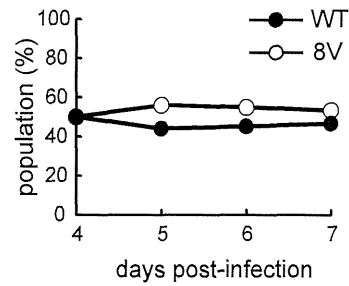


Supplementary Figure 1:

Recognition of TI8 (WT) and TV8 (8V) peptides by TI8-specific CTL clones.

Cytotoxic activity toward C1R-B*51:01 cells prepulsed with TI8 or TV8 peptide at concentrations of 1 to 100 nM was determined. The cytotoxic activity was measured at an E:T ratio of 2:1.



Supplementary Figure 2:

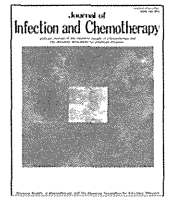
Replication capacity of WT and the 8V mutant viruses.

A competitive proliferation assay using WT and the 8V mutant viruses. T1 cells were infected with WT and the 8V mutant viruses at a ratio of 1:1. The culture supernatants were collected from day 4 to day 7 post infection. Viral RNA extracted from the supernatants was subjected to RT-PCR followed by nested PCR for direct sequencing. The population change in the viral mixture was determined by the relative peak height on the sequencing electrogram.



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Original article

Low body weight and tenofovir use are risk factors for renal dysfunction in Vietnamese HIV-infected patients. A prospective 18-month observation study



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ABSTRACT

Background: The use of tenofovir has been rapidly increasing in Vietnam. Several studies identified low body weight as a risk factor for tenofovir-induced nephrotoxicity. However, little is known about the impact of tenofovir on renal function in HIV-infected Vietnamese with generally low weight.

Methods: An observational single-center cohort of adult HIV-infected patients on antiretroviral therapy at National Hospital of Tropical Diseases, Hanoi. Patients on tenofovir or with creatinine clearance ≤ 60 ml/min at baseline were excluded. The incidence of renal dysfunction was compared between patients who switched to tenofovir and those who did not. Renal dysfunction was defined as 25% decline of creatinine clearance from baseline. Time to renal dysfunction was analyzed by the Kaplan–Meier method between the two groups. The Cox hazard model was used to determine risk factors for renal dysfunction in uni- and multivariate analyses.

Results: Of 556 patients enrolled in this study, 403 were non-tenofovir group while 153 were the tenofovir-switched group. Renal dysfunction occurred at a higher rate in the tenofovir-switched group (92.5 per 1000 person-years) than the non-tenofovir group (47.8 per 1000 person-years) ($p = 0.023$, Log-rank test). Multivariate analysis confirmed that tenofovir use, low body weight and glucosuria were significant risk factors for renal dysfunction (hazard ratio = 1.980; 95% confidential interval, 1.094–3.582, HR = 1.057; 95%CI, 1.016–1.098, HR = 5.202; 95%CI, 1.245–21.738, respectively).

Conclusions: Tenofovir use, low body weight and glucosuria were significant risk factors for renal dysfunction. We suggest close monitoring of renal function in patients with these risk factors even in resource-limited setting.

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Key points

Treatment with TDF and low body weight were significant risk factors for renal dysfunction in Vietnamese HIV-treated patients. Given that the average body weight of Vietnamese is small, close monitoring of renal function in HIV-1-infected patients is important during treatment with TDF.

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1. Introduction

Although renal dysfunction is an important cause of morbidity and mortality in HIV-infected patients [1–7], only limited information is available on renal function in Vietnamese HIV-infected patients. Along with the 2010 WHO guidelines which phased out stavudine and recommended tenofovir (TDF) (URL: http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf), the use of TDF had been increasing in Vietnam in recent years.

TDF-associated nephrotoxicity is well known adverse effect. However, a meta-analysis study that evaluated the safety of TDF concluded that TDF-associated nephrotoxicity can be considered negligible and thus there is no need to restrict TDF use even when regular observation of renal function is not feasible [8]. Other experimental and clinical studies, however; provide a different scenario: one study of rhesus macaques described a dose-dependent nephrotoxic effect for TDF [9] and several studies reported cases of TDF-associated nephrotoxicity in low-body-weight HIV-infected patients [10,11]. Our group also reported that low body weight and use of TDF were significantly associated with chronic kidney dysfunction in Vietnamese HIV-infected patients in a cross-sectional study [12]. Since Vietnamese have a considerably smaller body weight compared with Caucasians, and the use of TDF in Vietnam is increasing throughout the country, the potential risk for TDF-related nephrotoxicity is a concern in Vietnam. This is also true in all countries in the region since the Asian population is, in general, of low body weight. To examine this issue in more detail, we conducted a longitudinal study to evaluate the incidence of renal dysfunction in Vietnamese HIV-infected patients and the risk factors of such morbidity, including use of TDF and low body weight.

2. Patients and methods

2.1. Study design

We performed a prospective observational study of a single-center cohort of Vietnamese HIV-infected patients on antiretroviral therapy (ART) to evaluate the impact of TDF and low body weight on renal function. This cohort was established in 2007 at the National Hospital of Tropical Disease (NHTD) in Hanoi, one of the biggest outpatient clinics for HIV infected-patients in Vietnam. The population of the cohort consists of Vietnamese HIV-infected patients on ART aged more than 17 years referred to NHTD.

To evaluate renal function, serum creatinine had been measured since October 2011, which is the baseline of this study. Entry criteria were patients who were registered in this cohort on October 2011. Patients taking TDF or with serum creatinine clearance (CrCl) of ≤ 60 ml/min at baseline were excluded. Also excluded from the study were patients whose creatinine was not obtained twice at least. The follow-up period was 18 months (between October 2011 and April 2013). All patients of this cohort received ART at baseline. ART included Zidovudine (AZT)/Lamivudine (3 TC), Stavudine (d4T)/3 TC or TDF/3 TC as nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) in combination with efavirenz (EFV), Nevirapine (NVP) or ritonavir boosted lopinavir (LPV/r). To estimate the incidence of renal dysfunction in this population, patients were divided into those who switched to TDF and those who did not. Laboratory data, including serum creatinine, were measured twice a year (in April and October) in this cohort. The study was approved by the Human Research Ethics Committee of NHTD. Each patient included in this study provided a written informed consent for the clinical and laboratory data to be used for publication. The study was conducted according to the principles expressed in the Declaration of Helsinki.

2.2. Measurements

Clinical and laboratory data included demographic variables (age, sex and weight), serum creatinine (mg/dl, measured by Jaffe method), CD4 cell count (cell/mm³, measured by flow cytometry), plasma HIV-RNA (copies/ml, measured by the Roche COBAS Taq-Man HIV monitor assay), complete history of ART, use of cotrimoxazole, date of HIV diagnosis, and presence of other comorbidities such as hepatitis B and C virus, diabetes mellitus and AIDS defining diseases. Renal dysfunction was defined as 25% decline in CrCl estimated by the Cockcroft–Gault formula, relative to the baseline.

2.3. Statistical analysis

Baseline characteristics were compared between case patients and control patients by the Student's *t*-test for continuous variables and by either the χ^2 test or Fisher's exact test for categorical variables. The time from baseline to renal dysfunction was analyzed by the Kaplan–Meier method for patients who switched to TDF and those who did not, and the log-rank test was used to determine the statistical significance. Censored cases represented those who died, dropped out, or were referred to other facilities before the end of follow-up period. The Cox proportional hazards regression analysis was used to estimate the impact of TDF use on the incidence of renal dysfunction. The impact of basic demographics, baseline laboratory data, and other medical conditions was also estimated with univariate Cox proportional hazards regression. Variables significantly associated with renal dysfunction in univariate analysis ($p < 0.05$) were entered into multivariate analysis. Statistical significance was defined at two-sided p value < 0.05 . We used the hazard ratio (HR) and 95% confidence interval (95%CI) to estimate the association of each variable with renal dysfunction. All analyses were performed in SPSS (version 22.0).

3. Results

At baseline, 793 Vietnamese HIV-infected patients on ART were registered in this study. However, 237 patients were excluded from the study due to existing renal dysfunction at baseline (CrCl < 60 ml/min, $n = 72$), had already been treated with TDF at baseline ($n = 143$), and lack of repeated measurements of CrCl ($n = 22$). Thus, 556 patients who received ART met the study criteria and were included in the study. Of these, 153 patients were switched to TDF during the study period, while 403 patients continued treatment with non-TDF-containing regimen. The criteria for switch to TDF were adverse event caused by ART or induction of treatment for chronic hepatitis B virus infection.

Table 1 compares the baseline demographics and clinical variables of patients of the TDF-switched group and the non-TDF group. The TDF-switched group was significantly more likely to be males, hepatitis B virus S antigen-positive and hepatitis C virus antibody-positive compared to the non-TDF group. The TDF-switched group had marginally significant trend to be older and have diabetes mellitus. Body weight, serum creatinine, CD4 count, HIV RNA viral load, duration of ART, frequency of proteinuria and glucosuria, use of ritonavir boosted lopinavir (LPV/r) and cotrimoxazole, and history of AIDS-defining disease were not significantly different between the two groups. The mean CD4 count was >300 /mm³ and the mean HIV RNA load was <100 copies/ml in both groups.

During the observation period, renal dysfunction, defined as 25% decline in CrCl, was observed in 19 (12.4%) of the TDF-switched group and 27 (6.7%) of the non-TDF group, with an estimated incidence of 92.5 and 47.8 per 1000 person-years, respectively. Fig. 1 depicts the time from the baseline to the development of

Table 1
Baseline characteristic of Vietnamese patients treated with or without TDF.

Variables	Without TDF	With TDF	P value
Number of patients (%)	403 (72.5)	153 (27.5)	
Age, years	35.6 ± 7.0	36.9 ± 6.8	0.064
Women, n (%)	167 (41.4)	45 (29.4)	0.009
Body weight	55.7 ± 8.3	56.5 ± 8.2	0.284
Serum creatinine, mg/dl	0.93 ± 0.13	0.93 ± 0.12	0.668
CD4+ cell count, cell/μl	394 ± 197	385 ± 166	0.651
Log 10 HIV-RNA level, copies/ml	1.48 ± 0.55	1.42 ± 0.41	0.190
Proteinuria, n (%)	48 (11.9)	21 (13.7)	0.522
Glucosuria, n (%)	3 (0.7)	2 (1.3)	0.617
HBVAg (+), n (%)	22 (5.5)	29 (18.9)	<0.001
HCVAb (+), n (%)	153 (38.0)	69 (45.1)	0.014
Duration of ART, years	1.14 ± 1.35	1.20 ± 1.47	0.650
Use of ritonavir boosted lopinavir, n (%)	7 (1.7)	5 (3.3)	0.326
Use of cotrimoxazole drug, n (%)	136 (33.7)	45 (29.4)	0.330
Prior AIDS defining disease, n (%)	36 (8.9)	12 (7.8)	0.683
Diabetes mellitus (+), n (%)	31 (7.7)	19 (12.4)	0.082

Data are expressed as mean ± SD.

ART = Antiretroviral therapy; TDF = tenofovir.

renal dysfunction by Kaplan–Meier method in the two groups. The incidence of renal dysfunction was significantly higher in the TDF-switched group, compared with the non-TDF group ($p = 0.023$, Log-rank test). With regard to the time of switch to TDF, 109 (71.5%) patients of the TDF-switched group switched their nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) to TDF within 3 months from the baseline and additional 31 (20.0%) switched between 3 and 6 months. Furthermore, of the 19 patients of the TDF-switched group who developed renal dysfunction, 13 (71.2%) switched to TDF within 3 months from the baseline and additional 5 (23.5%) switched to TDF between 3 and 6 months.

Table 2 shows the results of the Cox proportional hazards regression model. Univariate analysis identified body weight per 1 kg-decrement, use of TDF, and glucosuria as factors significantly associated with renal dysfunction. After adjustment by multivariate analysis, body weight per 1 kg-decrement (HR = 1.057; 95%CI, 1.016–1.098; $p = 0.006$), use of TDF (HR = 1.980; 95%CI, 1.094–3.582; $p = 0.024$), and glucosuria (HR = 5.202; 95%CI, 1.245–21.738; $p = 0.024$) were still associated significantly with renal dysfunction.

We also compared the incidence of renal dysfunction in the TDF-switched group according to body weight. Fig. 2 shows the time from baseline to renal dysfunction in patients with body weight of <55 kg, representing the average weight of this study population, and in those with ≥55 kg of the TDF-switched group by Kaplan–Meier method. Patients of the <55 kg group were significantly more likely to develop renal dysfunction [12/66 cases (18.2%), 145.3/1000 person-year] compared to patients of the ≥55 kg group [7/87 cases (8.0%), 57.0/1000 person-year] ($p = 0.040$, Log-rank test).

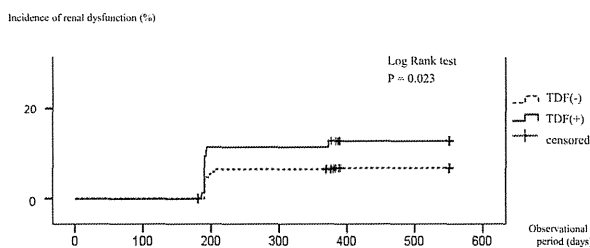


Fig. 1. Kaplan–Meier curve showing the time to renal dysfunction in patients of TDF-switched group and non-TDF-containing groups. Compared to patients of the non-TDF group, those of the TDF-switched group were significantly more likely to develop renal dysfunction ($p = 0.023$, Log-rank test).

Table 2
Risk factors for 25% decline in creatinine clearance estimated by uni- and multivariate analyses.

	Univariate analysis			Multivariate analysis		
	HR	95%CI	P value	HR	95%CI	P value
Age, per year	1.022	0.984–1.061	0.259			
Women	1.484	0.832–2.646	0.181			
Body weight per 1 kg decrease	1.053	1.013–1.094	0.008	1.057	1.016–1.098	0.006
Serum creatinine >1.1 mg/dl	0.397	0.096–1.636	0.201			
CD4+ cell count per cell/μl	1.001	0.999–1.002	0.227			
HIV-RNA level per log 10 copies/ml	0.887	0.446–1.764	0.733			
Proteinuria	0.474	0.147–1.528	0.211			
Glucosuria	5.372	1.301–22.176	0.020	5.202	1.245–21.738	0.024
HBVAg (+)	1.466	0.622–3.458	0.382			
HCVAb (+)	0.949	0.521–1.728	0.864			
Duration of ART per year	1.151	0.970–1.367	0.108			
Use of tenofovir	1.927	1.071–3.465	0.029	1.980	1.094–3.582	0.024
Use of ritonavir boosted lopinavir	2.024	0.491–8.349	0.329			
Use of cotrimoxazole	0.663	0.337–1.305	0.234			
Prior AIDS defining disease	0.043	0.000–4.144	0.177			
Diabetes mellitus (+)	0.952	0.341–2.654	0.925			

HR = hazard ratio; CI = confidence interval; ART = antiretroviral therapy.

The mean serum creatinine was higher in the TDF-switched group compared with the non-TDF group, and the difference in the mean serum creatinine between the two groups increased from 0 mg/dl at baseline, to 0.4 mg/dl at 6 month, 0.5 mg/dl at 12 months and 0.6 mg/dl at 18 months from the baseline.

4. Discussion

In this 18-month prospective study of a single-center cohort, we evaluated the impact of TDF on renal function in Vietnamese HIV-infected patients with low body weight of approximately 55 kg. The Kaplan–Meier curve showed that the cumulative incidence of renal dysfunction was significantly higher among the patients who switched to TDF than among those who did not ($p = 0.023$). Cox proportional hazards regression model identified the use of TDF, low body weight and glucosuria as significant high risk factors for renal dysfunction. In sub-analysis of the TDF-switched group, we confirmed that the cumulative incidence of renal dysfunction was significantly higher in patients with body weight <55 kg compared to those weighing ≥55 kg.

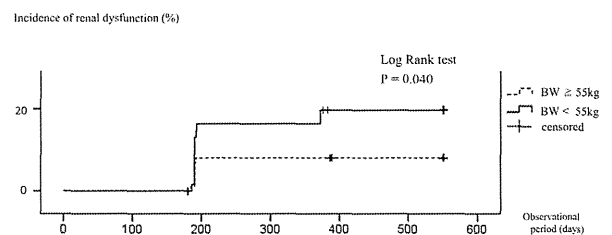


Fig. 2. Kaplan–Meier curve showing the time to renal dysfunction in patients of TDF-switched group classified according to body weight. Compared to patients with body weight ≥55 kg, those weighing <55 kg were significantly likely to develop renal dysfunction ($p = 0.040$, Log-rank test).

We reported previously that low body weight and TDF use were factors significantly associated with chronic kidney disease in a cross-sectional study of this cohort in Hanoi [12]. The present study confirmed that TDF exposure and low body weight bear a causative relationship to renal dysfunction. We also reported low body weight (<59 kg) as a risk factor for renal dysfunction in Japanese patients treated with TDF [10], whereas high body weight of >67 kg was not the risk, similar to the body weight of the patients reported by Cooper et al. [8]. In light of the fact that the average body weight of the patients in this cohort was 55 kg, which is around 30 kg lighter than that of average American males (88 kg) (URL:<http://www.cdc.gov/nchs/data/nhsr/nhsr010.pdf>), the impact of these risk factors on renal function remain unknown in patients with low body weight in the long-run, thus, observational studies will need to be continued for a longer term.

In addition to low body weight, the presence of glucosuria at baseline was identified as a risk factor for renal dysfunction. This result is consistent with the most recent WHO guidelines which suggest urinary glucose as one of the cost-effective screening test for serious TDF-induced kidney injury (URL: http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf). Since the number of patients with glucosuria was small in this study (about 1% of total population), and glucosuria was not followed until the end of the observation period, further evaluation of this factor is necessary.

Other risk factors for renal dysfunction described in previous studies, such as cotrimoxazole, LPV/r, hepatitis C virus co-infection and diabetes mellitus [13–16] were not identified as risk factors in this study. This discrepancy could be explained by the fact that patients who could be affected by these factors were already excluded according to the study design, which excluded patients with renal dysfunction. With regard to the use of LPV/r, which is known as a risk for renal dysfunction [14,17], especially in cases of co-use with TDF, a number of patients with LPV/r were excluded from the study since most of the patients with LPV/r were co-treated with TDF at baseline. Thus, the impact of co-use of LPV/r and TDF on renal function could be underestimated in this study. Given that LPV/r is used as a salvage regimen and often administered with TDF in Vietnam, long-term monitoring of renal function is required in patients treated with both LPV/r and TDF.

The present study has several limitations. First, data on hypertension, which is a risk factor for renal dysfunction, were not available in this study. Although the average age of patients in this study was around 36 years and the prevalence of hypertension may not high, measurement of blood pressure could lead to better management of renal dysfunction and hypertension should be evaluated for potential risk. Regarding diabetes mellitus as well, the degree of diabetes mellitus was not checked in detail. However, severe patients such as insulin dependence were not in this study, thus, the lack of data could be limited. Second, the observation period of 18 months is relatively short to evaluate long-term adverse event for renal function as mentioned above. Some studies advocated stabilization of decline in eGFR later after the first 6 months of TDF exposure [18] and reversibility of eGFR decline after cessation of TDF therapy [19], while several studies argued incomplete reversibility of eGFR decline following TDF exposure [20–22]. In this study, most of the patients who developed the decline in CrCl continued the same ART regimen because of their moderate and/or stabilized renal dysfunction. However, the observational period of the present study is relatively short compared to other studies, thus, whether or not the stabilization and reversibility will be observed in this cohort of averagely small body weight should be evaluated in the longer period.

Third, the timing of switch to TDF and total duration of ART were not unified in the present study, since the study was an

observational cohort in which patients were already on ART at enrollment. The reasons for switch to TDF were mainly related to adverse events caused by d4T and AZT or treatment for HBV infection, thus the timing of switch to TDF was not strictly controlled. However, more than 70% and 90% of the patients were switched to TDF within 3 and 6 months from baseline, respectively, thus influence of this limitation on the result of this study could be restricted.

Despite concern on nephrotoxicity, TDF remains an important drug with enough anti-HIV potency and less mitochondrial toxicity among NRTIs. In order to use it safely in the long term, serum creatinine should be monitored in patients with aforementioned risk factors even in resource-limited situations. Further longitudinal studies are required to determine the impact of TDF, low body weight and glucosuria on renal function in Vietnamese and other Asian people with low body weight.

Conflict 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.; 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., and ViiV Healthcare, Co. All other authors declare no conflict of interest.

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References

- [1] Wyatt CM, Winston JA, Malvestutto CD, Fishbein DA, Barash I, Cohen AJ, et al. Chronic kidney disease in HIV infection: an urban epidemic. *AIDS* 2007;21:2101–3.
- [2] Mocroft A, Kirk O, Gatell J, Reiss P, Gargalianos P, Zilmer K, et al. Chronic renal failure among HIV-1-infected patients. *AIDS* 2007;21:1119–27.
- [3] Deti EK, Thiebaut R, Bonnet F, Lawson-Ayayi S, Dupon M, Neau D, et al. Prevalence and factors associated with renal impairment in HIV-infected patients, ANRS C03 Aquitaine Cohort, France. *HIV Med* 2010;11:308–17.
- [4] Menezes AM, Torelly Jr J, Real L, Bay M, Poeta J, Sprinz E. Prevalence and risk factors associated to chronic kidney disease in HIV-infected patients on HAART and undetectable viral load in Brazil. *PLoS One* 2011;6:e26042.
- [5] Wyatt CM, Arons RR, Klotman PE, Klotman ME. Acute renal failure in hospitalized patients with HIV: risk factors and impact on in-hospital mortality. *AIDS* 2006;20:561–5.
- [6] Franceschini N, Napravnik S, Eron Jr JJ, Szczech LA, Finn WF. Incidence and etiology of acute renal failure among ambulatory HIV-infected patients. *Kidney Int* 2005;67:1526–31.
- [7] Schwartz EJ, Szczech LA, Ross MJ, Klotman ME, Winston JA, Klotman PE. Highly active antiretroviral therapy and the epidemic of HIV+ end-stage renal disease. *J Am Soc of Nephrol JASN* 2005;16:2412–20.
- [8] Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis* 2010;51:496–505.
- [9] Van Rompay KK, Durand-Gasselín L, Brignolo LL, Ray AS, Abel K, Cihlar T, et al. Chronic administration of tenofovir to rhesus macaques from infancy through adulthood and pregnancy: summary of pharmacokinetics and biological and virological effects. *Antimicrob Agents Chemother* 2008;52:3144–60.
- [10] Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, Kinai E, et al. Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients. *PLoS One* 2011;6:e22661.

- [11] Chaisiri K, Bowonwatanuwong C, Kasettrat N, Kiertiburanakul S. Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. *Curr HIV Res* 2010;8:504–9.
- [12] Mizushima D, Tanuma J, Kanaya F, Nishijima T, Gatanaga H, Lam NT, et al. WHO antiretroviral therapy guidelines 2010 and impact of Tenofovir on chronic kidney disease in Vietnamese HIV-infected patients. *PLoS One* 2013;8:e79885.
- [13] Peters L, Grint D, Lundgren JD, Rockstroh JK, Soriano V, Reiss P, et al. Hepatitis C virus viremia increases the incidence of chronic kidney disease in HIV-infected patients. *AIDS* 2012;26:1917–26.
- [14] Ryom L, Mocroft A, Kirk O, Worm SW, Kamara DA, Reiss P, et al. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J Infect Dis* 2013;207:1359–69.
- [15] Kalayjian RC, Lau B, Mehekano RN, Crane HM, Rodriguez B, Salata RA, et al. Risk factors for chronic kidney disease in a large cohort of HIV-1 infected individuals initiating antiretroviral therapy in routine care. *AIDS* 2012;26:1907–15.
- [16] Choi AI, Li Y, Parikh C, Volberding PA, Shlipak MG. Long-term clinical consequences of acute kidney injury in the HIV-infected. *Kidney Int* 2010;78:478–85.
- [17] Mwafongo A, Nkanaunena K, Zheng Y, Hogg E, Samaneka W, Mulenga L, et al. Renal events among women treated with tenofovir/emtricitabine in combination with either lopinavir/ritonavir or nevirapine: analysis from the AIDS clinical trial group A5208 trial. *AIDS* 2014;28:1135–42.
- [18] Gallant JE, Moore RD. Renal function with use of a tenofovir-containing initial antiretroviral regimen. *AIDS* 2009;23:1971–5.
- [19] Bonjoch A, Echeverria P, Perez-Alvarez N, Puig J, Estany C, Clotet B, et al. High rate of reversibility of renal damage in a cohort of HIV-infected patients receiving tenofovir-containing antiretroviral therapy. *Antiviral Res* 2012;96:65–9.
- [20] Jose S, Hamzah L, Campbell LJ, Hill T, Fisher M, Leen C, et al. Incomplete reversibility of estimated glomerular filtration rate decline following Tenofovir disoproxil fumarate exposure. *J Infect Dis* 2014;213:363–73.
- [21] Wever K, van Agtmael MA, Carr A. Incomplete reversibility of tenofovir-related renal toxicity in HIV-infected men. *J Acquir Immune Defic Syndr* 2010;55:78–81.
- [22] Yoshino M, Yagura H, Kushida H, Yonemoto H, Bando H, Ogawa Y, et al. Assessing recovery of renal function after tenofovir disoproxil fumarate discontinuation. *J Infect Chemother* 2012;18:169–74.

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

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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 m² relative to the baseline, more than 25% decrement in eGFR, and eGFR lower than 60 ml/min per 1.73 m² 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 m² 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 m² 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 m², 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

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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 m², 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 m² [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⁺ 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 days 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)]²}, history of AIDS, route of HIV-1 transmission, baseline laboratory data (CD4⁺ 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 m² 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 m² 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⁺ 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 m² 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⁺ 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, κ is 0.7 for women and 0.9 for men, α is -0.329 for women and -0.411 for men, min indicates the minimum of SCr/ κ or 1, and max indicates the maximum of SCr/ κ 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 ^a	36 (31–43)	36 (31–43)	36 (31–44)	0.23
Weight (kg) ^a	63 (57.8–70.4)	62.9 (57.2–69.8)	63.8 (58.0–71.4)	0.25
BMI (kg/m ²) ^a	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 ²) ^a	95.7 (84–110)	96.5 (84.7–111.5)	95.4 (83.7–108.6)	0.32
Serum creatinine (mg/dl) ^a	0.74 (0.66–0.82)	0.73 (0.66–0.82)	0.74 (0.67–0.83)	0.27
CD4 ⁺ cell count (/μl) ^a	189 (78–266)	199 (85–281)	183 (73–241)	0.002
HIV RNA viral load (log ₁₀ /ml) ^a	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) ^a	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.

^aMedian (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/m² (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⁺ 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 m² 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⁺ cell count also increased the risk of higher than 10 ml/min per 1.73 m² 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 m² 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 m² 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 m² 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 m² at 1 year of TDF to –23.7 ml/min per 1.73 m² at 5 years of TDF exposure, and in the control, from –8.0 ml/min per 1.73 m² at 1 year to –13.5 ml/min per 1.73 m² 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 m² 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 ² 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 ⁺ 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 m², at 2 years -3.6 ml/min per 1.73 m², at 3 years -5.5 ml/min per 1.73 m², at 4 years -6.6 ml/min per 1.73 m², and at 5 years -10.3 ml/min per 1.73 m² (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 m² 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 ²	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 ⁺ 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 m² 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 m² at 1 year to 15.0 ml/min per 1.73 m² at 5 years of TDF exposure, and in the control, from -4.1 ml/min per 1.73 m² at 1 year to -8.3 ml/min per 1.73 m² 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 m², 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-naïve 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 m² 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 m². 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 m² at 1 year of TDF exposure to -5.5 ml/min per 1.73 m² at 3 years, and to -10.3 ml/min per 1.73 m² 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 m²; 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 ²	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 ⁺ 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.

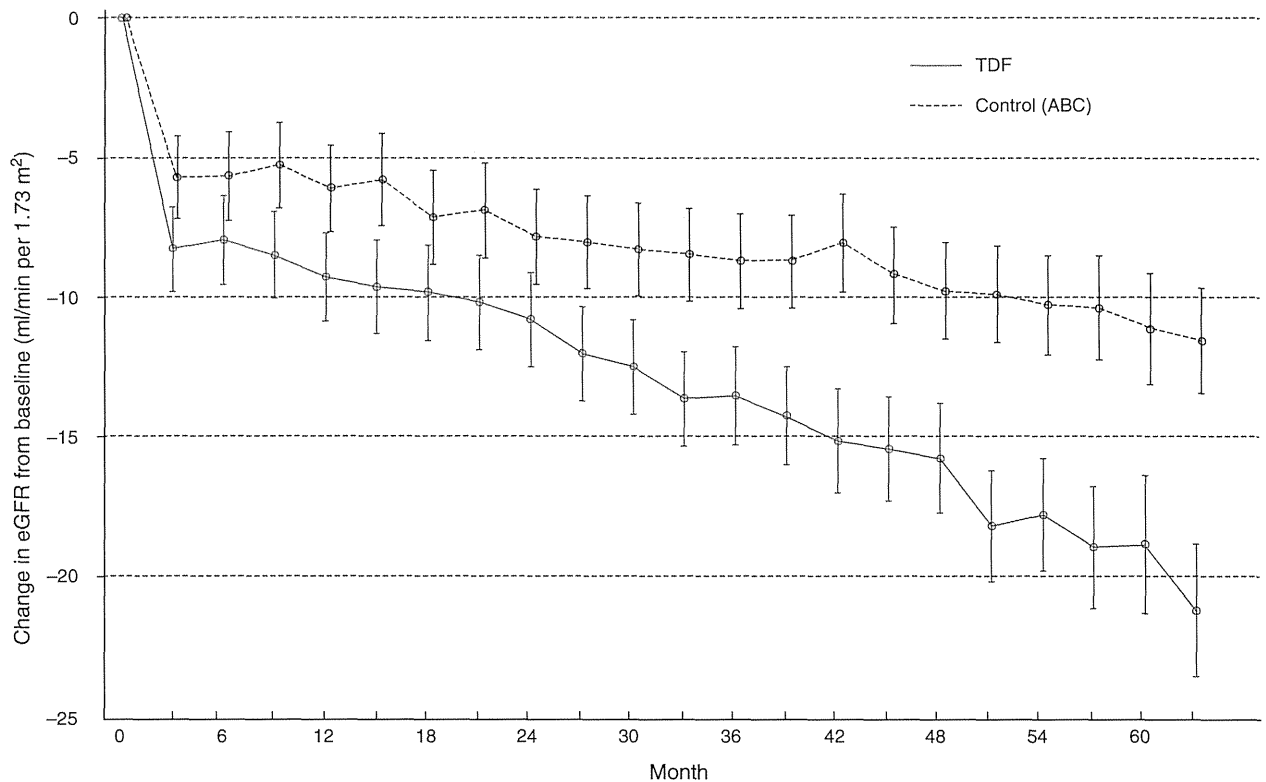


Fig. 1. Adjusted mean change in estimated glomerular filtration rate from baseline to 5 years in treatment-naïve patients treated with tenofovir disoproxil fumarate-containing antiretroviral therapy (red line) and controls (patients treated with abacavir-containing ART) (black line). Least-square means 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⁺ 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 m² 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 m², 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 m² 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.

References

1. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>. [Accessed 18 December 2013].
2. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf. [Accessed 19 January 2014].

3. Fung S, Kwan P, Fabri M, Horban A, Pelemis M, Hann HW, *et al.* Randomized comparison of tenofovir disoproxil fumarate vs emtricitabine and tenofovir disoproxil fumarate in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology* 2013; **146**:980–988.
4. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, *et al.* Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N Engl J Med* 2008; **359**:2442–2455.
5. Thigpen MC, Kebaabetswe PM, Paxton LA, Smith DK, Rose CE, Segolodi TM, *et al.* Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *N Engl J Med* 2012; **367**:423–434.
6. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, *et al.* Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N Engl J Med* 2012; **367**:399–410.
7. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougnot B, Girard PM, *et al.* Fanconi syndrome and renal failure induced by tenofovir: a first case report. *Am J Kidney Dis* 2002; **40**:1331–1333.
8. Schaaf B, Aries SP, Kramme E, Steinhoff J, Dalhoff K. Acute renal failure associated with tenofovir treatment in a patient with acquired immunodeficiency syndrome. *Clin Infect Dis* 2003; **37**:e41–e43.
9. Rollot F, Nazal EM, Chauvelot-Moachon L, Kelaidi C, Daniel N, Saba M, *et al.* Tenofovir-related Fanconi syndrome with nephrogenic diabetes insipidus in a patient with acquired immunodeficiency syndrome: the role of lopinavir-ritonavir-didanosine. *Clin Infect Dis* 2003; **37**:e174–e176.
10. Peyriere H, Reynes J, Rouanet I, Daniel N, de Boever CM, Mauboussin JM, *et al.* Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. *J Acquir Immune Defic Syndr* 2004; **35**:269–273.
11. Gallant JE, Winston JA, DeJesus E, Pozniak AL, Chen SS, Cheng AK, *et al.* The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue-containing regimen in antiretroviral-naïve patients. *AIDS* 2008; **22**:2155–2163.
12. Laprise C, Baril JG, Dufresne S, Trottier H. Association between tenofovir exposure and reduced kidney function in a cohort of HIV-positive patients: results from 10 years of follow-up. *Clin Infect Dis* 2013; **56**:567–575.
13. Nishijima T, Gatanaga H, Komatsu H, Tsukada K, Shimbo T, Aoki T, *et al.* Renal function declines more in tenofovir- than abacavir-based antiretroviral therapy in low-body weight treatment-naïve patients with HIV infection. *PLoS One* 2012; **7**:e29977.
14. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis* 2010; **51**:496–505.
15. Gallant JE, Moore RD. Renal function with use of a tenofovir-containing initial antiretroviral regimen. *AIDS* 2009; **23**:1971–1975.
16. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B, *et al.* The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. *AIDS* 2007; **21**:1273–1281.
17. Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, Kinai E, *et al.* Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients. *PLoS One* 2011; **6**:e22661.
18. Chaisiri K, Bowonwatanuwong C, Kasettrat N, Kiertiburanakul S. Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. *Curr HIV Res* 2010; **8**:504–509.
19. Gatanaga H, Tachikawa N, Kikuchi Y, Teruya K, Genka I, Honda M, *et al.* Urinary beta2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. *AIDS Res Hum Retroviruses* 2006; **22**:744–748.
20. Reid A, Stohr W, Walker AS, Williams IG, Kityo C, Hughes P, *et al.* Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. *Clin Infect Dis* 2008; **46**:1271–1281.
21. Ziagen (abacavir sulfate): highlights of prescribing information. Research Triangle Park, NC: GlaxoSmithKline, 2008. http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/020977s019,020978s022lbl.pdf. [Accessed 2 February 2014].
22. The guidelines for the treatment of HIV infection, March 2013 version. The Japanese Ministry of Health, Labour and Welfare. 1–154. <http://www.haart-support.jp/pdf/guideline2013.pdf>. [Accessed 20 December 2013].
23. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, *et al.* Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53**:982–992.
24. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**:604–612.
25. Horio M, Imai E, Yasuda Y, Watanabe T, Matsuo S. Performance of GFR equations in Japanese subjects. *Clin Exp Nephrol* 2013; **17**:352–358.
26. Evidence-based clinical practice guidelines for CKD. The Japanese Society of Nephrology. http://www.jsn.or.jp/guideline/pdf/CKD_evidence2013/all.pdf. [Accessed 1 February 2014].
27. Gupta SK, Eustace JA, Winston JA, Boydston II, Ahuja TS, Rodriguez RA, *et al.* Guidelines for the management of chronic kidney disease in HIV-infected patients: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis* 2005; **40**:1559–1585.
28. Goicoechea M, Liu S, Best B, Sun S, Jain S, Kemper C, *et al.* Greater tenofovir-associated renal function decline with protease inhibitor-based versus nonnucleoside reverse-transcriptase inhibitor-based therapy. *J Infect Dis* 2008; **197**:102–108.
29. Roldan V, Marin F, Fernandez H, Manzano-Fernandez S, Gallego P, Valdes M, *et al.* Renal impairment in a 'real-life' cohort of anticoagulated patients with atrial fibrillation (implications for thromboembolism and bleeding). *Am J Cardiol* 2013; **111**:1159–1164.
30. Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D, Beniowski M, *et al.* Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. *AIDS* 2010; **24**:1667–1678.
31. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology* 2009; **20**:488–495.
32. Horio M, Imai E, Yasuda Y, Watanabe T, Matsuo S. Modification of the CKD epidemiology collaboration (CKD-EPI) equation for Japanese: accuracy and use for population estimates. *Am J Kidney Dis* 2010; **56**:32–38.
33. Wetzels JF, Kiemeneij LA, Swinkels DW, Willems HL, den Heijer M. Age- and gender-specific reference values of estimated GFR in Caucasians: the Nijmegen Biomedical Study. *Kidney Int* 2007; **72**:632–637.
34. UNAIDS adults and children estimated to be living with HIV 2012. http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/201309_epi_core_en.pdf. [Accessed 19 January 2014].
35. Mugenyi P, Walker AS, Hakim J, Munderi P, Gibb DM, Kityo C, *et al.* Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised noninferiority trial. *Lancet* 2010; **375**:123–131.
36. Patel KK, Patel AK, Ranjan RR, Patel AR, Patel JK. Tenofovir-associated renal dysfunction in clinical practice: an observational cohort from western India. *Indian J Sex Transm Dis* 2010; **31**:30–34.
37. Mizushima D, Tanuma J, Kanaya F, Nishijima T, Gatanaga H, Lam NT, *et al.* WHO antiretroviral therapy guidelines 2010 and impact of tenofovir on chronic kidney disease in Vietnamese HIV-infected patients. *PLoS One* 2013; **8**:e79885.
38. Praditpornsilpa K, Avihingsanon A, Chaiwatanarat T, Chaiyachong P, Wongsabut J, Wibolyam S, *et al.* Comparisons between validated estimated glomerular filtration rate equations and isotopic glomerular filtration rate in HIV patients. *AIDS* 2012; **26**:1781–1788.
39. Perkovic V, Cass A, Patel AA, Suriyawongpaisal P, Barzi F, Chadban S, *et al.* High prevalence of chronic kidney disease in Thailand. *Kidney Int* 2008; **73**:473–479.
40. Rym L, Mocroft A, Kirk O, Worm SW, Kamara DA, Reiss P, *et al.* Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J Infect Dis* 2013; **207**:1359–1369.

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

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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.¹ However, some case reports/series have documented nephrolithiasis containing atazanavir,^{2–5} 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.^{6–8} 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.^{9–11}

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,¹² whereas urine is usually mildly acidic.⁶ 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.^{7,13}

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.^{14,15} 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.¹² SNPs in the *NR1I2* gene, which encodes the nuclear receptor pregnane X receptor (PXR), regulate the expression of CYP3A4¹⁶ and *ABCB1*,¹⁷ and also influence atazanavir concentration.¹⁸ 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.^{19–22} 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.²³

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 2012,⁷ 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:^{6,7} 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.^{4,5,24–27} They included age, sex, body weight, BMI (body weight (kg)/[height (m)]²), 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.²⁸ 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.⁷ 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.^{15,18,20,23,29–32} The allele frequency data for the Japanese were obtained from the Japanese SNP (JSNP) database.³³ 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)₇TAA (UGT1A1*28), which is known to be less transcriptionally active than the common promoter with six TA repeats (UGT1A1*1),³⁴ 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'-TTTGCTCCTGCCAGAGGT T-5';³⁵ 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 χ^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 2×3 table (2×6 table for rs2032582) and the χ^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,⁷ 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)	P 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 ²), 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 ²), 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 ³), median (IQR)	230 (187–302)	232 (194–356)	229 (187–290)	0.63
HIV-1 load (log ₁₀ /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-naive, 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			Allele frequency			
		nephrolithiasis (n=31)	no nephrolithiasis (n=47)	P value ^a	nephrolithiasis (n=31)	no nephrolithiasis (n=47)	P value ^a	
<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	46 (74)	68 (72)	0.81
	A/G	14 (45)	14 (30)		G	16 (26)	26 (28)	
	G/G	1 (3)	6 (13)					
365 T→C, rs3213619				365 T→C, rs3213619				
	T/T	28 (90)	37 (79)	0.23	T	59 (95)	84 (89)	0.33
	T/C	3 (10)	10 (21)		C	3 (5)	10 (11)	
	C/C	0	0					
1236 T→C, rs1128503				1236 T→C, rs1128503				
	Gly412Gly			0.48	T	33 (53)	52 (55)	0.80
	T/T	7 (23)	15 (32)		C	29 (47)	42 (45)	
	T/C	19 (61)	22 (47)					
	C/C	5 (16)	10 (21)					
2677 T→A/G, rs2032582				2677T→A/G, rs2032582				
	A:Ser893Thr			0.45	T	26 (42)	30 (32)	0.20
	G:Ser893Ala	5 (16)	5 (11)		A	14 (23)	17 (18)	
	T/T	6 (19)	5 (11)		G	22 (35)	47 (50)	
	T/A	3 (10)	10 (21)					
	G/G	10 (32)	15 (32)					
	G/T	6 (19)	12 (26)					
	G/A	1 (3)	0					
	A/A							
3435 C→T, rs1045642				3435 C→T, rs1045642				
	Ile1145Ile			0.35	C	36 (58)	65 (69)	0.16
	C/C	10 (32)	22 (47)		T	26 (42)	29 (31)	
	C/T	16 (52)	21 (45)					
	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	20 (32)	35 (37)	0.53
	C/A	18 (58)	23 (49)		A	42 (68)	59 (63)	
	A/A	12 (39)	18 (38)					
370 G→A, rs3732359				370 G→A, rs3732359				
	G/G	8 (26)	16 (34)	0.37	G	35 (56)	53 (56)	1.00
	G/A	19 (61)	21 (45)		A	27 (44)	41 (44)	
	A/A	4 (13)	10 (21)					
522 C→T, rs3732360				522 C→T, rs3732360				
	C/C	8 (26)	15 (32)	0.60	C	35 (56)	53 (56)	1.00
	C/T	19 (61)	23 (49)		T	27 (44)	41 (44)	
	T/T	4 (13)	9 (19)					
1195 A→C, rs3814057				1195 A→C, rs3814057				
	A/A	4 (13)	11 (23)	0.39	A	27 (44)	44 (47)	0.69
	A/C	19 (61)	22 (47)		C	35 (56)	50 (53)	
	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)					
<i>UGT1A1</i>				<i>UGT1A1</i>			
*28 ^b				*28 ^b			
*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)					
<i>SLCO1B1</i>				<i>SLCO1B1</i>			
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)					
<i>CYP3A5</i>				<i>CYP3A5</i>			
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)					

^aBy Fisher's exact test.

^b*1, reference sequence A(TA)₆TAA; *28, A(TA)₇TAA.