Figure 1. Endoscopic features of amebic colitis, Japan, 2003–2009. A) Colonoscopy showing ulcers in the rectum. B) Colonoscopy showing multiple erosions with exudates surrounded by edematous mucosa in the sigmoid colon.

of amebic infection. The physician confirmed whether the patient had traveled in tropical areas, resided in a facility for the intellectually disabled, was a male or female commercial sex worker (CSW), or had contact with a CSW or MSM. For travel exposure, history of overseas travel in the past year was elicited. Patients to whom none of the above applied were treated as unknown.

Statistical Analysis

We assessed changes in annual prevalence by using the χ^2 test for linear trends. We summarized descriptive data for patients with and without amebic colitis. To determine risk factors for amebic colitis, we estimated the odds ratio (OR) between amebic colitis and clinical factors including age,

sex, sexual preference, and history of STDs. We divided patients into 2 age groups, ≥ 50 years and <50 years. We used a multiple logistic regression model with factors that showed $p<0.2$ by univariate analysis. A final model was then developed by backward selection of factors that showed $p<0.05$. The adequacy of this model was evaluated by using the Hosmer-Lemeshow goodness-of-fit test and a receiver operating characteristic area under the curve.

We also conducted subgroup analysis concerning HIV infection. We investigated interactions between the effect of HIV infection and risk factors for amebic colitis. In HIV-positive patients, the relationship between prevalence of amebic colitis and CD4 cell counts in 4 categories was evaluated by using the χ^2 test for linear trends. All statistical
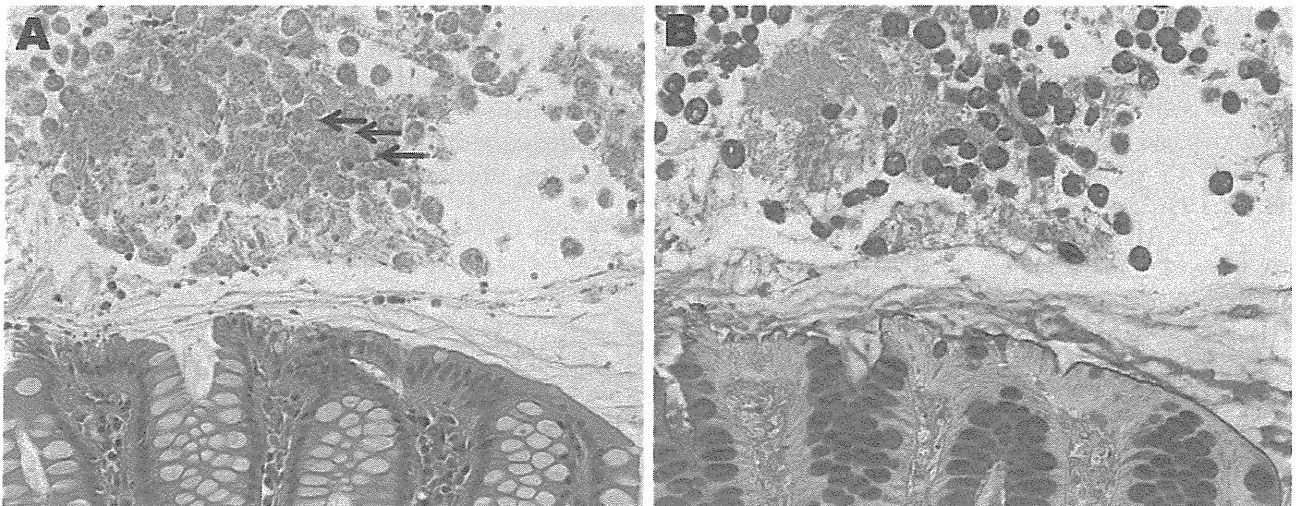

Figure 2. Histologic analysis of amebic colitis, Japan, 2003–2009. A) Trophozoites of *Entamoeba histolytica* ingesting erythrocytes (arrows) (hematoxylin and eosin stain). B) Numerous amebic trophozoites on the mucosal surface (periodic acid–Schiff stain). Original magnification ×200.

analyses were performed by using Stata version 10 software (StataCorp LP, College Station, TX, USA).

Results

Annual Prevalence of Amebic Colitis

Among 10,930 patients, 54 (0.5%) showed development of amebic colitis. Prevalence was 0.16% in 2003 but tended to increase over time (p<0.01 by trend test) (Figure 3). Prevalence was 5.6-fold higher in 2009 than in 2003.

Patient Characteristics

HIV-infected patients constituted 248 (2.3%) of 10,930 patients, and they had a median age of 43 years (interquartile range [IQR] 35–55 years) (Table 1). These HIV-infected patients were predominantly male (91.5%, 227/248). Median CD4 cell count was 230 cells/μL (IQR 89.5–401 cells/μL). Routes of HIV infection included homosexual (58.9%, 146/248), heterosexual (12.5%, 31/248), bisexual (10.5%, 26/248), unknown (12.1%, 30/248), untreated blood products (6.0%, 15/248), and drug use (0%).

Patients with a history of HBV infection constituted 184 (1.7%) of 10,390 patients, and they had a median age of 61 years (IQR 47.5–69 years). These patients were also predominantly male (69.0%, 127/184).

Patients with a history of syphilis constituted 266 (2.4%) of 10,390 patients, and they had a median age of 64 years (IQR 48–74 years). These patients were also predominantly male (76.3%, 203/266).

Risk Factors for Amebic Colitis

Risk factors for amebic colitis were age <50 years (OR 11.4, 95% CI 6.1–22.4), male sex (OR 18.5, 95% CI 4.9–156.7), HIV infection (OR 66.2, 95% CI 36.6–120.7), history of HBV infection (OR 9.0, 95% CI 3.4–20.4) and history of syphilis (OR 19.6, 95% CI 10.2–36.2) (Table 1). Multivariate analysis showed that age <50 years (OR 4.73, 95% CI 2.43–9.20, p<0.001), male sex (OR 8.39, 95% CI 1.99–35.40, p<0.01), HIV infection (OR 15.85, 95% CI 7.93–31.70, p<0.01), and history of syphilis (OR 2.90, 95% CI 1.40–5.99, p<0.01) were independent risk factors for amebic colitis. This logistic regression model was evaluated by using the Hosmer-Lemeshow test (p = 0.44) and receiver operating characteristic area under the curve (0.90).

Comparison of HIV-Positive and HIV-Negative Patients

Annual Prevalence of Amebic Colitis

Numbers of HIV-positive and HIV-negative patients have been increased annually during 2003–2009 in Japan

(Figure 4). Among HIV-positive patients, the prevalence in 2009 increased by 2.1-fold over that in 2003 (Figure 4, panel A). Among HIV-negative patients, the prevalence in 2009 increased by 7.1-fold over that in 2003 (Figure 4, panel B).

Risk Factors for Amebic Colitis

Among HIV-positive patients, age <50 years, history of syphilis, and MSM status were risk factors for amebic colitis (Table 2). Immunosuppressed status, such as CD4 cell count <100 cells/μL, was not associated with amebic colitis among HIV-positive patients (Table 2). As CD4 cell counts decreased, the prevalence of amebic decreased (OR 0.3; p = 0.08 by trend test).

Among HIV-negative patients, age <50 years, male sex, history of HBV infection, and history of syphilis were risk factors for amebic colitis (Table 2). No interactions were apparent between HIV infection and risk factors, such as age, sex, history of syphilis, and history of HBV infection.

Route of Amebic Infection

Among HIV-positive patients, all 31 patients with amebic infection were male (Table 3). Of these patients, 28 were MSM and 2 were male CSWs. No patients reported contact with CSWs. The route of infection was unknown for 3 patients.

Among HIV-negative patients, 2 patients were female and 21 were male. Both female patients were CSWs. Of the 21 male patients, 8 had had sexual contact with a female CSW and 7 patients were MSM (2 bisexual and 5 homosexual). The route of infection was unknown for 6 patients.

Discussion

Endoscopic examination combined with biopsy sample collection is a valuable method for confirming suspected amebic colitis, which is often misdiagnosed as inflammatory bowel disease or other forms of infectious colitis caused by the similarity of associated gastrointestinal symptoms (e.g.,
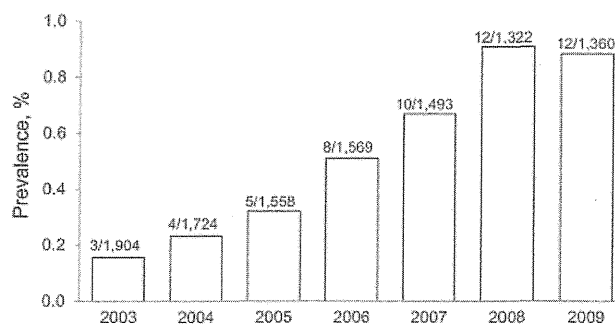

Figure 3. Annual prevalence of amebic colitis, Japan, 2003–2009. Values above bars are no. positive/no. tested.

Table 1. Characteristics and risk factors for 10,930 patients with amebic colitis, Japan, 2003–2009*

Characteristic	All, n = 10,930	Amebic colitis, n = 54	No amebic colitis, n = 10,876	Odds ratio (95% CI)
Median age (IQR)	64 (54–73)	41 (36–52)	65 (54–73)	NA
Age, y				
≥50	8,875 (81.2)	15 (27.7)	8,860 (81.5)	Referent
<50	2,055 (18.8)	39 (72.2)	2,016 (18.5)	11.4 (6.1–22.4)
Sex				
F	4,522 (41.4)	2 (3.7)	4,520 (39.1)	Referent
M	6,408 (58.6)	52 (96.3)	6,356 (58.4)	18.5 (4.9–156.7)
HIV infection				
Negative	10,682 (97.7)	23 (42.5)	10,659 (98.0)	Referent
Positive	248 (2.3)	31 (57.4)	217 (2.0)	66.2 (36.6–120.7)
HBV infection				
Negative	10,746 (98.3)	47 (87.0)	10,699 (84.0)	Referent
Positive	184 (1.7)	7 (13.0)	177 (1.6)	9.0 (3.4–20.4)
Syphilis				
Negative	10,664 (97.6)	37 (68.5)	10,627 (97.7)	Referent
Positive	266 (2.4)	17 (31.5)	249 (2.3)	19.6 (10.2–36.2)

*Values are no. (%) except as indicated. IQR, interquartile range; NA, not applicable; HBV, hepatitis B virus. p values for all comparisons were <0.05, by Mann-Whitney U test.

diarrhea, hematochezia, and abdominal pain) (14,22,23). However, only a few studies have included patients who had undergone endoscopy (17,22,23). In the present study, we performed a large number of endoscopic examinations. The prevalence of patients with amebic colitis was 0.5% (54/10,930) in this 7-year study. This prevalence was far lower than results from serum prevalence studies, which have shown prevalence in children of 8.4% in Mexico (24) and 4.2% in Bangladesh (25). However, the annual prevalence of the disease showed a tendency to increase to nearly 1% in recent years, and we assume the prevalence will continue to increase in the future.

In the past, amebic infection in Japan was reportedly caused by overseas travel to countries where epidemics occurred or where amebic infection was found in residents of facilities for the intellectually disabled (16,26). However, patients with these characteristics were not observed in this study. Multivariate analysis indicated that risk factors for amebic colitis in this study were male sex, age <50 years, and histories of syphilis and HIV infection.

The reason male sex was a risk factor might be related to specific sexual preference (8,10–15) because 52 male patients with amebic colitis often had contact with MSM (n = 35) or female CSWs (n = 8). In this study, MSM constituted 90% of men (OR 4.7 for patients with HIV infection), which is consistent with results of previous reports (8,10–15). However, HIV-negative male patients included heterosexual patients, and ≈35% of them had had contact with CSWs. We included CSWs as routes of infection for amebiasis because amebiasis among female CSWs has been reported in Japan (27). Therefore, new infection routes other than MSM, which has been considered a risk because of a diversity of sexual activities, should be considered.

Consistent with results of past reports (8,14,15), younger age was a risk factor. One possibility is that younger age represents a risk factor because younger persons are more sexually active, although this was not clarified in the present study.

Histories of syphilis or HIV infection have been noted as risk factors in previous case series (7,15,28). The present study included many patients with HIV infection or history of syphilis, which supports the hypothesis that these factors increase the risk for amebic colitis.
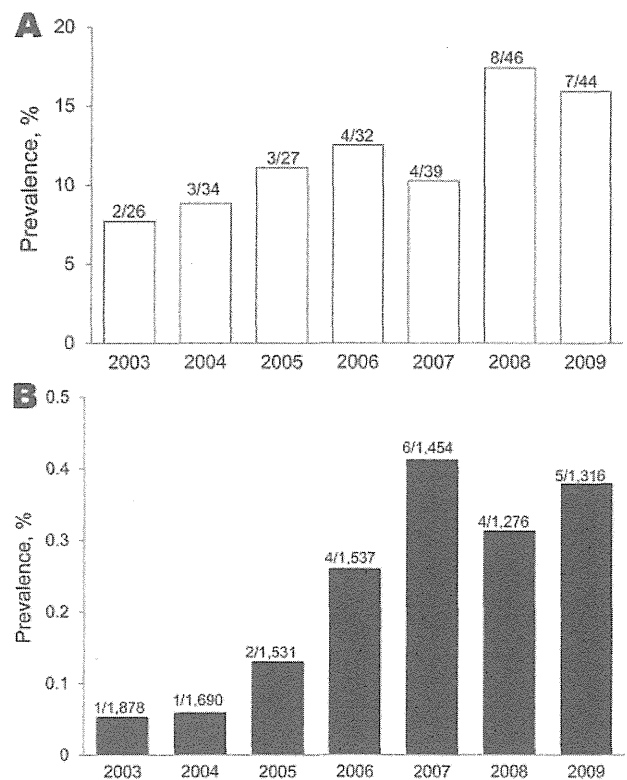

Figure 4. Annual prevalence of amebic colitis in persons with or without HIV infection, Japan, 2003–2009. A) HIV-positive patients. B) HIV-negative patients. Values above bars are no. positive/no. tested.

217

Table 2. Risk factors for amebic colitis among HIV-positive and -negative patients, Japan, 2003–2009*

Risk factor	HIV-positive patients				HIV-negative patients				p value for interaction
	Amebic colitis, n = 31	No amebic colitis, n = 217	OR (95% CI)	p value	Amebic colitis, n = 23	No amebic colitis, n = 10,659	OR (95% CI)	p value	
Age, y									
≥50	6	83	Referent		9	8,777	Referent		
<50	25	134	2.6 (1.0–8.0)	0.04	14	1,882	7.3 (2.9–19.0)	<0.01	0.11
Sex									
F	0	21	Referent		2	4499	Referent		
M	31	196	4.6† (0.8–∞)	0.11†	21	6,160	7.7 (1.9–67.5)	<0.01	0.56
HBV infection									
Negative	26	189	Referent		21	10,510	Referent		
Positive	5	28	1.3 (0.4–3.8)	0.62	2	149	6.7 (0.8–27.9)	<0.01	0.07
Syphilis									
Negative	16	163	Referent		21	10,464	Referent		
Positive	15	54	2.8 (1.2–6.5)	<0.01	2	195	5.1 (0.6–21.1)	0.01	0.48
Sexual preference									
Heterosexual	3	73	Referent		ND	ND	ND	ND	ND
MSM	28	144	4.7 (1.4–25.0)	<0.01	ND	ND	ND	ND	ND
CD4 cell count/µL									
>300	14	82	Referent	ND	ND	ND	ND	ND	ND
201–300	5	31	0.9 (0.3–2.8)	ND	ND	ND	ND	ND	ND
100–200	9	39	1.35 (0.5–3.4)	ND	ND	ND	ND	ND	ND
<100	3	65	0.3 (0.07–1.0)	0.15	ND	ND	ND	ND	ND

*OR, odds ratio; HBV, hepatitis B virus; ND, no applicable data; MSM, men who have sex with men.
†Analysis by using exact logistic regression model because number in cell was 0.

Among STDs, HIV infection showed the highest risk ratio, a ≈16-fold increase. HIV infection has been identified as a risk factor for invasive amebiasis in many studies (10–12,21), although many details of this risk remain unclear (19,29).

We presumed that compromised immune function increased the susceptibility of patients to invasive diseases. However, no relationship was seen between low CD4 cell counts and development of amebic colitis. Under existing conditions, the reason for HIV infection representing a risk factor for amebic colitis is considered the preference for oral–anal sex as a common risk factor for both infectious conditions.

We compared prevalence and risk factors between amebic colitis patients with and without HIV infection. An incidence of 0.1% (4/5,193) has been reported in studies of HIV-negative patients with positive results for occult blood in feces (17), and our results were similar. However, annual prevalence increased in 2009 (0.38%, 5/1,316) compared with 2003 (0.05%, 1/1,878), and the rate of increase was higher than that for HIV-positive patients. This result calls

Table 3. Route of amebic infection for 54 persons, Japan, 2003–2009*

Route	HIV positive, no. (%), n = 31	HIV negative, no. (%), n = 23
Travelers from tropical areas	0	0
Residents of facilities for intellectually disabled	0	0
MSM, male CSW	28, 2 (90.3)	7 (30.4)
Female CSW	0	2 (8.7)
Contact with female CSW	0	8 (34.8)
Unknown	3 (9.7)	6 (26.1)

*MSM, men who have sex with men; CSW, commercial sex worker.

for careful attention in hospitals in which patients with HIV infection are not commonly encountered. In terms of risk factors, ORs for age, sex, and history of HBV infection or syphilis in our study did not vary according to HIV infection status.

Some limitations need to be considered in this study. First, Japan has not had epidemics of amebiasis, and data in this study were obtained from a metropolitan area. In addition, our hospital treats the largest number of patients with HIV infection in Japan. Second, selection bias was present because participants were patients who had undergone endoscopic examinations, which are highly likely to be performed for healthy patients. In addition, patients suspected before examination of having amebiasis might have been more likely to be actively included in the study. Third, the number of patients with amebic colitis was small; thus, the statistical power of the study might have been low. Fourth, a retrospective design was used for this investigation. With regard to HBV infection or history of syphilis, judgments had to be made for using results of serologic testing in some cases. In addition, determination of sexual preferences and overseas travel had to be based on the self-reports of patients.

In recent years, infectious diseases caused by E. histolytica and HIV have been increasing in Japan (15,16,30). HIV infection is a particularly serious problem because its incidence is consistently increasing in Japan while decreasing in western countries (30,31).

Numbers of patients with both infectious diseases studied are predicted to increase because little is known about measures to prevent infection in association with

218

a diversity of sexual activities. Amebic infection, in particular, is scarcely recognized as a sexually acquired infection, and improved education is needed to prevent these diseases. In Japan, measures to prevent the spread of HIV and amebic infections are urgently needed.

In conclusion, although this study was conducted at 1 center and involved retrospective analysis of a relatively small number of cases of amebic infection, the results suggest that the number of amebic colitis patients with or without HIV infection is tending to increase in Japan. Younger men with syphilis and HIV infections are at increased risk for amebic colitis. Route of infection differed slightly in that contact with CSWs was more frequent among HIV-negative patients than among HIV-positive patients. Among HIV-positive patients, homosexual intercourse, and not immunosuppressed status, seems to be a risk factor for amebic colitis.

Dr. Nagata is a gastroenterologist at the NCGM in Tokyo, Japan. His research interests include gastrointestinal infections such as esophageal candidiasis, cytomegalovirus-related disease, mycobacterial infections, intestinal amebiasis, intestinal spirochetosis, chlamydial infection, and HIV-related gastrointestinal disease.

References

1. Stanley SL Jr. Amoebiasis. Lancet. 2003;361:1025–34. http://dx.doi.org/10.1016/S0140-6736(03)12830-9
2. Li E, Stanley SL Jr. Protozoa. Amebiasis. Gastroenterol Clin North Am. 1996;25:471–92. http://dx.doi.org/10.1016/S0889-8553(05)70259-4
3. Petri WA Jr, Singh U. Diagnosis and management of amebiasis. Clin Infect Dis. 1999;29:1117–25. http://dx.doi.org/10.1086/313493
4. Allason-Jones E, Mindel A, Sargeaunt P, Williams P. *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. N Engl J Med. 1986;315:353–6. http://dx.doi.org/10.1056/NEJM198608073150603
5. Reed SL, Wessel DW, Davis CE. *Entamoeba histolytica* infection and AIDS. Am J Med. 1991;90:269–71.
6. Rivera WL, Tachibana H, Kanbara H. Field study on the distribution of *Entamoeba histolytica* and *Entamoeba dispar* in the northern Philippines as detected by the polymerase chain reaction. Am J Trop Med Hyg. 1998;59:916–21.
7. Gathiram V, Jackson TF. A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*. S Afr Med J. 1987;72:669–72.
8. Takeuchi T, Okuzawa E, Nozaki T, Kobayashi S, Mizokami M, Minoshima N, et al. High seropositivity of Japanese homosexual men for amebic infection. J Infect Dis. 1989;159:808. http://dx.doi.org/10.1093/infdis/159.4.808
9. Haque R, Huston CD, Hughes M, Houpt E, Petri WA Jr. Amebiasis. N Engl J Med. 2003;348:1565–73. http://dx.doi.org/10.1056/NEJMra022710
10. Hung CC, Deng HY, Hsiao WH, Hsieh SM, Hsiao CF, Chen MY, et al. Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. Arch Intern Med. 2005;165:409–15. http://dx.doi.org/10.1001/archinte.165.4.409
11. Tsai JJ, Sun HY, Ke LY, Tsai KS, Chang SY, Hsieh SM, et al. Higher seroprevalence of *Entamoeba histolytica* infection is associated with human immunodeficiency virus type 1 infection in Taiwan. Am J Trop Med Hyg. 2006;74:1016–9.
12. Park WB, Choe PG, Jo JH, Kim SH, Bang JH, Kim HB, et al. Amebic liver abscess in HIV-infected patients, Republic of Korea. Emerg Infect Dis. 2007;13:516–7. http://dx.doi.org/10.3201/eid1303.060894
13. Stark D, van Hal SJ, Matthews G, Harkness J, Marriott D. Invasive amebiasis in men who have sex with men, Australia. Emerg Infect Dis. 2008;14:1141–3. http://dx.doi.org/10.3201/eid1407.080017
14. Ohnishi K, Murata M. Present characteristics of symptomatic amebiasis due to *Entamoeba histolytica* in the east-southeast area of Tokyo. Epidemiol Infect. 1997;119:363–7. http://dx.doi.org/10.1017/S0950268897008236
15. Ohnishi K, Kato Y, Imamura A, Fukayama M, Tsunoda T, Sakaue Y, et al. Present characteristics of symptomatic *Entamoeba histolytica* infection in the big cities of Japan. Epidemiol Infect. 2004;132:57–60. http://dx.doi.org/10.1017/S0950268803001389
16. Amebiais in Japan 2003–2006. Infectious Agents Surveillance Report. 2007;28:103–64.
17. Okamoto M, Kawabe T, Ohata K, Togo G, Hada T, Katamoto T, et al. Amebic colitis in asymptomatic subjects with positive fecal occult blood test results: clinical features different from symptomatic cases. Am J Trop Med Hyg. 2005;73:934–5.
18. Yoshikawa I, Murata I, Yano K, Kume K, Otsuki M. Asymptomatic amebic colitis in a homosexual man. Am J Gastroenterol. 1999;94:2306–8. http://dx.doi.org/10.1111/j.1572-0241.1999.01325.x
19. Morán P, Ramos F, Ramiro M, Curiel O, Gonzalez E, Valadez A, et al. Infection by human immunodeficiency virus-1 is not a risk factor for amebiasis. Am J Trop Med Hyg. 2005;73:296–300.
20. Seeto RK, Rockey DC. Amebic liver abscess: epidemiology, clinical features, and outcome. West J Med. 1999;170:104–9.
21. Mitarai S, Nagai H, Satoh K, Hebisawa A, Shishido H. Amebiasis in Japanese homosexual men with human immunodeficiency virus infection. Intern Med. 2001;40:671–5. http://dx.doi.org/10.2169/internalmedicine.40.671
22. Blumencranz H, Kasen L, Romeu J, Waye JD, LeLeiko NS. The role of endoscopy in suspected amebiasis. Am J Gastroenterol. 1983;78:15–8.
23. Pai SA. Amebic colitis can mimic tuberculosis and inflammatory bowel disease on endoscopy and biopsy. Int J Surg Pathol. 2009;17:116–21. http://dx.doi.org/10.1177/1066896908316380
24. Caballero-Salcedo A, Viveros-Rogel M, Salvatierra B, Tapia-Conyer R, Sepulveda-Amor J, Gutierrez G, et al. Seroepidemiology of amebiasis in Mexico. Am J Trop Med Hyg. 1994;50:412–9.
25. Haque R, Faruque AS, Hahn P, Lyerly DM, Petri WA Jr. *Entamoeba histolytica* and *Entamoeba dispar* infection in children in Bangladesh. J Infect Dis. 1997;175:734–6. http://dx.doi.org/10.1093/infdis/175.3.734
26. Nozaki T. Current problems of amebiasis in Japan and recent advances in amebiasis research. Jpn J Infect Dis. 2000;53:229–37.
27. Amebic dysentery April 1999–December 2002. Infectious Agents Surveillance Report. 2003;24:79–80.
28. Takeuchi T, Kobayashi S, Asami K, Yamaguchi N. Correlation of positive syphilis serology with invasive amebiasis in Japan. Am J Trop Med Hyg. 1987;36:321–4.

219

29. Lowther SA, Dworkin MS, Hanson DL. *Entamoeba histolytica/ Entamoeba dispar* infections in human immunodeficiency virus–infected patients in the United States. Clin Infect Dis. 2000;30:955–9. http://dx.doi.org/10.1086/313811

30. HIV/AIDS in Japan in 2009. Infectious Agents Surveillance Report. 2010;31:226–7.

31. Joint United Nations Program on HIV/AIDS. UNAIDS report on the global AIDS epidemic changes in the incidence rate of HIV infection, 2001 to 2009, selected countries [cited 2011 Apr 15]. http://www.unaids.org/globalreport/Global_report.htm

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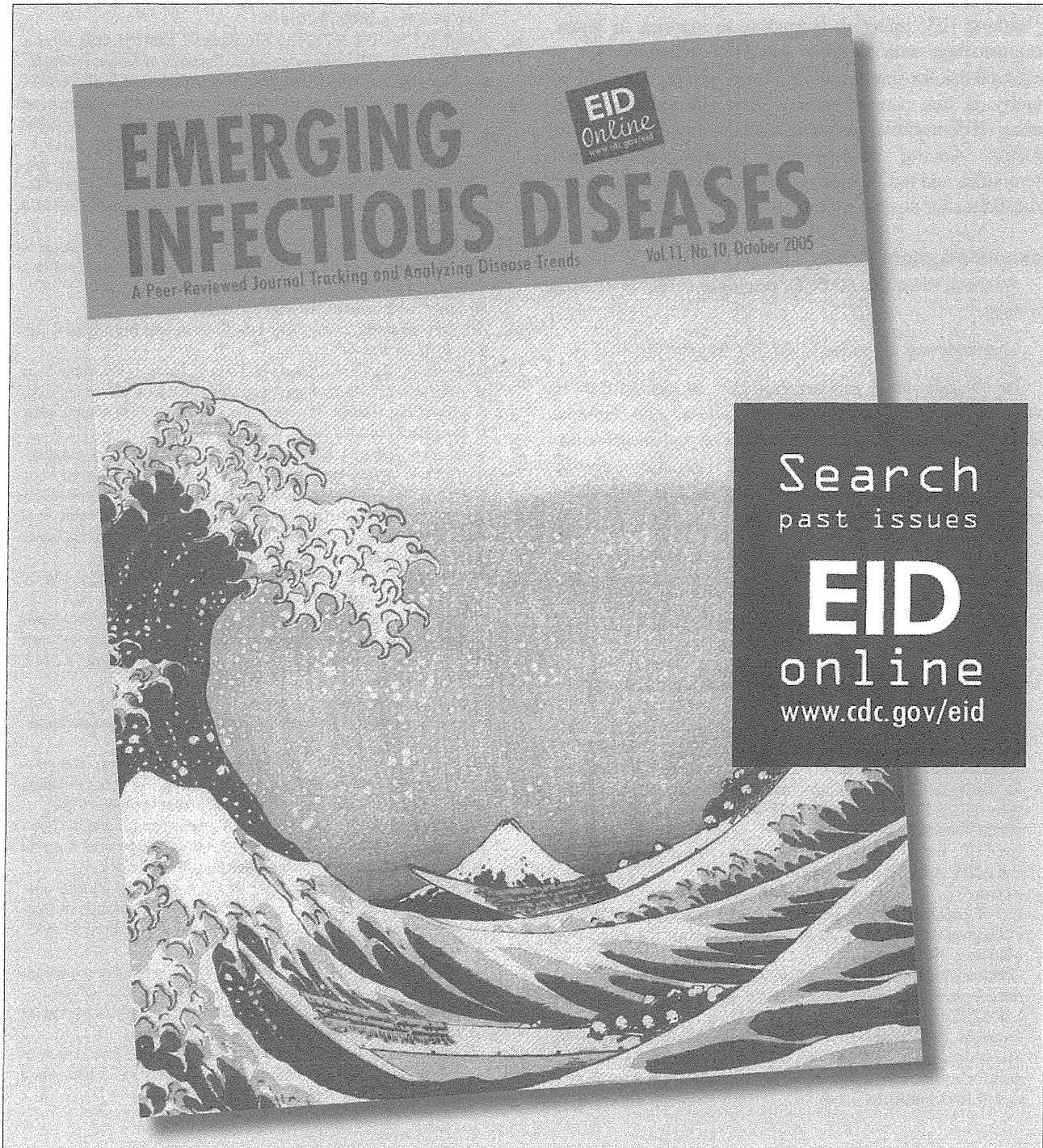
High Incidence of Renal Stones Among HIV-Infected Patients on Ritonavir-Boosted Atazanavir Than in Those Receiving Other Protease Inhibitor–Containing Antiretroviral Therapy

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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].

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 m² 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
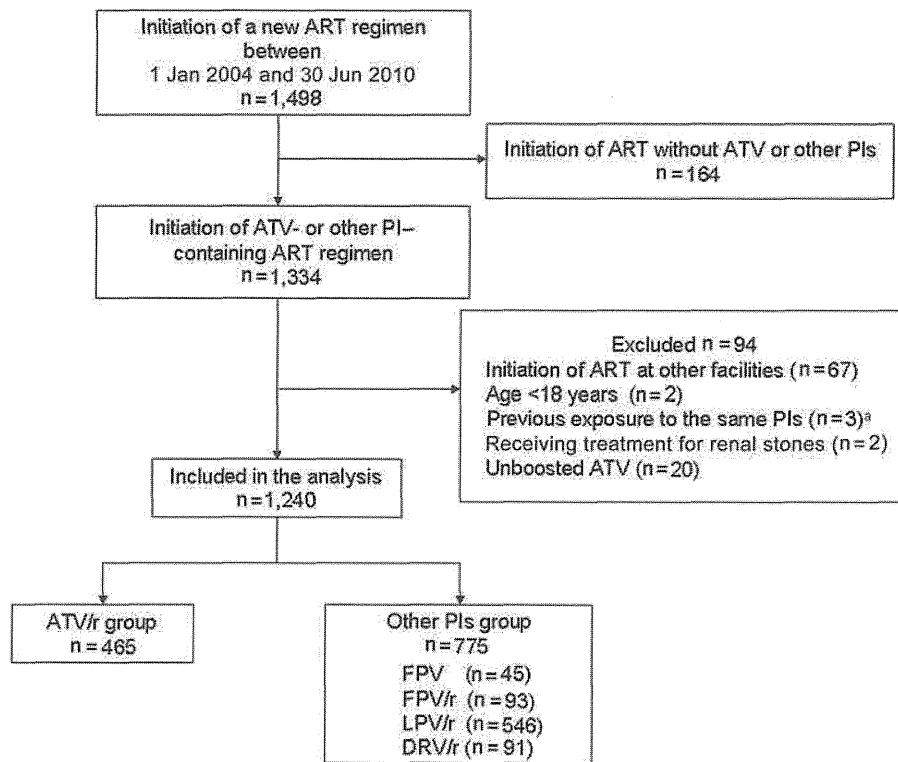
Figure 1. Flow diagram of patient selection. [a]Three patients were excluded for past lopinavir/ritonavir (LPV/r) exposure and commencement of LPV/r in the study. Abbreviations: ART, antiretroviral treatment; ATV, atazanavir; ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; FPV, 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.
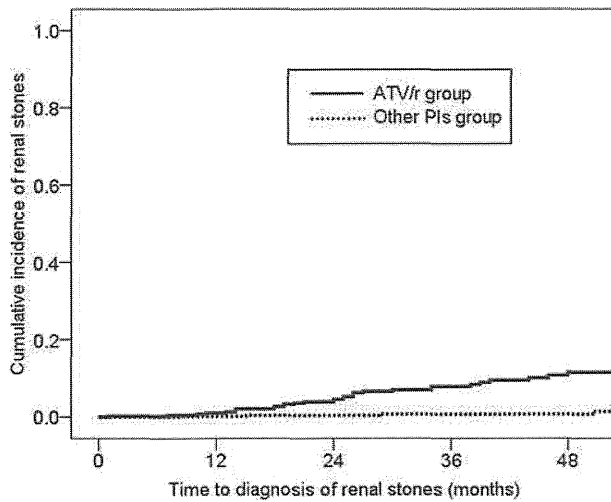
Figure 2. Kaplan-Meier curve showing time to diagnosis of renal stones. Abbreviations: ATV/r, ritonavir-boosted atazanavir; PIs, protease inhibitors.

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 m² 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,
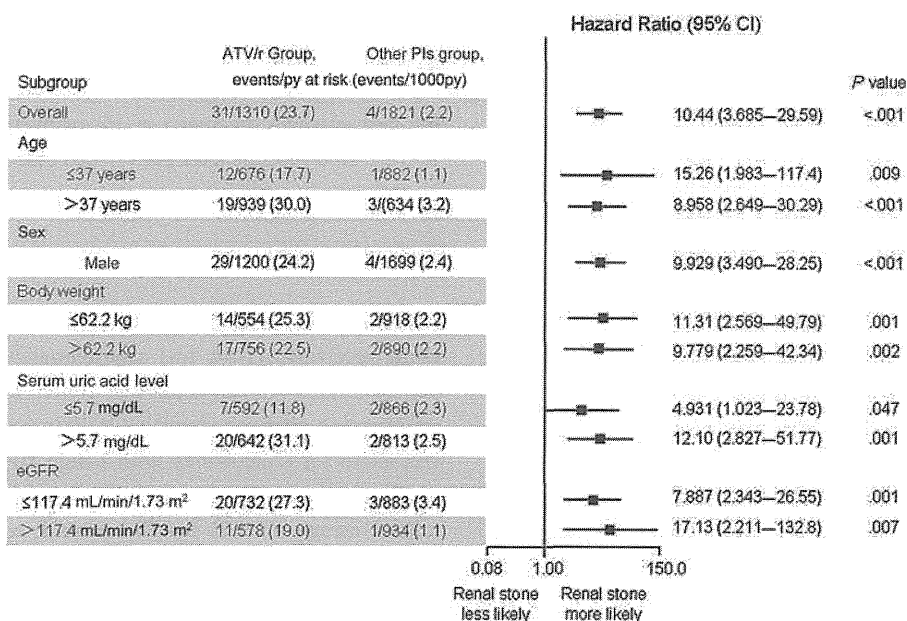

Figure 3. Estimated effect of ritonavir-boosted atazanavir, compared with other protease inhibitors on the hazard of renal stone formation, according to baseline characteristics. Abbreviations: ATV/r, ritonavir-boosted atazanavir; CI, confidence interval; eGFR, estimated glomerular filtration rate; PI,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

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.

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 $\beta2$-microglobulin, urinary $\alpha1$-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].

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 = $[(140 - age) \times$ weight (kg)]/(serum creatinine $\times$ 72)($\times$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.
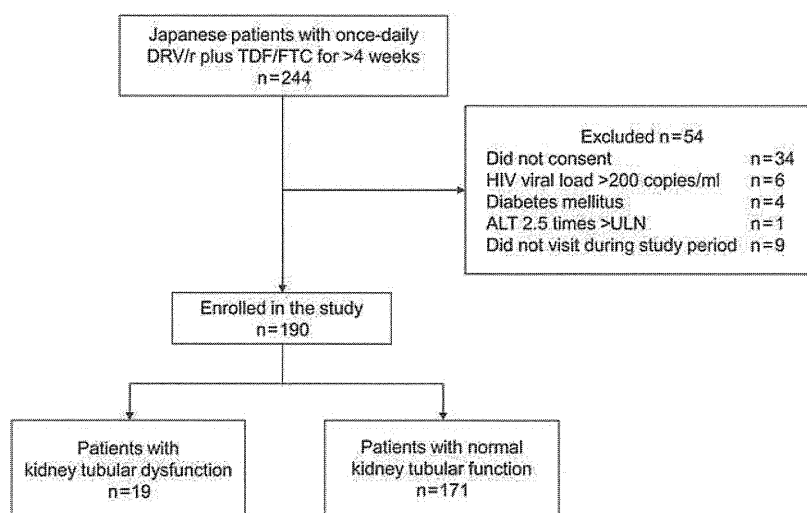

Figure 1. Patient enrollment. Abbreviations: ALT, alanine transaminase; DRV/r, ritonavir-boosted darunavir; HIV, human immunodeficiency virus; TDF/FTC, tenofovir/emtricitabine; ULN, upper limit of normal.

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 m²)[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