline to 5 years in treatment-naïve patients treated with tenofovir disoproxil fumarate-containing antiretroviral therapy (red line) and controls (patients treated with abacavir-containing ART) (black line).** Least-square means and their 95% confidence intervals were estimated by the linear mixed model. The x-axis is labeled ‘Months’ to make the figure visually understandable; however, 30 days is labeled here as 1 month. Thus, 3 months equals to 90 days and so on. The model included five fixed effects (assigned treatment, baseline eGFR, baseline body weight, nephrotoxic drug use and ritonavir-boosted protease use) in this figure. ABC, abacavir; ART, antiretroviral therapy; gGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate.

Third, the study employed the Japanese equation developed by the JSN for the calculation of eGFR [23,26]. Because commonly used methods, such as MDRD and CKD-EPI equations, were validated mostly in whites and African Americans, they are probably not appropriate for people of other ethnicity or of different body stature [23,38,39]. With regard to body stature, CKD-EPI was derived from datasets of people with mean weight of 79–82 kg [24], whereas the Japanese equation was derived from the set of people with mean weight of 60.4–61 kg [23]. Accordingly, clinicians are usually encouraged to validate their own equation or use MDRD or CKD-EPI equation with ethnic coefficient [25,38]. In the present study, using the Japanese equation for eGFR for Japanese patients probably yielded a better estimate of the effect of long-term TDF use on renal function [25]. Furthermore, additional analyses with use of CKD-EPI equation adjusted with the Japanese coefficient again showed that TDF exposure increased the risk of renal dysfunction and the loss in eGFR relative to the control increased continuously up to 5 years.

Apart from the above strengths, the present study has several limitations. First, because of its observational nature, there is a potential for channeling bias by indication for TDF use. Indeed, control patients were more likely to have risks for renal dysfunction, such as diabetes mellitus, hypertension, concurrent nephrotoxic drugs, and lower CD4<sup>+</sup> cell count [16,27], than patients who started TDF-containing ART. Thus, the incidence of TDF nephrotoxicity might have been underestimated in the present study. The median observation period of the control group was longer than that of the TDF group, and this might as well contribute to underestimation of TDF nephrotoxicity. Second, a high percentage of our study population used PI/r, which is considered a risk for TDF nephrotoxicity [28]. Although it is difficult to completely exclude the effect of concurrent PI/r, it should be noted that PI/r use itself (even without concurrent TDF) has been considered a risk for CKD [30,40], and the percentage of PI/r use was similarly high in both the TDF and control group, suggesting that PI/r affected renal function of the control patients to some extent as well. Furthermore, the use of PI/rs did not correlate with any of the three renal outcomes in this study (Tables 2–4). Third, all study participants were Japanese and we had a small number of women. Further studies are needed to determine whether the findings of this study are also applicable to women and patients of different racial background.

In conclusion, this long-term observational study of HIV-1-infected patients with predominantly low body weight demonstrated that initiation of TDF-containing ART doubled the risk of higher than 10 ml/min per 1.73 m<sup>2</sup> decrement and more than 25% decrement in eGFR, and also four-fold increased the risk of deterioration of eGFR to lower than 60 ml/min per 1.73 m<sup>2</sup>, compared with the controls who started ABC-containing ART. The loss in

eGFR in the TDF group relative to the control increased continuously over time and reached –10 ml/min per 1.73 m<sup>2</sup> at 5 years of TDF exposure. The results of the study certainly warrant regular and long-term monitoring of renal function in patients with low body weight who start TDF-containing ART. Further larger studies are needed to confirm the long-term renal prognosis with TDF use in patients with low body weight.

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## Conflicts of interest

S.O. has received honoraria and research grants from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K.; has received honoraria from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Co., Dainippon Sumitomo Pharma, Co., GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, Co., Torii Pharmaceutical, Co., and ViiV Healthcare. H.G. has received honoraria from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Torii Pharmaceutical, Co., Roche Diagnostics K.K., and ViiV Healthcare, Co.

The remaining authors declare no conflict of interest.

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## Single-nucleotide polymorphisms in the UDP-glucuronosyltransferase 1A-3' untranslated region are associated with atazanavir-induced nephrolithiasis in patients with HIV-1 infection: a pharmacogenetic study

Takeshi Nishijima<sup>1,2</sup>, Kiyoto Tsuchiya<sup>1</sup>, Noriko Tanaka<sup>3</sup>, Akane Joya<sup>1</sup>, Yohei Hamada<sup>1</sup>, Daisuke Mizushima<sup>1,2</sup>, Takahiro Aoki<sup>1</sup>, Koji Watanabe<sup>1</sup>, Ei Kinai<sup>1</sup>, Haruhito Honda<sup>1</sup>, Hirohisa Yazaki<sup>1</sup>, Junko Tanuma<sup>1</sup>, Kunihisa Tsukada<sup>1</sup>, Katsuji Teruya<sup>1</sup>, Yoshimi Kikuchi<sup>1</sup>, Shinichi Oka<sup>1,2</sup> and Hiroyuki Gatana<sup>1,2\*</sup>

<sup>1</sup>AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan; <sup>2</sup>Center for AIDS Research, Kumamoto University, Kumamoto, Japan; <sup>3</sup>Biostatistics Section, Department of Clinical Research and Informatics, Clinical Science Center, National Center for Global Health and Medicine, Tokyo, Japan

\*Corresponding author. AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan.  
Tel: +81-3-3202-7181; Fax: +81-3-5273-6483; E-mail: higtana@acc.ncgm.go.jp

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**Objectives:** Ritonavir-boosted atazanavir (atazanavir/ritonavir) is a widely used antiretroviral drug, though it can potentially cause nephrolithiasis. The aim of this study was to determine the relationship between polymorphisms in genes encoding proteins involved in metabolism and transportation of atazanavir, and atazanavir/ritonavir-induced nephrolithiasis in HIV-1-infected patients treated with atazanavir/ritonavir.

**Methods:** Nineteen SNPs in the *ABCB1*, *NR1I2*, *UGT1A1*, *SLCO1B1* and *CYP3A5* genes were examined in case patients with atazanavir/ritonavir-induced nephrolithiasis ( $n=31$ ) and controls ( $n=47$ ). Case patients were those with a clinical diagnosis of nephrolithiasis while on atazanavir/ritonavir, based on new-onset acute flank pain plus one of the following: (i) new-onset haematuria; (ii) documented presence of stones by either abdominal ultrasonography or CT; or (iii) confirmed stone passage. Control patients were consecutively enrolled among those with  $>2$  years of atazanavir/ritonavir exposure free of nephrolithiasis. Genotyping was performed by allelic discrimination using TaqMan 5'-nuclease assays with standard protocols. Associations between alleles and atazanavir/ritonavir-induced nephrolithiasis were tested by univariate and multivariate logistic regression analyses.

**Results:** Multivariate analysis showed a significant association between atazanavir/ritonavir-induced nephrolithiasis and genotype T/C versus C/C at position c.211 (adjusted OR=3.7; 95% CI, 1.13–11.9;  $P=0.030$ ), genotype G/C versus C/C at 339 (adjusted OR=5.8; 95% CI, 1.56–21.3;  $P=0.009$ ) and genotype G/G or G/C versus C/C at 440 (adjusted OR=5.8; 95% CI, 1.56–21.3;  $P=0.009$ ) of the UGT1A-3' untranslated region (UTR).

**Conclusions:** This is the first known study to identify the association between SNPs in the UGT1A-3'-UTR and atazanavir-induced nephrolithiasis. Further studies are warranted to confirm this association and to elucidate how these SNPs might influence atazanavir exposure.

**Keywords:** atazanavir sulphate, renal stones, SNPs

### Introduction

Ritonavir-boosted atazanavir (atazanavir/ritonavir) is a widely used protease inhibitor for the treatment of HIV-1 infection.<sup>1</sup> However, some case reports/series have documented nephrolithiasis containing atazanavir,<sup>2–5</sup> and cohort studies demonstrated that the incidence of nephrolithiasis is substantially higher in patients on atazanavir/ritonavir-containing ART than

patients on other protease inhibitor- or efavirenz-containing ART.<sup>6–8</sup> The development of renal stones, even a single episode, is a risk factor for significant decrement in renal function, which could affect the prognosis of patients.<sup>9–11</sup>

The mechanism of atazanavir-induced nephrolithiasis is not fully understood. However, unchanged atazanavir is reported to be excreted in urine at 7% of the administered dose, and strong acidity (e.g. pH 1.9) is required to achieve optimal dissolution of



atazanavir,<sup>12</sup> whereas urine is usually mildly acidic.<sup>6</sup> These characteristics of atazanavir are similar to those of indinavir, an old protease inhibitor well known for its precipitation and renal stone formation, and could explain the high incidence of nephrolithiasis in patients treated with atazanavir/ritonavir.<sup>7,13</sup>

A number of proteins are considered to take part in the metabolism or transportation of atazanavir, and thus can affect atazanavir exposure. Atazanavir is mainly metabolized by cytochrome P450 (CYP3A), including CYP3A4 and CYP3A5, and their variants can affect the concentration and clearance of atazanavir.<sup>14,15</sup> Minor biotransformation pathways for atazanavir or its metabolites include glucuronidation, suggesting that UDP-glucuronosyltransferase 1A1 (*UGT1A1*), known for its association with atazanavir-induced unconjugated hyperbilirubinaemia, is also involved in the metabolism of atazanavir.<sup>12</sup> SNPs in the *NR1I2* gene, which encodes the nuclear receptor pregnane X receptor (PXR), regulate the expression of *CYP3A4*<sup>16</sup> and *ABCB1*,<sup>17</sup> and also influence atazanavir concentration.<sup>18</sup> With regard to atazanavir transportation, P-glycoprotein is a membrane protein expressed on the cells of the intestine, hepatocytes and renal proximal tubules. Encoded by the *ABCB1* gene, P-glycoprotein regulates atazanavir intestinal absorption, and thus affects exposure to atazanavir.<sup>19–22</sup> The organic anion-transporting polypeptide 1B1 (*OATP1B1*), encoded by the gene *SLCO1B1*, is another protein involved in influx transportation of protease inhibitors and unconjugated bilirubin. SNPs in *SLCO1B1* also modify atazanavir concentration.<sup>23</sup>

To our knowledge, there are no published studies that investigated the association between genetic variants in the genes that encode proteins involved in the metabolism or transport of atazanavir and atazanavir-induced nephrolithiasis. Based on the above background, the present study was designed to elucidate the association between polymorphisms in genes encoding the abovementioned proteins and atazanavir-induced nephrolithiasis.

## Methods

### Ethics statement

The 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 written informed consent for genetic testing and publication of the clinical data. The study was conducted according to the principles expressed in the Declaration of Helsinki.

### Study design

We performed a case-control study to elucidate the association between SNPs in genes encoding proteins that take part in the metabolism of atazanavir and drug transporters and atazanavir-induced nephrolithiasis in a single-centre cohort.

### Study subjects

The eligible subjects were HIV-1-infected Japanese patients, aged >17 years, who commenced treatment with atazanavir/ritonavir-containing ART between 1 January 2004 and 30 June 2012,<sup>7</sup> including both treatment-naïve and treatment-experienced patients. Patients were excluded if they had (i) commenced atazanavir/ritonavir-containing ART during the study period at other facilities or (ii) been prescribed

unboosted atazanavir. Case patients were those in whom nephrolithiasis occurred while on atazanavir/ritonavir-containing ART. Nephrolithiasis was defined as described in previous studies:<sup>6,7</sup> cases with a clinical diagnosis by the attending physician based on new onset of acute flank pain plus one of the following: (i) new-onset haematuria confirmed by urine dipstick test; (ii) documented presence of stones or radiological findings suggestive of renal stones, such as hydronephrosis or obstruction or dilatation of the ureter, by either abdominal ultrasonography or CT; or (iii) stone passage confirmed by either the patient or the attending physician. Control patients were consecutively enrolled HIV-1-infected patients with >2 years of atazanavir/ritonavir experience who were free of nephrolithiasis based on the chart review. Enrolment took place from September 2012 to February 2013.

### Measurements

The potential risk factors for nephrolithiasis were determined according to previous studies and collected from the medical records, together with basic demographics.<sup>4,5,24–27</sup> They included age, sex, body weight, BMI (body weight (kg)/[height (m)]<sup>2</sup>), baseline laboratory data [CD4 cell count, HIV viral load, estimated glomerular filtration rate (eGFR) and serum uric acid] and the presence or absence of other medical conditions (concurrent use of tenofovir, past history of nephrolithiasis, previous exposure to indinavir, diabetes mellitus defined by using antidiabetic agents or fasting plasma glucose >126 mg/dL or plasma glucose >200 mg/dL on two different days, hypertension defined by current treatment with antihypertensive agents or two successive measurements of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg at the clinic, infection with hepatitis B virus defined by positive hepatitis B surface antigen, and infection with hepatitis C virus defined by positive hepatitis C viral load). eGFR was calculated using the equation of the four-variable Modification of Diet in Renal Diseases (MDRD) study.<sup>28</sup> We used the data on or closest to and preceding the day of starting atazanavir/ritonavir-containing ART by no more than 180 days, except for serum uric acid level, which were collected within 180 days from the day of starting ART.<sup>7</sup> The value of serum total bilirubin was collected in two ways: for patients who continued atazanavir/ritonavir for >2 years, the value of total bilirubin closest to 2 years after initiation of atazanavir/ritonavir was collected. For patients who discontinued atazanavir/ritonavir within 2 years, the value closest to and preceding the day of discontinuation was used. At our clinic, body weight and blood pressure were measured on every visit.

### Genetic polymorphisms

SNPs in genes encoding proteins that take part in the metabolism of atazanavir and drug transport were selected based on their functional significance, findings of previously published reports and/or reported minor-allele frequencies >5% in the Japanese.<sup>15,18,20,23,29–32</sup> The allele frequency data for the Japanese were obtained from the Japanese SNP (JSNP) database.<sup>33</sup> The 19 selected SNPs were: (i) *ABCB1* (encodes P-glycoprotein) 2677T→A/G (A:Ser893Thr, G:Ser893Ala; rs2032582); 1236T→C (Gly412Gly; rs1128503); 3435C→T (Ile1145Ile; rs1045642); 193A→G [in the 3' untranslated region (UTR)]; (rs3842)]; 365T→C (5'-UTR; rs3213619); (ii) *NR1I2* (encodes PXR) 370G→A (3'-UTR; rs3732359); 522C→T (3'-UTR; rs3732360); 131C→A (5'-UTR; rs1523127); 1232T→C (3'-UTR; rs3814058); 1195A→C (3'-UTR; rs3814057); 63396T→C (intron; rs2472677); 44477T→C (5'-UTR; rs1523130); (iii) *UGT1A1* 211G→A (Gly71Arg; rs4148323); c.211T→C (3'-UTR; rs10929303), 339G→C (3'-UTR; rs1042640); 440G→C (3'-UTR; rs8330); (iv) *SLCO1B1* (encodes OATP1B1) 521T→C (Val174Ala; rs4149056); 388A→G (Asn130Asp; rs2306283); and (v) *CYP3A5* (encodes cytochrome P450 3A5) 14T→C (3'-UTR; rs15524). We did not find appropriate SNPs in *CYP3A4* to examine. The *UGT1A1* variant that contains seven thymine adenine (TA) nucleotide

repeats, A (TA)<sub>7</sub>TAA (UGT1A1\*28), which is known to be less transcriptionally active than the common promoter with six TA repeats (UGT1A1\*1),<sup>34</sup> was also examined.

**Pharmacogenetic analyses**

Genomic DNA was extracted from peripheral blood leucocytes using the QIAamp DNA MiniKit and the protocol provided by the manufacturer (Qiagen, Valencia, CA, USA). All genotyping was performed by allelic discrimination using TaqMan 5'-nuclease assays with standard protocols (TaqMan SNP Genotyping Assays; Applied Biosystems, Foster City, CA, USA). The primer and probe sequences are available on request. Primer sequences for PCR amplification of the TATA box of the UGT1A1 promoter were 5'-GTCACGTGACACAGTCAAAC-3' and 3'-TTTGCTCTGCCAGAGGT T-5';<sup>35</sup> the PCR conditions were as follows: 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 58°C for 30 s and 72°C for 30 s, and 72°C for 7 min.

**Statistical analysis**

Baseline characteristics were compared between case patients and control patients by Student's *t*-test for continuous variables and by either the  $\chi^2$  test or Fisher's exact test for categorical variables. Differences in genotype frequencies and allele frequencies between the two groups were assessed by Fisher's exact test using a 2x3 table (2x6 table for rs2032582) and the  $\chi^2$  test, respectively. Associations between genotypes and atazanavir-induced nephrolithiasis were tested by univariate and multivariate logistic regression analyses. The risk of atazanavir-induced nephrolithiasis of other variables was estimated with univariate analysis and the variables with *P*<0.10 were incorporated into multivariate analysis as covariates, in addition to the basic demographics, such as age and sex. Statistical significance was defined as a two-sided *P* value of <0.05. We used ORs and 95% CIs to estimate the strength of association between nephrolithiasis and each variable. Haploview software was used to test for Hardy-Weinberg equilibrium and to estimate the linkage

disequilibrium measure *D'*. All other statistical analyses were performed with the Statistical Package for Social Sciences version 21.0 (SPSS, Chicago, IL, USA).

**Results**

Of 37 patients diagnosed with nephrolithiasis while on atazanavir/ritonavir-containing ART,<sup>7</sup> 31 provided written informed consent, and thereby constituted the case patients. Furthermore, 47 consecutive control patients who continued atazanavir/ritonavir for >2 years were enrolled in the study. The baseline characteristics and laboratory data of patients in the two groups are listed in Table 1. The basic demographics (sex and age) and established risk factors for nephrolithiasis (weight, BMI, serum uric acid, hypertension, diabetes mellitus, history of nephrolithiasis and history of indinavir use) were not different between the two groups, except for hepatitis C infection, which was more common among case patients (*P*=0.034). Serum total bilirubin was higher in case patients than the controls (*P*<0.001).

Table 2 summarizes the distribution of genotypes and allele frequencies at the *ABCB1*, *NR1I2*, *UGT1A1* (including *UGT1A1*\*28), *SLCO1B1* and *CYP3A5* genes in the two groups. The genotype distributions for all polymorphisms were in Hardy-Weinberg equilibrium with a cut-off *P* value of 0.001. In single SNP analysis, a higher percentage of patients with nephrolithiasis had genotype T/C versus C/C at position c.211 (*P*=0.025), genotype G/C versus C/C at position 339 (*P*=0.007) and genotype G/G and G/C versus C/C at position 440 (*P*=0.009) of the UGT1A-3'-UTR. These results were consistent with allele frequency analysis, as case patients were more likely to possess allele T versus C at c.211 (*P*=0.033), allele G versus C at position 339 (*P*=0.012) and allele G versus C at 440 (*P*=0.006) of the UGT1A-3'-UTR, respectively. These three SNPs were in linkage disequilibrium with each other (*D'*>0.9). Figure 1

**Table 1.** Characteristics of patients with and without nephrolithiasis

	Total patients	Patients with nephrolithiasis (n=31)	No nephrolithiasis (n=47)	<i>P</i> value
Male, n (%)	71 (91)	29 (94)	42 (89)	0.70
Age (years), median (IQR)	40 (35-47)	39 (35-45)	40 (35-49)	0.40
Weight (kg), median (IQR)	65.6 (58.2-72.3)	64.8 (57.6-71.9)	66.5 (58.2-73.2)	0.60
BMI (kg/m <sup>2</sup> ), median (IQR)	22.7 (20.3-24.6)	22.7 (19.9-24.9)	22.7 (20.4-24.6)	0.58
eGFR (mL/min/1.73 m <sup>2</sup> ), median (IQR)	88.3 (76.8-98.4)	85.8 (69.7-97.9)	88.5 (78.4-98.6)	0.81
Serum creatinine (mg/dL), median (IQR)	0.75 (0.68-0.89)	0.75 (0.72-0.93)	0.75 (0.66-0.85)	0.25
CD4 cell count (cells/mm <sup>3</sup> ), median (IQR)	230 (187-302)	232 (194-356)	229 (187-290)	0.63
HIV-1 load (log <sub>10</sub> /mL), median (IQR)	4.19 (3.38-4.77)	3.96 (2.25-4.79)	4.22 (3.41-4.77)	0.65
Serum uric acid (mg/dL), median (IQR)	6.1 (5.1-7.0)	6.3 (5.5-7.7)	5.9 (4.9-6.7)	0.15
History of nephrolithiasis, n (%)	6 (8)	4 (13)	2 (4)	0.21
Hypertension, n (%)	9 (12)	3 (10)	6 (13)	1.00
Diabetes mellitus, n (%)	3 (4)	1 (3)	2 (4)	1.00
Hepatitis C infection, n (%)	6 (8)	5 (16)	1 (2)	0.034
Hepatitis B infection, n (%)	2 (3)	1 (3)	1 (2)	1.00
Treatment-naïve, n (%)	50 (64)	18 (58)	32 (68)	0.47
Co-administration of TDF, n (%)	26 (33)	9 (29)	17 (36)	0.63
History of indinavir use, n (%)	6 (8)	3 (10)	3 (6)	0.68
Serum total bilirubin (mg/dL)	1.9 (1.4-2.4)	2.3 (1.8-3.4)	1.7 (1.3-1.9)	<0.001

TDF, tenofovir disoproxil fumarate.

**Table 2.** Genotype/allele frequencies for *ABCB1*, *NR1I2*, *UGT1A1*, *SLCO1B1* and *CYP3A5* in patients with and without nephrolithiasis

	Amino acid	Genotype frequency			P value <sup>a</sup>	nephrolithiasis (n=31)	no nephrolithiasis (n=47)	nephrolithiasis (n=31)
		nephrolithiasis (n=31)	no nephrolithiasis (n=47)	nephrolithiasis (n=31)				
<i>ABCB1</i> (P-glycoprotein)							<i>ABCB1</i> (P-glycoprotein)	
193 A→G, rs3842							193 A→G, rs3842	
A/A		16 (52)	27 (57)	0.24			A	
A/G		14 (45)	14 (30)				G	
G/G		1 (3)	6 (13)					
365 T→C, rs3213619							365 T→C, rs3213619	
T/T		28 (90)	37 (79)	0.23			T	
T/C		3 (10)	10 (21)				C	
C/C		0	0					
1236 T→C, rs1128503	Gly412Gly						1236 T→C, rs1128503	
T/T		7 (23)	15 (32)	0.48			T	
T/C		19 (61)	22 (47)				C	
C/C		5 (16)	10 (21)					
2677 T→A/G, rs2032582	A:Ser893Thr G:Ser893Ala						2677T→A/G, rs2032582	
T/T		5 (16)	5 (11)	0.45			T	
T/A		6 (19)	5 (11)				A	
G/G		3 (10)	10 (21)				G	
G/T		10 (32)	15 (32)					
G/A		6 (19)	12 (26)					
A/A		1 (3)	0					
3435 C→T, rs1045642	Ile1145Ile						3435 C→T, rs1045642	
C/C		10 (32)	22 (47)	0.35			C	
C/T		16 (52)	21 (45)				T	
T/T		5 (16)	4 (9)					
<i>NR1I2</i> (PXR)							<i>NR1I2</i> (PXR)	
131 A→C, rs1523127							131 A→C, rs1523127	
C/C		1 (3)	6 (13)	0.38			C	
C/A		18 (58)	23 (49)				A	
A/A		12 (39)	18 (38)					
370 G→A, rs3732359							370 G→A, rs3732359	
G/G		8 (26)	16 (34)	0.37			G	
G/A		19 (61)	21 (45)				A	
A/A		4 (13)	10 (21)					
522 C→T, rs3732360							522 C→T, rs3732360	
C/C		8 (26)	15 (32)	0.60			C	
C/T		19 (61)	23 (49)				T	
T/T		4 (13)	9 (19)					
1195 A→C, rs3814057							1195 A→C, rs3814057	
A/A		4 (13)	11 (23)	0.39			A	
A/C		19 (61)	22 (47)				C	
C/C		8 (26)	14 (30)					

1232 T→C, rs3814058				1232 T→C, rs3814058			
T/T	4 (13)	10 (21)		T	27 (44)	41 (44)	1.00
T/C	19 (61)	21 (45)	0.37	C	35 (56)	53 (56)	
C/C	8 (26)	16 (34)					
44477 T→C, rs1523130				44477 T→C, rs1523130			
T/T	1 (3)	6 (13)		T	19 (31)	34 (36)	0.48
T/C	17 (55)	22 (47)	0.38	C	43 (69)	60 (64)	
C/C	13 (42)	19 (40)					
63396 T→C, rs2472677				63396 T→C, rs2472677			
T/T	18 (58)	24 (51)		T	45 (73)	68 (72)	0.98
T/C	9 (29)	20 (43)	0.38	C	17 (27)	26 (28)	
C/C	4 (13)	3 (6)					
UGT1A1				UGT1A1			
*28 <sup>b</sup>				*28 <sup>b</sup>			
*1/*1	27 (87)	44 (94)		*1	57 (92)	91 (97)	0.21
*1/*28	3 (10)	3 (6)	0.50	*28	5 (8)	3 (3)	
*28/*28	1 (3)	0					
211 G→A, rs4148323	Gly71Arg			211 G→A, rs4148323			
G/G	19 (61)	30 (64)		G	49 (79)	73 (78)	0.85
G/A	11 (36)	13 (28)	0.61	A	13 (21)	21 (22)	
A/A	1 (3)	4 (9)					
c.211 T→C, rs10929303				c.211 T→C, rs10929303			
T/T	0	0		T	11 (18)	6 (6)	0.033
T/C	11 (35)	6 (13)	0.025	C	51 (82)	88 (94)	
C/C	20 (65)	41 (87)					
339 G→C, rs1042640				339 G→C, rs1042640			
G/G	0	0		G	11 (18)	4 (4)	0.012
G/C	11 (35)	4 (9)	0.007	C	51 (82)	90 (96)	
C/C	20 (65)	43 (91)					
440 G→C, rs8330				440 G→C, rs8330			
G/G	1 (3)	0		G	12 (19)	4 (4)	0.006
G/C	10 (32)	4 (9)	0.007	C	50 (81)	90 (96)	
C/C	20 (65)	43 (91)					
SLCO1B1				SLCO1B1			
388 A→G, rs2306283	Asn130Asp			388 A→G, rs2306283			
A/A	14 (45)	24 (51)		A	42 (68)	68 (72)	0.54
A/G	14 (45)	20 (43)	0.83	G	20 (32)	26 (28)	
G/G	3 (10)	3 (6)					
521 T→C, rs4149056	Val174Ala			521 T→C, rs4149056			
T/T	20 (65)	31 (66)		T	51 (82)	75 (80)	0.71
T/C	11 (36)	13 (28)	0.34	C	11 (18)	19 (20)	
C/C	0	3 (6)					
CYP3A5				CYP3A5			
14 T→C, rs15524				14 T→C, rs15524			
T/T	16 (52)	25 (53)		T	46 (74)	67 (71)	0.70
T/C	14 (45)	17 (36)	0.50	C	16 (26)	27 (29)	
C/C	1 (3)	5 (11)					

<sup>a</sup>By Fisher's exact test.

<sup>b</sup>\*1, reference sequence A(TA)<sub>6</sub>TAA; \*28, A(TA)<sub>7</sub>TAA.