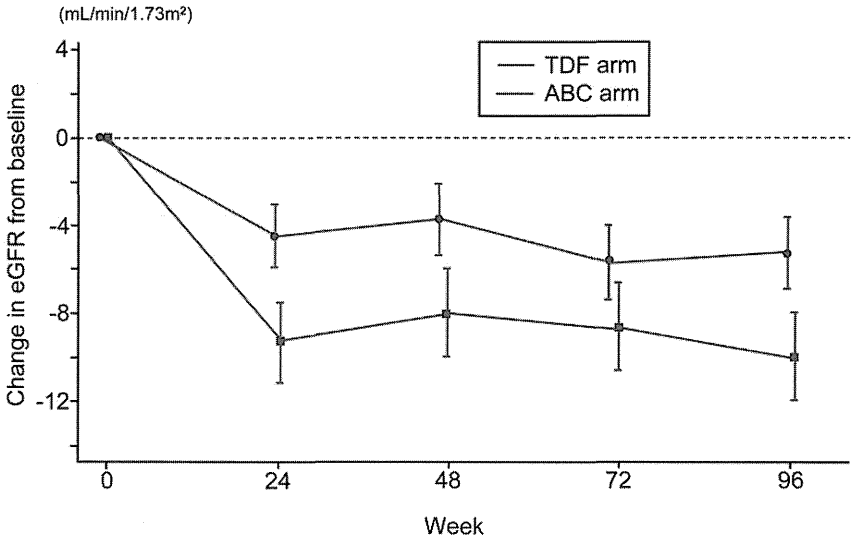

Figure 2. Changes in eGFR in patients treated with TDF or ABC between baseline and 96 weeks. The fall in eGFR was significantly greater in the TDF group than the ABC group (p = 0.003). Data are adjusted mean ±95% confidence interval. eGFR: estimated glomerular filtration rate, TDF: tenofovir, ABC: abacavir.
doi:10.1371/journal.pone.0029977.g002

results of the present study on TDF-related nephrotoxicity differ from the findings of randomized clinical trials that demonstrated no major change in renal function of TDF- and ABC-treated patients over 48–96 week follow-up [2,10,11]. The discrepant results might arise from differences between observational cohort and clinical trials, since observational studies tend to express the results in "real world setting" whereas clinical trials include patients who fulfill more strict criteria, therefore with better profile [9]. The discrepant results could be also due to the use of different definitions for renal dysfunction in these studies. However, the discrepant results could also reflect the difference in median body weight between the present study and these clinical trials. The results of our subgroup analysis support this hypothesis by showing that the effect of TDF on renal dysfunction was more evident in patients with low body weight. Apart from being low-body-weighted, the patients in this study did not appear to have many of other established risks for TDF-related nephrotoxicity; they were comparatively young, had relatively stable CD4 count, and had only a few co-morbidities (Table 1). Although the majority concurrently used ritonavir-boosted PIs, which are a probable risk for TDF-related nephrotoxicity, ritonavir-boosted PIs were not significantly associated with renal dysfunction in our cohort (Table 2) [24].

Changes in eGFR in those patients treated with TDF-containing ART were characterized by a rapid decline during the first 24 weeks of therapy, followed by a plateau until 96 weeks (Fig. 2). This finding is consistent with that reported from the Johns Hopkins group [9,28]. Together with the finding that the median time from commencement of ART to the >25% decline in eGFR in the TDF-treated patients was 246 days, these results suggest that careful monitoring of renal function is particularly warranted in the first year of TDF-based therapy. Thus, we suggest that renal function should be monitored by measurement of serum creatinine at least once annually in resource-limited settings and twice annually in resource-rich settings in patients starting TDF-containing ART, especially those with baseline body weight <60 kg.

The Department of Health and Human Services guideline for the treatment of HIV infection in the U.S. lists ABC as an alternative NRTI because it can potentially cause serious hypersensitivity reaction and cardiovascular diseases (URL:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). However, some international guidelines consider both TDF and ABC as the preferred NRTIs under the condition that ABC should be used with caution in patients with viral load >100,000 copies/mL, based on the low incidence of ABC-related hypersensitivity among HLA-B*5701-negative population and the controversial association between ABC and cardiovascular diseases [1,29–32] (URL: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf) (http://www.haart-support.jp/guideline2011.pdf. in Japanese). The present study, together with our previous analysis that demonstrated preferential TDF-related nephrotoxicity in patients with low body weight, emphasize the advantage of ABC over TDF with regard to prognosis of renal function in low body weight patients [16].

TDF is the prodrug of acyclic nucleotide analog tenofovir, which is excreted by both glomerular filtration and active tubular secretion. Tenofovir is considered to cause mitochondrial damage in proximal renal tubular cells [33]. The concentration of tenofovir in the proximal renal tubules could be augmented with the complex interactions of pharmacological, environmental, and genetic factors, including small body weight, consequently resulting in renal tubular dysfunction [34]. Body weight has been identified as an important factor in TDF-related nephrotoxicity not only in clinical trials, but also in *in vitro* and pharmacokinetic studies [35–37].

The present study has several limitations. First, because of its retrospective nature, it was not possible to control the baseline characteristics of the enrolled patients. Thus, it is possible that patients with potential risk for TDF-related nephrotoxicity were not prescribed TDF. A proportion of patients treated with ABC had low CD4 count and others were hypertensive, both conditions are known risk factors for renal dysfunction [23,25]. However, for these reasons, the incidence of TDF-associated renal dysfunction might have been underestimated. Second, the definition of TDF-related nephrotoxicity, especially the criteria used to evaluate proximal renal tubular damage, is not uniformly established in the field and is different in the published studies. Accordingly, we

decided to adopt changes in eGFR, instead of parameters for proximal renal tubular damage. Using the eGFR as a marker for TDF-associated renal dysfunction, our results might have underestimated the incidence of TDF-related renal tubular dysfunction. However, the result of this study could be informative to resource-limited settings, where it is difficult to evaluate renal tubular markers. The rational and limitation of adopting more than 25% decrement in eGFR as the criterion for renal dysfunction were discussed in detail in our previous study [16]. Third, our cohort was characterized by the high prevalence of ritonavir-boosted PI use, which is considered by some groups a risk for TDF-related nephrotoxicity [24]. While it is difficult to completely exclude the impact of concurrent ritonavir-boosted PI in this study, it should be noted that the use of ritonavir-boosted PIs did not correlate with renal dysfunction in univariate analysis in this cohort (Table 2). Fourth, the study subjects were mainly men (mostly men who have sex with men and very few injection drug users). Further studies are needed to determine whether the findings of this study are also applicable to females, patients with different route of transmissions, and patients of different racial background.

In conclusion, the present study demonstrated a high incidence of renal dysfunction with TDF use, compared to ABC, among treatment-naïve patients with low body weight. TDF use was identified as an independent risk for renal dysfunction in a statistical model that included TDF as a primary exposure. At 96 weeks, patients with TDF showed greater eGFR decrement than patients treated with ABC. TDF is certainly a drug of choice in the treatment of HIV infection, but the importance of close monitoring of renal function in patients with small body weight, especially those with baseline body weight <60 kg should be emphasized for early detection of TDF-related nephrotoxicity. Further studies are warranted to elucidate the long-term prognosis of renal function with TDF use in patients with low body weight.

Acknowledgments

The authors thank Fumihiko Hinoshita, Ai Hori, Daisuke Tasato, Mahoko Kamimura, Kunio Yanagisawa, Daisuke Mizushima, Yohei Hamada, Aki Hashimoto, Akio Chiba, Yuko Yamauchi, Taiichiro Kobayashi, Kumi Tamura, and all other clinical staff at the AIDS Clinical Center for their help in completion of this study.

Author Contributions

Conceived and designed the experiments: TN HK HG TS EK JT SO. Performed the experiments: TN HK TS TA KW EK MH. Analyzed the data: TN HK HG TS HH HY K. Tsukada MH K. Teruya YK. Contributed reagents/materials/analysis tools: TA KW HH JT HY K. Tsukada MH K. Teruya YK. Wrote the paper: TN HK HG TS EK SO.

References

1. Mallal S, Phillips E, Carosi G, Molina JM, Workman C, et al. (2008) HLA-B*5701 screening for hypersensitivity to abacavir. N Engl J Med 358: 568–579.
2. Sax PE, Tierney C, Collier AC, Fischl MA, Mollan K, et al. (2009) Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy. N Engl J Med 361: 2230–2240.
3. Peyriere H, Reynes J, Rouanet I, Daniel N, de Boever CM, et al. (2004) Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. J Acquir Immune Defic Syndr 35: 269–273.
4. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, et al. (2002) Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis 40: 1331–1333.
5. Winston A, Amin J, Mallon P, Marriott D, Carr A, et al. (2006) Minor changes in calculated creatinine clearance and anion-gap are associated with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy. HIV Med 7: 105–111.
6. Fux CA, Simcock M, Wolbers M, Bucher HC, Hirschel B, et al. (2007) Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. Antivir Ther 12: 1165–1173.
7. Gallant JE, Winston JA, DeJesus E, Pozniak AL, Chen SS, et al. (2008) The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue-containing regimen in antiretroviral-naive patients. AIDS 22: 2155–2163.
8. Kinai E, Hanabusa H (2009) Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. AIDS Res Hum Retroviruses 25: 387–394.
9. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, et al. (2010) Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis 51: 496–505.
10. Post FA, Moyle GJ, Stellbrink HJ, Domingo P, Podzamczer D, et al. (2010) Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr 55: 49–57.
11. Smith KY, Patel P, Fine D, Bellos N, Sloan L, et al. (2009) Randomized, double-blind, placebo-matched, multicenter trial of abacavir/lamivudine or tenofovir/emtricitabine with lopinavir/ritonavir for initial HIV treatment. AIDS 23: 1547–1556.
12. Gayet-Ageron A, Ananworanich J, Jupimai T, Chetchotisakd P, Prasithsirikul W, et al. (2007) No change in calculated creatinine clearance after tenofovir initiation among Thai patients. J Antimicrob Chemother 59: 1034–1037.
13. Chaisiri K, Bowonwatanuwong C, Kasettratat N, Kiertiburanakul S (2010) Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. Curr HIV Res 8: 504–509.
14. Reid A, Stohr W, Walker AS, Williams IG, Kityo C, et al. (2008) Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. Clin Infect Dis 46: 1271–1281.
15. Gatanaga H, Tachikawa N, Kikuchi Y, Teruya K, Genka I, et al. (2006) Urinary beta2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. AIDS Res Hum Retroviruses 22: 744–748.
16. Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, et al. (2011) Impact of Small Body Weight on Tenofovir-Associated Renal Dysfunction in HIV-Infected Patients: A Retrospective Cohort Study of Japanese Patients. PLoS One 6: e22661.
17. Bygrave H, Kranzer K, Hilderbrand K, Jouquet G, Goemaere E, et al. (2011) Renal safety of a tenofovir-containing first line regimen: experience from an antiretroviral cohort in rural Lesotho. PLoS One 6: e17609.
18. De Beaudrap P, Diallo MB, Landman R, Gueye NF, Ndiaye I, et al. (2010) Changes in the renal function after tenofovir-containing antiretroviral therapy initiation in a Senegalese cohort (ANRS 1215). AIDS Res Hum Retroviruses 26: 1221–1227.
19. Brennan A, Evans D, Maskew M, Naicker S, Ive P, et al. (2011) Relationship between renal dysfunction, nephrotoxicity and death among HIV adults on tenofovir. AIDS 25: 1603–1609.
20. Bash LD, Coresh J, Kottgen A, Parekh RS, Fulop T, et al. (2009) Defining incident chronic kidney disease in the research setting: The ARIC Study. Am J Epidemiol 170: 414–424.
21. Chue CD, Edwards NC, Davis LJ, Steeds RP, Townend JN, et al. (2011) Serum phosphate but not pulse wave velocity predicts decline in renal function in patients with early chronic kidney disease. Nephrol Dial Transplant.
22. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, et al. (2006) Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 145: 247–254.
23. Gupta SK, Eustace JA, Winston JA, BoydstunII, Ahuja TS, et al. (2005) 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 40: 1559–1585.
24. Goicoechea M, Liu S, Best B, Sun S, Jain S, et al. (2008) Greater tenofovir-associated renal function decline with protease inhibitor-based versus nonnucleoside reverse-transcriptase inhibitor-based therapy. J Infect Dis 197: 102–108.
25. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, et al. (2007) The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. AIDS 21: 1273–1281.
26. Garrow JS, Webster J (1985) Quetelet's index (W/H2) as a measure of fatness. Int J Obes 9: 147–153.
27. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16: 31–41.
28. Gallant JE, Moore RD (2009) Renal function with use of a tenofovir-containing initial antiretroviral regimen. AIDS 23: 1971–1975.
29. Bedimo RJ, Westfall AO, Drechsler H, Vidiella G, Tebas P (2011) Abacavir use and risk of acute myocardial infarction and cerebrovascular events in the highly active antiretroviral therapy era. Clin Infect Dis 53: 84–91.
30. Nolan D, Gaudieri S, Mallal S (2003) Pharmacogenetics: a practical role in predicting antiretroviral drug toxicity? J HIV Ther 8: 36–41.
31. Sun HY, Hung CC, Lin PH, Chang SF, Yang CY, et al. (2007) Incidence of abacavir hypersensitivity and its relationship with HLA-B*5701 in HIV-infected patients in Taiwan. J Antimicrob Chemother 60: 599–604.
32. Gatanaga H, Honda H, Oka S (2008) Pharmacogenetic information derived from analysis of HLA alleles. Pharmacogenomics 9: 207–214.

33. Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, et al. (2009) Tenofovir renal toxicity targets mitochondria of renal proximal tubules. Lab Invest 89: 513–519.
34. Post FA, Holt SG (2009) Recent developments in HIV and the kidney. Curr Opin Infect Dis 22: 43–48.
35. Jullien V, Treluyer JM, Rey E, Jaffray P, Krivine A, et al. (2005) Population pharmacokinetics of tenofovir in human immunodeficiency virus-infected patients taking highly active antiretroviral therapy. Antimicrob Agents Chemother 49: 3361–3366.
36. Van Rompay KK, Durand-Gasselin L, Brignolo LL, Ray AS, Abel K, et al. (2008) Chronic administration of tenofovir to rhesus macaques from infancy through adulthood and pregnancy: summary of pharmacokinetics and biological and virological effects. Antimicrob Agents Chemother 52: 3144–3160.
37. Kiser JJ, Fletcher CV, Flynn PM, Cunningham CK, Wilson CM, et al. (2008) Pharmacokinetics of antiretroviral regimens containing tenofovir disoproxil fumarate and atazanavir-ritonavir in adolescents and young adults with human immunodeficiency virus infection. Antimicrob Agents Chemother 52: 631–637.

HIV/AIDS MAJOR ARTICLE

High Incidence of Renal Stones Among HIV-Infected Patients on Ritonavir-Boosted Atazanavir Than in Those Receiving Other Protease Inhibitor–Containing Antiretroviral Therapy

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(See the Brief Report by Rakotondravelo et al on pages 1270–2.)

Background. Little information is available on the incidence of renal stones with ritonavir-boosted atazanavir (ATV/r) use.

Methods. In a single-center study, the incidence of renal stones was compared between human immunodeficiency virus (HIV)–infected patients who commenced ritonavir-boosted atazanavir (ATV/r)–containing antiretroviral (ARV) therapy (the ATV/r group) and those who were receiving other protease inhibitors (the other PIs group). The effects of ATV/r were estimated by univariate and multivariate Cox proportional hazards regression models. Other possible risk factors were evaluated by univariate analysis, and those found to be significant were entered into multivariate analysis.

Results. Renal stones were diagnosed in 31 patients (23.7 cases per 1000 person-years) in the ATV/r group (n = 465) and 4 in patients (2.2 cases per 1000 person-years) in the other PIs group (n = 775). ATV/r use was significantly associated with renal stones, by univariate and multivariate analyses (adjusted hazard ratio, 10.44; 95% confidence interval [CI], 3.685–29.59; $P < .001$). ATV/r remained a significant risk factor for renal stones in all subgroups stratified by the median values of baseline variables. In the 31 patients receiving ATV/r who developed renal stones, the median time from commencement of ATV/r to diagnosis was 24.5 months (interquartile range, 14.7–34.6 months). Of the 18 patients who continued ATV/r despite the diagnosis of renal stones, 6 (33.3%) experienced recurrence. No patient who discontinued ATV/r experienced recurrence during the observation period (250.6 person-months).

Conclusions. The incidence of renal stones was substantially higher among patients in the ATV/r group, compared with patients in the other PIs group. Continuation of ATV/r after diagnosis of renal stones was associated with a high rate of recurrence. Switching ATV/r to other ARVs is warranted in patients who develop renal stones.

Ritonavir-boosted atazanavir (ATV/r) is a widely used protease inhibitor (PI) in combination with other antiretroviral drugs for patients infected with human immunodeficiency virus type 1 (HIV). According to the present guidelines, ATV/r is one of the key first-line drugs because of its high efficacy, tolerability, favorable lipid profile, and once-daily dosing [1–4]. However, renal stone formation has been reported in patients receiving ATV/r-containing antiretroviral therapy (ART) [5, 6].

Received 11 April 2012; accepted 19 June 2012; electronically published 19 July 2012.

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Clinical Infectious Diseases 2012;55(9):1262–9

DOI: 10.1093/cid/cis621

Urolithiasis is a well-known side effect of indinavir (IDV), and its etiology is considered to be drug crystallization in the urine [7]. Previous studies identified ATV-containing urolithiasis, suggesting a similar etiology [5, 6, 8, 9]. However, there is virtually no information on the incidence of ATV/r-induced renal stones, although ATV/r is one of the most frequently prescribed PIs. It is important to elucidate the incidence of ATV/r-associated renal stones, since renal stones are risk factors for chronic kidney diseases (CKD), an important comorbidity associated with AIDS and death [10–12].

On the basis of this background, we conducted a retrospective study to compare the incidence of renal stones among patients receiving an ATV/r-containing regimen with the incidence among patients receiving one of the following commonly used PIs: unboosted fosamprenavir (FPV), ritonavir-boosted fosamprenavir (FPV/r), lopinavir/ritonavir (LPV/r), and ritonavir-boosted darunavir (DRV/r).

METHODS

Ethics Statement

This study was approved by the Human Research Ethics Committee of our hospital, the National Center for Global Health and Medicine, Tokyo. Each participant provided a written informed consent. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study Subjects

We performed a retrospective, single-center cohort study of HIV-infected patients using the medical records at our hospital. Our facility is one of the largest clinics for patients with HIV infection in Japan, with >2700 registered patients. The study population was HIV-infected patients aged >17 years who commenced treatment with ART containing ATV/r, FPV/r, FPV, LPV/r, or DRV/r between 1 January 2004 and 30 June 2010. Both treatment-naive and treatment-experienced patients were included. The follow-up period started at the time of commencement of ART that contained the above-mentioned drug for the first time during the study period, and patients were followed until 30 June 2011. Patients were excluded if (1) they had started the above-mentioned ART during the study period at other facilities, (2) they were prescribed unboosted ATV, or (3) they were receiving treatment for urolithiasis at the time they commenced the above-mentioned ART. Patients with previous exposure to one of the above-mentioned drugs before the present study and commenced the same drug in this study were also excluded from the analysis.

The attending physician selected ATV/r, FPV, FPV/r, LPV/r, or DRV/r at baseline. The use of these drugs was based on the Japanese guidelines, which placed all of the above-mentioned drugs as the preferred choice, at least for 3 years during the study period [13]. The attending physician also selected the concurrent drugs, including nucleoside reverse-transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs), integrase inhibitors, and CCR5 inhibitors. None of the patients received 2 PIs during the study period.

Measurements

The primary investigator (Y. H.) reviewed the medical records of all study patients who started new key drugs during the study period, to identify renal stone cases. Then 2 experienced HIV physicians (T. N. and K. W.) reviewed the set of medical records of each renal stone case to determine whether the cases fit into the following predefined criteria for renal stones: cases with a clinical diagnosis by the attending physician based on new onset of acute flank pain, plus one of the following: (1) new-onset hematuria confirmed by urine dipstick test; (2) 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 computed tomography; or (3) stone passage confirmed by either the patient or the attending physician. Patients with acute flank pain due to etiologies other than renal stones were excluded. In case of disagreement between the 2 reviewers, a third independent reviewer (H. K.) evaluated the deidentified document set by the same criteria to make the final determination. At the time of diagnosis of renal stones, the attending physician selected either continuation or modification of ART. In our clinic, it is customary for the patient to visit the clinic every month before the initiation of ART and until suppression of HIV load, but the visit interval is extended up to every 3 months after viral load suppression.

In this study, the primary exposure variable was ATV/r use over other PIs (FPV, FPV/r, LPV/r, and DRV/r). The potential risk factors for renal stones were determined according to previous studies and were collected from the medical records, together with the basic demographic characteristics [8, 9, 14]. They included age, sex, body weight, body mass index (BMI; defined as the weight in kilograms divided by the square of the height in meters), baseline laboratory data (CD4 cell count, HIV load, estimated glomerular filtration rate [eGFR], serum uric acid, and urine pH), and presence or absence of other medical conditions (ie, concurrent use of tenofovir [TDF]; past history of renal stones; previous exposure to IDV; coinfection with hepatitis B virus [HBV], defined by detection of HBV surface antigen; and coinfection with hepatitis C virus [HCV], defined by detection of HCV load). eGFR was calculated using the equation from the 4-variable Modification of Diet in Renal Diseases study [15]. Among patients receiving ATV/r-containing ART, the total serum bilirubin level measured on the day of stone diagnosis (for patients with renal stones) or 2 years after ATV/r initiation (for patients without

renal stones) was used. For patients who discontinued ATV/r within 2 years, the value closest to the day of discontinuation was used. At our clinic, weight was 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 ≤180 days.

Statistical Analysis

Baseline characteristics were compared using the unpaired Student *t* test or the χ^2 test (ie, the Fisher exact test) for quantitative or qualitative variables, respectively. The time to the diagnosis of urolithiasis was calculated from the date of commencement of predefined PI-containing ART to the date of diagnosis for urolithiasis. Censored cases represented those who discontinued the PIs, dropped out, were referred to other facilities, or at the end of the follow-up period. The time from the start of ART to the diagnosis of renal stones was analyzed by the Kaplan-Meier method for patients who started ATV/r (the ATV/r group) and those who started other PIs (the other PIs group), and the log-rank test was used to determine the statistical significance. The Cox proportional hazards regression analysis was used to estimate the impact of ATV/r use, compared with other PIs, on the incidence of renal stones. The impact of basic demographic characteristics, baseline laboratory data, and other medical conditions listed above was also estimated with univariate Cox proportional hazards regression. To estimate the unbiased prognostic impact of ATV/r use over other PIs for renal stones, we conducted 3 models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for ATV/r use over other PIs. Model 2 included age, sex, and weight plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with *P* values of < .05 in univariate analysis after adjustment (these included eGFR per 10 mL/min/1.73 m^2 and serum uric acid per 1 mg/dL). Possible risk factors for ATV/r-induced renal stones identified in previous studies were also added to model 3 (these included past history of renal stones and prior exposure to IDV) [8, 9].

To elucidate whether the impact of ATV/r on renal stones persist in subgroups, we divided patients into 2 groups on the basis of sex, age, baseline body weight, eGFR, and serum uric acid level, using the respective median value of each parameter. Then, the above-mentioned univariate analysis was conducted for each subgroup. In addition, to examine the association between total serum bilirubin level during ATV/r-containing ART and the incidence of renal stones, the median total serum bilirubin levels were compared between stone cases and nonstone cases, using the Mann-Whitney *U* test.

To explore the impact of urolithiasis on renal function, the change in eGFR was compared between stone cases (ie, the eGFR change between baseline and the diagnosis of renal stones) and nonstone cases (ie, the eGFR between baseline and 2 years after initiation of ATV/r) in patients receiving ATV/r, using the Student *t* test.

Statistical significance was defined as a 2-sided *P* value of < .05. We used hazard ratios (HRs) and 95% confidence intervals (CIs) to estimate the impact of each variable on renal stones. All statistical analyses were performed with SPSS, version 17.0 (SPSS, Chicago, IL).

RESULTS

A total of 1498 patients commenced or switched key drugs (PIs, NNRTIs, or an integrase inhibitor) between 1 January 2004 and 30 June 2010. Of the 1240 patients who were included in the analysis, 465 (37.5%) started ATV/r-containing ART, while 775 (62.5%) started other PI–containing ART (Figure 1). Table 1 shows the baseline characteristics of the study population. The majority of the study population was male, of East Asian origin, and comparatively young. The ATV/r group included significantly more patients of East Asian origin ($P = .015$) and had a significantly higher body weight ($P < .001$), higher CD4 cell count ($P < .001$), lower viral load ($P < .001$), higher baseline serum uric acid level ($P = .034$), and lower eGFR ($P = .012$). In contrast, patients in the other PIs group were significantly more likely to be treatment naive ($P < .001$) and significantly less likely to have had previous exposure to IDV ($P = .036$). However, all other major background parameters were similar in the 2 groups (Table 1).

The primary investigator (Y. H.) identified 37 renal stone cases, and 2 of these were excluded by the reviewers. Thirty-five patients fulfilled the predefined criteria for renal stones. Renal stones were identified in 31 patients (6.7%) from the ATV/r group and in 4 (0.52%) from the other PIs group, with an estimated incidence of 23.7 cases and 2.20 cases per 1000 person-years, respectively. The incidence of renal stones in the ATV/r group was approximately 10 times the incidence in the other PIs group. Of those renal stone cases, 4 and 14 patients were diagnosed on the basis of hematuria and stone passage, respectively, as defined above. Furthermore, 17 cases were diagnosed on the basis of radiological findings, of which renal calcification was identified in 4 cases. Figure 2 is a Kaplan-Meier curve of the time from initiation or switching of PIs defined above to the diagnosis of renal stones in the 2 groups. Patients in the ATV/r group were significantly more likely to develop renal stones, compared with those of the other PIs group ($P < .001$, by the log-rank test). The median time from the commencement of ART to the diagnosis of renal stones was 24.5 months (interquartile range [IQR], 14.7–34.6 months) for the ATV/r group and 21.9 months (IQR, 10.1–45.1 months) for the other PIs group. The total observation period was 1310.1 patient-years (median duration, 31.0 months; IQR, 15.0–48.7 months) for the

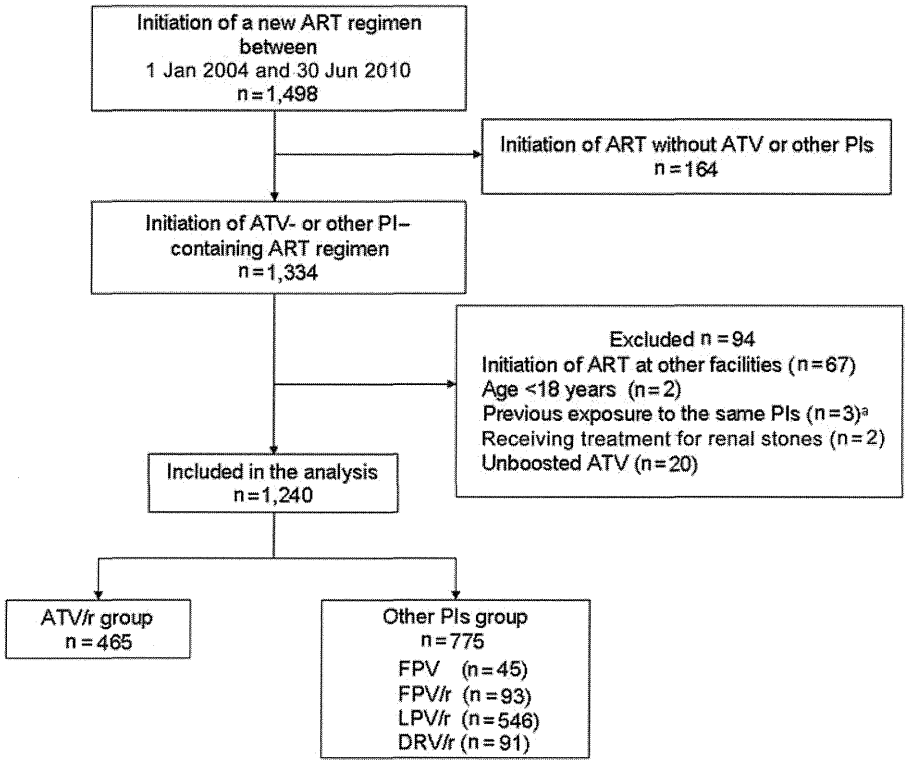

Figure 1. Flow diagram of patient selection. [a]Three patients were excluded for past lopinavir/ritonavir (LPV/r) exposure and commencement of LPV/r in the study. Abbreviations: ART, antiretroviral treatment; ATV, atazanavir; ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; FPV, fosamprenavir; FPV/r, ritonavir-boosted fosamprenavir; LPV/r, lopinavir/ritonavir; PIs, protease inhibitors.

Table 1. Baseline Demographic Characteristics and Laboratory Data for 1240 Patients Who Received Ritonavir-Boosted Atazanavir– or Other Protease Inhibitor–Containing Antiretroviral Therapy

Variable	ATV/r (n = 465)	Other PIs (n = 775)	P^a
Age, years	39.0 ± 10.6	40.0 ± 11.5	.125
Male sex	433 (93.1)	712 (91.9)	.424
Race (East Asian origin)	448 (96.3)	721 (93.0)	.015
Body weight, kg	65.0 ± 10.5	62.1 ± 10.7	<.001
BMI, kg/m^2	22.7 ± 3.14	21.7 ± 3.25	<.001
CD4 cell count, cells/μL	303.9 ± 184.7	176.4 ± 170.9	<.001
HIV load, $\log_{10}$ copies/mL	3.58 ± 1.38	4.42 ± 1.40	<.001
Treatment naive	282 (60.6)	555 (71.6)	<.001
Tenofovir use	177 (38.1)	326 (42.1)	.165
eGFR, mL/min/1.73 m^2	117.4 ± 25.8	121.7 ± 33.6	.012
Serum uric acid level, mg/dL	5.90 ± 1.31	5.71 ± 1.64	.034
Urine pH	6.30 ± 0.67	6.32 ± 0.62	.759
HBV or HCV coinfection	57 (12.3)	111 (14.3)	.304
Past history of urinary stone	35 (7.5)	41 (5.3)	.114
Previous exposure to IDV	43 (9.2)	47 (6.1)	.036

Data are No. (%) of patients or mean ± standard deviation.

Abbreviations: ATV/r, ritonavir-boosted atazanavir; BMI, body mass index; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDV, indinavir; PI, protease inhibitor.

[a] The χ^2 test or Fisher exact test was used for categorical data, and the Student *t* test was used for continuous variables.

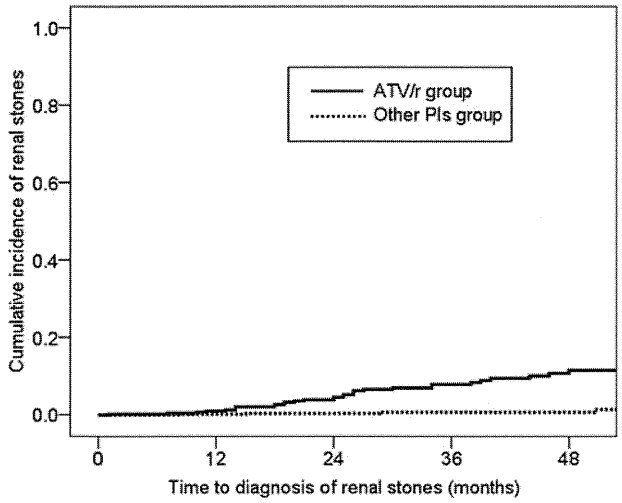

Figure 2. Kaplan-Meier curve showing time to diagnosis of renal stones. Abbreviations: ATV/r, ritonavir-boosted atazanavir; PIs, protease inhibitors.

ATV/r group and 1821.3 patient-years (median duration, 23.0 months; IQR, 10.3–42.4 months) for the other PIs group.

Univariate analysis showed a significant relationship between ATV/r use and renal stones (HR, 10.44; 95% CI, 3.685–29.59; $P < .001$; Table 2). Lower baseline eGFR (HR, 1.180; 95% CI, 1.042–1.336; $P = .009$) and higher serum uric acid level (HR, 1.334; 95% CI, 1.085–1.640; $P = .006$) were also significantly associated with the development of renal stones. On the other hand, body weight, BMI, history of IDV use, and past history of renal stones were not associated with renal stones (Table 2). Multivariate analysis identified ATV/r use as a significant risk for renal stones after adjustment for age, sex, and weight (adjusted HR, 9.339; 95% CI, 3.254–26.80; $P < .001$; Table 3, model 2) and also after adjustment for other risk factors (adjusted HR, 10.08; 95% CI, 3.487–29.17; $P < .001$; Table 3, model 3).

Figure 3 shows subgroup analysis of the patients stratified by sex and the median of the above-mentioned baseline variables. In all the subgroups, ATV/r remained an independent risk for renal stones. The median total bilirubin values in stone cases and nonstone cases were not significantly different (2.4 mg/dL [IQR, 1.8–3.4 mg/dL] and 2.3 mg/dL [IQR, 1.6–3.1 mg/dL], respectively; $P = .376$).

Of the 31 patients who developed renal stones in the ATV/r group, 13 discontinued ATV/r. Of the 18 patients who continued ATV/r despite the diagnosis of renal stones, 6 (33.3%) experienced recurrence of renal stones. The median time from the first episode of renal stones to recurrence was 4.9 months (IQR, 1.5–12.2 months). No patient required invasive procedures, such as lithotripsy. None of the 13 patients who discontinued ATV/r experienced recurrence during the observation period (total observation period, 250.6 person-months).

Table 2. Univariate Analysis to Estimate the Risk of Various Factors on Renal Stone Formation

	Hazard Ratio	95% CI	*P*
ATV/r use	10.44	3.685–29.59	<.001
Age, per 1 year increase	1.012	.981–1.043	.456
Male sex	1.380	.331–5.754	.659
Race (East Asian origin)	1.927	.264–14.08	.518
Body weight, per 1 kg increase	0.994	.962–1.028	.740
BMI per 1 kg/m^2 increase	0.997	.900–1.105	.954
CD4 cell count, per 10 cells/μL increase	1.013	.998–1.028	.096
HIV load, per $\log_{10}$/mL increase	0.909	.729–1.133	.395
Treatment naive	0.565	.291–1.099	.092
Tenofovir use	0.623	.299–1.299	.207
Baseline eGFR, per 10 mL/min/1.73 m^2 decrease	1.180	1.042–1.336	.009
Baseline serum uric acid level, per 1 mg/dL increase	1.334	1.085–1.640	.006
Baseline urine pH, per 1 increase	0.385	.133–1.119	.080
HBV or HCV coinfection	1.629	.712–3.729	.248
Past history of renal stone	2.109	.818–5.438	.122
Previous exposure to IDV	2.072	.860–4.996	.105

Abbreviations: BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDV, indinavir.

The mean eGFR decreased more significantly in the stone cases than in nonstone cases (30.7 vs 8.1 mL/min/1.73 m^2; $P < .001$). In the 13 patients who discontinued ATV/r after the first episode, the mean eGFR recovery was 20.1 mL/min/1.73 m^2 in 6 months after ATV/r discontinuation.

DISCUSSION

In the present study, the incidence of renal stones among patients receiving ATV/r was approximately 10 times the incidence among those receiving other PIs. Univariate and multivariate analyses identified ATV/r use as an independent risk factor for renal stones, with a high HR.

This study estimates the incidence of ATV/r-induced renal stones, using clinically feasible criteria: acute flank pain with clinical diagnosis of renal stones by the attending physician, confirmed by radiological findings, new-onset hematuria, or confirmation of stone passage. A single previous report compared the incidence of renal stones among patients receiving ATV/r and those receiving other antiretrovirals [16]. However, the diagnosis of renal stones in that study was based only on

Table 3. Multivariate Analysis to Estimate the Risk of Ritonavir-Boosted Atazanavir– or Other Protease Inhibitor–Containing Antiretroviral Therapy on Renal Stone Formation

	Model 1, Crude (n = 1240)		Model 2, Adjusted (n = 1115)		Model 3, Adjusted (n = 1115)	
	HR	95% CI	HR	95% CI	HR	95% CI
ATV/r use	10.44	3.685–29.59	9.339	3.254–26.80	10.08	3.487–29.12
Age, per 1 year increase	…	…	1.012	.980–1.046	1.002	.965–1.040
Male sex	…	…	1.731	.378–7.932	1.222	.257–5.799
Body weight, per 1 kg increase	…	…	0.980	.944–1.018	0.965	.927–1.004
Baseline eGFR, per 10 mL/min/1.73 m^2 decrease	…	…	…	…	1.157	.968–1.382
Baseline serum uric acid level, per 1 mg/dL increase	…	…	…	…	1.423	1.091–1.856
Past history of renal stone	…	…	…	…	1.182	.310–4.501
Past exposure to IDV	…	…	…	…	1.265	.415–3.859

Abbreviations: ATV/r, ritonavir-boosted atazanavir; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; IDV, indinavir.

radiological findings. It is likely that the incidence of ATV/r-induced renal stones was underestimated in that study, because radiological studies were not necessarily performed on all patients suspected of renal stones. Accordingly, the reported incidence of ATV/r-induced renal stones was much lower (7.3 cases per 1000 person-years), compared with 23.7 cases per 1000 person-years in our study. Thus, our results more likely reflect the true incidence of ATV/r-induced renal stones.

The development of renal stones is a risk factor for CKD [10, 11]. Many studies have also demonstrated that ATV/r use is a risk factor for renal dysfunction or CKD [17–19]. The high incidence of renal stones with ATV/r use may in part contribute to ATV/r being a risk factor for CKD. Thus, ATV/r should be carefully introduced to patients with concomitant predisposing factors for CKD.

Six of the 18 patients who continued ATV/r despite the diagnosis of renal stones experienced recurrence. In contrast,

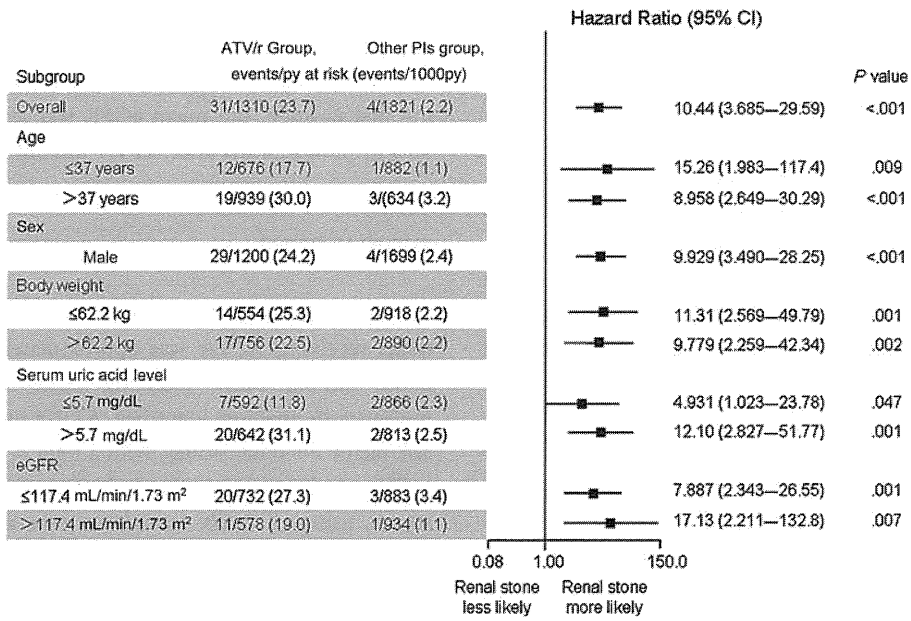

Figure 3. Estimated effect of ritonavir-boosted atazanavir, compared with other protease inhibitors on the hazard of renal stone formation, according to baseline characteristics. Abbreviations: ATV/r, ritonavir-boosted atazanavir; CI, confidence interval; eGFR, estimated glomerular filtration rate; PI, protease inhibitor; py, person-years.

none who discontinued ATV/r experienced recurrence. Thus, replacement of ATV/r with other drugs should be considered for patients who receive a diagnosis of renal stones, to prevent further deterioration in renal function.

Subgroup analysis showed that ATV/r was a risk factor for renal stones in all subgroups. Thus, we could not find any alleviating or aggravating factors for ATV/r-induced renal stones. Previous reports suggested several risk factors for ATV-induced renal stones, such as chronic renal impairment, coinfection with hepatitis virus, and past history of renal stones [9, 16]. However, the statistical methods used in those studies were inadequate to elucidate risks for ATV/r-induced renal stones. Our study did not add new findings to the risk for ATV/r-induced stones because of the small number of patients, leading to a low statistical power in subgroup analysis.

The mechanism of ATV/r-induced renal stone formation is not fully understood. However, like IDV stones, the precipitation of pure ATV is suggested as a possible etiology [9]. About 7% of ATV and 20% of IDV is excreted unchanged in the urine, which may contribute to the stone formation [24, 25]. In contrast, urolithiasis associated with other PIs, such as LPV/r, nelfinavir, and amprenavir, is rare, and this could be due to the minimal (<3%) excretion of these PIs [20–23]. Rockwood et al [16] found a close association between hyperbilirubinemia and the development of renal stones. This may be explained by the previously reported data that plasma ATV concentrations correlate with serum bilirubin level [26]. However, our data showed no relation between serum bilirubin level and the occurrence of renal stones. The concomitant use of TDF lowers plasma concentrations of ATV [1], and it is of interest whether the incidence of ATV/r-stones is lower among patients with concomitant use of TDF than among those without concomitant TDF use. Nevertheless, the present study did not find concomitant TDF to be a protective factor against ATV-renal stones.

There are several limitations to our study. First, because of the retrospective nature of the study, the baseline characteristics of the enrolled patients were not controlled. Thus, it is possible that more patients with potential risks for renal stones were included in the ATV/r group. Patients in the ATV/r group had hyperuricemia, which is a known risk factor for renal stones. However, ATV/r use remained a strong risk factor by multivariate analysis, even after adjustment for possible risk factors, including hyperuricemia. Second, the definition of renal stones in our study did not necessarily require radiological confirmation in all cases. However, the definition used in our study is well suited to cover clinically significant renal stone cases, especially considering that many ATV-induced renal stones are radiolucent [9]. Third, none of the patients with renal stones had stone composition analysis performed. Therefore, it is possible that renal stones with other etiologies were included. Fourth, because the number of individuals receiving efavirenz or raltegravir was small in our cohort, they were not included in the analysis, and we thus could not compare the effect of ATV/r to effect of these widely used antiretroviral drugs on the development of renal stones (Figure 1). Last, since most of the patients were of East Asian origin, our results may not be applicable to other populations.

In conclusion, the present study demonstrated a high incidence of renal stones among patients receiving ATV/r-containing ART, compared with those receiving other PI–containing ART. ATV/r use was an independent risk for renal stones in a robust statistical model that included ATV/r use as a primary exposure. ATV/r should be carefully prescribed to patients with predisposing factors for renal stone formation or those with CKD. For those who develop ATV/r-induced renal stones, discontinuation of ATV/r is warranted because of the high risk of recurrence.

Notes

Acknowledgments. We thank Akiko Nakano, for supporting this study as a research coordinator, and all other clinical staff at the AIDS Clinical Center, for their help in the completion of this study.

Financial support. This work was supported by a grant-in-aid for AIDS research from the Ministry of Health, Labor, and Welfare of Japan (H23-AIDS-001) and by the Global Center of Excellence Program, the Ministry of Education, Science, Sports, and Culture of Japan.

Potential conflicts of interest. S. O. has received honoraria and research grants from MSD, Abbott Japan, Janssen Pharmaceutical, Pfizer, and Roche Diagnostics and has received honoraria from Astellas Pharmaceutical, Bristol-Myers, Daiichisankyo, Dainippon Sumitomo Pharma, GlaxoSmithKline, Taisho Toyama Pharmaceutical, Torii Pharmaceutical, and ViiV Healthcare. H. G. has received honoraria from MSD, Abbott Japan, Janssen Pharmaceutical, Torii Pharmaceutical, and ViiV Healthcare. All other authors report no potential conflicts.

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

References

1. Adolescents PoAGfAa. Panel on antiretroviral guidelines for adults and adolescents. guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Available at: http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed 3 April 2012.
2. European AIDS Clinical Society. Guidelines for the clinical management and treatment of HIV infected adults in Europe. Available at: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf. Accessed 3 April 2012.
3. Squires K, Lazzarin A, Gatell JM, et al. Comparison of once-daily atazanavir with efavirenz, each in combination with fixed-dose zidovudine and lamivudine, as initial therapy for patients infected with HIV. J Acquir Immune Defic Syndr **2004**; 36:1011–9.
4. Molina JM, Andrade-Villanueva J, Echevarria J, et al. Once-daily atazanavir/ritonavir compared with twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 96-week efficacy and safety results of the CASTLE study. J Acquir Immune Defic Syndr **2010**; 53:323–32.

5. Chang HR, Pella PM. Atazanavir urolithiasis. N Engl J Med **2006**; 355:2158–9.
6. Anderson PL, Lichtenstein KA, Gerig NE, Kiser JJ, Bushman LR. Atazanavir-containing renal calculi in an HIV-infected patient. AIDS **2007**; 21:1060–2.
7. Nadler RB, Rubenstein JN, Eggener SE, Loor MM, Smith ND. The etiology of urolithiasis in HIV infected patients. J Urol **2003**; 169:475–7.
8. Chan-Tack KM, Truffa MM, Struble KA, Birnkrant DB. Atazanavir-associated nephrolithiasis: cases from the US Food and drug administration's adverse event reporting system. AIDS **2007**; 21:1215–8.
9. Couzigou C, Daudon M, Meynard JL, et al. Urolithiasis in HIV-positive patients treated with atazanavir. Clin Infect Dis **2007**; 45:e105–8.
10. Rule AD, Bergstralh EJ, Melton LJ 3rd, Li X, Weaver AL, Lieske JC. Kidney stones and the risk for chronic kidney disease. Clin J Am Soc Nephrol **2009**; 4:804–11.
11. Jungers P, Joly D, Barbey F, Choukroun G, Daudon M. ESRD caused by nephrolithiasis: prevalence, mechanisms, and prevention. Am J Kidney Dis **2004**; 44:799–805.
12. Gupta SK, Eustace JA, Winston JA, 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**; 40:1559–85.
13. The Japanese Ministry of Health, Labour, and Welfare. Guidelines for antiretroviral treatment for patients with HIV infection [in Japanese]. Available at: http://www.haart-support.jp/guideline2011.pdf. Accessed 3 April 2012.
14. Parmar MS. Kidney stones. BMJ **2004**; 328:1420–4.
15. Levey AS, Coresh J, Greene T, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med **2006**; 145:247–54.
16. Rockwood N, Mandalia S, Bower M, Gazzard B, Nelson M. Ritonavir-boosted atazanavir exposure is associated with an increased rate of renal stones compared with efavirenz, ritonavir-boosted lopinavir and ritonavir-boosted darunavir. AIDS **2011**; 25:1671–3.
17. Mocroft A, Kirk O, Reiss P, et al. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. AIDS **2010**; 24:1667–78.
18. Albini L, Cesana BM, Motta D, et al. A randomized, pilot trial to evaluate glomerular filtration rate by creatinine or cystatin C in naive HIV-infected patients after tenofovir/emtricitabine in combination with atazanavir/ritonavir or efavirenz. J Acquir Immune Defic Syndr **2012**; 59:18–30.
19. Young J, Schafer J, Fux CA, et al. Renal function in patients with HIV starting therapy with tenofovir and either efavirenz, lopinavir or atazanavir. AIDS **2012**; 26:567–75.
20. Engeler DS, John H, Rentsch KM, Ruef C, Oertle D, Suter S. Nelfinavir urinary stones. J Urol **2002**; 167:1384–5.
21. Feicke A, Rentsch KM, Oertle D, Strebel RT. Same patient, new stone composition: amprenavir urinary stone. Antivir Ther **2008**; 13:733–4.
22. Doco-Lecompte T, Garrec A, Thomas L, Trechot P, May T, Rabaud C. Lopinavir-ritonavir (Kaletra) and lithiasis: seven cases. AIDS **2004**; 18:705–6.
23. Kaletra (lopinavir/ritonavir): full prescription information [package insert]. Barcelona, PR: Abbott Pharmaceuticals PR, **2012**.
24. Reyataz (atazanavir sulfate): full prescription information [package insert]. Princeton, NJ: Bristol-Myers Squibb, **2012**.
25. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. JAMA **1997**; 277:145–53.
26. Rodriguez Novoa S, Barreiro P, Rendon A, et al. Plasma levels of atazanavir and the risk of hyperbilirubinemia are predicted by the 3435C → T polymorphism at the multidrug resistance gene 1. Clin Infect Dis **2006**; 42:291–5.

HIV/AIDS MAJOR ARTICLE

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

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

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

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

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

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

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

Received 20 May 2012; accepted 24 August 2012; electronically published 5 September 2012.

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Clinical Infectious Diseases 2012;55(11):1558–67

DOI: 10.1093/cid/cis772

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

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

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

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

METHODS

Ethics Statement

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

Study Design

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

Study Subjects

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

Measurements

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

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

Definition of Renal Proximal Tubular Dysfunction

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

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

Genetic Polymorphisms

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

Pharmacogenetic Analyses

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

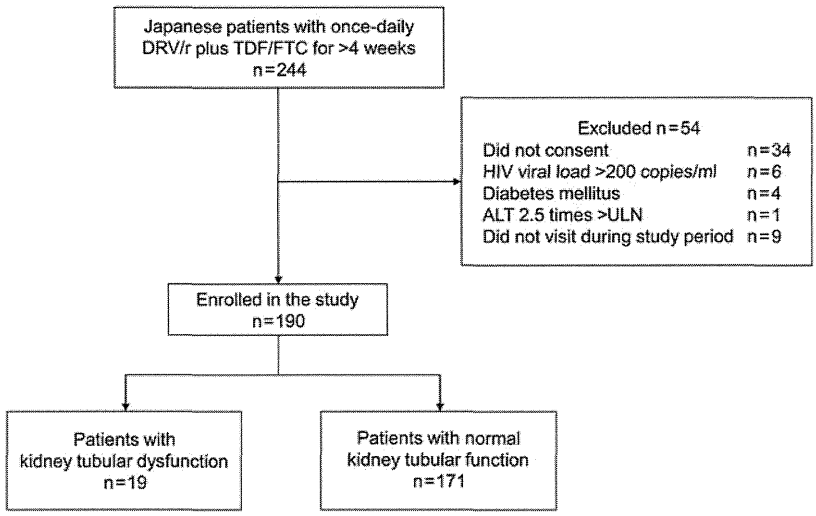

Figure 1. Patient enrollment. Abbreviations: ALT, alanine transaminase; DRV/r, ritonavir-boosted darunavir; HIV, human immunodeficiency virus; TDF/FTC, tenofovir/emtricitabine; ULN, upper limit of normal.

Statistical Analysis

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

RESULTS

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

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

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

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

[a] Median (interquartile range).

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

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

Table 2 continued.

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

Abbreviation: KTD, kidney tubular dysfunction.

[a] By Fisher exact test.

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

Association of Genotypes with KTD

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

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

Association of Haplotypes at ABCC2 and ABCC4 with KTD

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

polymorphisms in tenofovir-induced KTD and confirm the effect of the SNPs in *ABCC2* gene in tenofovir-induced KTD.

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

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

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

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

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

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

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

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

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

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

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

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

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

Notes

Acknowledgments. The authors thank Ryo Yamada, Takuro Shimbo, Fumihiko Hinoshita, Yoshimi Kikuchi, Katsuji Teruya, Kunihisa Tsukada, Junko Tanuma, Hirohisa Yazaki, Haruhito Honda, Ei Kinai, Koji

Watanabe, Takahiro Aoki, Daisuke Mizushima, Yohei Hamada, Michiyo Ishisaka, Mikiko Ogata, Mai Nakamura, Akiko Nakano, Fumihide Kanaya, and all other staff at the AIDS Clinical Center for their help in completion of this study.

Financial support. This work was supported by a Grant-in-Aid for AIDS research from the Japanese Ministry of Health, Labour, and Welfare (H23-AIDS-001), and the Global Center of Excellence Program (Global Education and Research Center Aiming at the Control of AIDS) from the Japanese Ministry of Education, Science, Sports, and Culture.

Potential conflicts of interest. S. O. has received honorariums and research grants from MSD K.K., Abbott Japan, Janssen Pharmaceutical K.K., Pfizer, and Roche Diagnostics K.K.; has received honorariums from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Dainippon Sumitomo Pharma, GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, Torii Pharmaceutical, and ViiV Healthcare. H. G. has received honorariums from MSD K.K., Abbott Japan, Janssen Pharmaceutical K.K., Torii Pharmaceutical, Roche Diagnostics K.K., and ViiV Healthcare. The remaining authors declare no conflict of interest.

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

References

1. Sax PE, Tierney C, Collier AC, et al. Abacavir/lamivudine versus tenofovir DF/emtricitabine as part of combination regimens for initial treatment of HIV: final results. J Infect Dis **2011**; 204:1191–201.
2. Post FA, Moyle GJ, Stellbrink HJ, et al. Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr **2010**; 55:49–57.
3. Arribas JR, Pozniak AL, Gallant JE, et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naive patients: 144-week analysis. J Acquir Immune Defic Syndr **2008**; 47:74–8.
4. de Vries-Sluijs TE, Reijnders JG, Hansen BE, et al. Long-term therapy with tenofovir is effective for patients co-infected with human immunodeficiency virus and hepatitis B virus. Gastroenterology **2010**; 139:1934–41.
5. Izzedine H, Hulot JS, Vittecoq D, et al. Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. Nephrol Dial Transplant **2005**; 20:743–6.
6. Nelson MR, Katlama C, Montaner JS, et al. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. AIDS **2007**; 21:1273–81.
7. 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.
8. Verhelst D, Monge M, Meynard JL, et al. Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis **2002**; 40:1331–3.
9. 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:e41–3.
10. Peyriere H, Reynes J, Rouanet I, et al. Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. J Acquir Immune Defic Syndr **2004**; 35:269–73.
11. Kohler JJ, Hosseini SH, Hoying-Brandt A, et al. Tenofovir renal toxicity targets mitochondria of renal proximal tubules. Lab Invest **2009**; 89:513–9.
12. Herlitz LC, Mohan S, Stokes MB, Radhakrishnan J, D'Agati VD, Markowitz GS. Tenofovir nephrotoxicity: acute tubular necrosis with distinctive clinical, pathological, and mitochondrial abnormalities. Kidney Int **2010**; 78:1171–7.
13. Izzedine H, Hulot JS, Villard E, et al. Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. J Infect Dis **2006**; 194:1481–91.
14. Kiser JJ, Aquilante CL, Anderson PL, King TM, Carten ML, Fletcher CV. Clinical and genetic determinants of intracellular tenofovir diphosphate concentrations in HIV-infected patients. J Acquir Immune Defic Syndr **2008**; 47:298–303.
15. Rodriguez-Novoa S, Labarga P, Soriano V, et al. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. Clin Infect Dis **2009**; 48:e108–16.
16. Uwai Y, Ida H, Tsuji Y, Katsura T, Inui K. Renal transport of adefovir, cidofovir, and tenofovir by SLC22A family members (hOAT1, hOAT3, and hOCT2). Pharm Res **2007**; 24:811–5.
17. Mallants R, Van Oosterwyck K, Van Vaeck L, Mols R, De Clercq E, Augustijns P. Multidrug resistance-associated protein 2 (MRP2) affects hepatobiliary elimination but not the intestinal disposition of tenofovir disoproxil fumarate and its metabolites. Xenobiotica **2005**; 35:1055–66.
18. Imaoka T, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal elimination of the antiviral drugs adefovir and tenofovir. Mol Pharmacol **2007**; 71:619–27.
19. Kohler JJ, Hosseini SH, Green E, et al. Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. Lab Invest **2011**; 91:852–8.
20. Ray AS, Cihlar T, Robinson KL, et al. Mechanism of active renal tubular efflux of tenofovir. Antimicrob Agents Chemother **2006**; 50:3297–304.
21. Pushpakom SP, Liptrott NJ, Rodriguez-Novoa S, et al. Genetic variants of ABCC10, a novel tenofovir transporter, are associated with kidney tubular dysfunction. J Infect Dis **2011**; 204:145–53.
22. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA **2000**; 97:3473–8.
23. Horinouchi M, Sakaeda T, Nakamura T, et al. Significant genetic linkage of MDR1 polymorphisms at positions 3435 and 2677: functional relevance to pharmacokinetics of digoxin. Pharm Res **2002**; 19:1581–5.
24. Kurata Y, Ieiri I, Kimura M, et al. Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. Clin Pharmacol Ther **2002**; 72:209–19.
25. Han WK, Waikar SS, Johnson A, et al. Urinary biomarkers in the early diagnosis of acute kidney injury. Kidney Int **2008**; 73:863–9.
26. Kiser JJ, Carten ML, Aquilante CL, et al. The effect of lopinavir/ritonavir on the renal clearance of tenofovir in HIV-infected patients. Clin Pharmacol Ther **2008**; 83:265–72.
27. Goicoechea M, Liu S, Best B, et al. Greater tenofovir-associated renal function decline with protease inhibitor-based versus nonnucleoside reverse-transcriptase inhibitor-based therapy. J Infect Dis **2008**; 197:102–8.
28. Rodriguez-Novoa S, Labarga P, Soriano V. Pharmacogenetics of tenofovir treatment. Pharmacogenomics **2009**; 10:1675–85.
29. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron **1976**; 16:31–41.
30. Salem MA, el-Habashy SA, Saeid OM, el-Tawil MM, Tawfik PH. Urinary excretion of n-acetyl-beta-D-glucosaminidase and retinol binding protein as alternative indicators of nephropathy in patients with type 1 diabetes mellitus. Pediatr Diabetes **2002**; 3:37–41.
31. Ezinga M, Wetzels J, van der Ven A, Burger D. Kidney tubular dysfunction is related to tenofovir plasma concentration, abstract 603. In: Program and abstracts of the 19th Conference on Retroviruses and Opportunistic Infections. 5–8 March 2012, Seattle, Washington.
32. Gupta SK, Eustace JA, Winston JA, 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**; 40:1559–85.