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Efficacy and safety of once-daily ritonavir-boosted darunavir and abacavir/lamivudine for treatment-naïve patients: a pilot study

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The efficacy and safety of once-daily darunavir/ritonavir and fixed-dose abacavir/lamivudine was examined in 22 treatment-naïve patients with HIV-1 infection. Three patients discontinued antiretroviral therapy due to mild adverse events. Among 18 patients who continued therapy, 66.7% had viral load less than 50 copies/ml at week 48. Only two patients experienced virologic failure with the emergence of resistant virus. This pilot

study demonstrated the viral efficacy and safety of darunavir/ritonavir and abacavir/lamivudine.

Only very little information is available on the efficacy and safety of the combination antiretroviral therapy (ART) of ritonavir-boosted darunavir (DRV/r) and fixed-dose abacavir/lamivudine (ABC/3TC) [1]. DRV/r is a protease inhibitor with proven efficacy and safety as well as with a high barrier to drug resistance [2,3]. ABC/3TC is an alternative choice of nucleoside reverse transcriptase inhibitor (NRTI) backbone in the American Department of Health and Human Services (DHHS) Guidelines and is the other preferred backbone regimen for treatment-naïve patients in other international guidelines [4,5]. In this pilot study, we evaluated the efficacy and safety of DRV/r and ABC/3TC for treatment-naïve patients in a single-center, observational cohort.

The participants of this retrospective study were all treatment-naïve patients with HIV infection who commenced once-daily DRV/r and fixed-dose ABC/3TC from November 2009 (when the first patient commenced such regimen at our clinic) to November 2010 at our clinic (AIDS Clinical Center, Tokyo, Japan). All patients were followed for at least 48 weeks after commencement of treatment at our facility. Baseline data, including age, sex, mode of infection, ethnicity, CD4 cell count, and HIV viral load, were collected from the medical charts. The Cobas TaqMan HIV-1 real-time PCR version 1.0 assay (Roche Diagnostics, NJ) was used to measure HIV-1 viral load throughout the research period. For those who discontinued either DRV/r or fixed-dose ABC/3TC before reaching 48 weeks, the reasons for discontinuation were collected. All patients provided written informed consent for the data to be published. Primary outcomes were the proportion of patients with viral load less than 50 copies/ml at 24 and 48 weeks. Safety parameters through 48 weeks were also collected.

The study included 22 patients [1 (4.6%) female] of East Asian origin, with a median age of 34.5 years [interquartile range (IQR) 27.5–43.8]. The route of transmission was homosexual intercourse 86.3%, heterosexual 9%, and unknown in one patient. HLA was examined in 20 patients and all were HLA-B*5701-negative. Twenty-one patients had HIV-1 drug-resistant testing before commencement of ART and none had resistant mutations related to NRTIs, protease inhibitors, or non-NRTIs. At baseline, the median CD4 cell count was 47/μl (IQR 27.5–187.8), whereas the HIV viral load was 5.61 $\log_{10}$ copies/ml (IQR 4.57–6.01 $\log_{10}$ copies/ml). In three patients, ART was either changed or discontinued during the study due to adverse events [skin rash ($n=1$), vomiting ($n=1$), and limb paresthesia ($n=1$)] and one patient changed the regimen due to a concern with drug interactions with antipsychotics before 48 weeks. The skin rash was due to darunavir, because the rash disappeared after switching darunavir to raltegravir, while continuing ABC/3TC. This patient was HLA-B*5701-negative. None presented with ABC-associated hypersensitivity or with grade 3 or 4 liver enzyme elevation.

On-treatment analysis of the 18 patients (excluding the above four patients who discontinued the regimen) showed 72.2% had viral load less than 50 copies/ml at week 24 (88.9% viral load <200 copies/ml), and 66.7% had viral load less than 50 copies/ml at week 48 (88.9% viral load <200 copies/ml). Intention-to-treatment analysis showed 59.0% with viral load less than 50 copies/ml at week 24 (77.3% viral load <200 copies/ml), and 54.6% with viral load less than 50 copies/ml at week 48 (72.7% viral load <200 copies/ml) (Fig. 1). Four patients showed rebounds greater than 200 copies/ml (<1000 copies/ml) after 24 weeks; two of them were single rebounds and were considered blips. The other two patients showed two consecutive viral load greater than 200 copies/ml, fulfilling the criteria of virological failure (11.1% at 48 weeks). The latter two patients underwent a genotypic resistance test that detected, in one case, the reverse transcriptase mutation M184V and, in the other, the protease mutation M46I.

In the 12 patients with baseline viral load above 100 000 copies/ml, on-treatment analysis showed viral load of less than 200 copies/ml at 24 weeks in 10 (83.3%) patients, and less than 50 copies/ml at both 24 and 48 weeks in seven (58.3%). In comparison, all six patients with baseline viral load below 100 000 copies/ml showed suppression of the load to below 50 copies/ml at both 24 and 48 weeks. The median increment in CD4 cell count at 48 weeks was 187/μl (IQR 82.5–264.5/μl).

Discussion

To our knowledge, this is the first published study on the efficacy and safety of the combination of once-daily DRV/r and fixed-dose ABC/3TC in treatment-naïve patients. This combination ART resulted in viral suppression, although the baseline viral load was above 100 000 copies/ml in 66.6% of the patients. Only 13.6% discontinued this regimen due to adverse events before 48 weeks and none of the adverse events was serious. Considering that most patients in this cohort were at an advanced stage of HIV infection with a low median baseline CD4 cell count of 47/μl, we conclude that DRV/r and ABC/3TC is a well tolerated and efficacious combination ART.

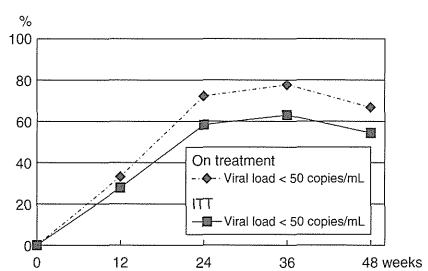

Fig. 1. Proportions of patients with viral load below 50 copies/ml at 48 weeks with on-treatment and intention-to-treat (ITT) analysis.

The DHHS guidelines for the treatment of HIV infection in the USA list ABC/3TC as alternative NRTIs since abacavir can potentially cause serious hypersensitivity reaction in 5–8% of the patients and its viral efficacy in patients with baseline viral load of above 100 000 copies/ml is inferior to fixed-dose tenofovir/emtricitabine (TDF/FTC) when used with efavirenz or ritonavir-boosted atazanavir as a key drug [4,6]. However, the incidence of ABC-related hypersensitivity is low among the HLA-B*5701-negative population, such as the Japanese [7,8]. Moreover, the HEAT study demonstrated that the viral efficacy of ABC/3TC was not inferior to that of TDF/FTC when used with lopinavir/ritonavir for treatment-naive patients [9]. Taking this background into account, once-daily DRV/r and ABC/3TC could be a good alternative, especially in patients with a low prevalence of HLA-B*5701 who cannot tolerate tenofovir due to its nephrotoxicity [10].

In conclusion, this single-center pilot study demonstrated the viral efficacy and safety of once-daily DRV/r and ABC/3TC in treatment-naïve patients with HIV-1 infection. This regimen could be a suitable alternative to DRV/r and tenofovir/emtricitabine or other first-line regimens. Nevertheless, the number of patients in this cohort is too small to allow firm conclusions and further studies of larger samples (ideally a clinical trial that compares the viral efficacy of TDF/FTC to ABC/3TC with once-daily DRV/r) are needed to elucidate this issue.

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Conflicts of interest

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Trends in early and late diagnosis of HIV-1 infections in Tokyoites from 2002 to 2010

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SUMMARY

Objective: The objective of this study was to delineate the trends in early and late diagnosis of HIV-1 infection in newly diagnosed Tokyoites.
Methods: The BED assay was used to identify cases diagnosed at an early stage of infection. BED-positive non-AIDS cases with a CD4 cell count ≥200/μl were defined as cases with recent infection. The rates of AIDS and recent infection in 809 newly diagnosed Tokyoites during 2002–2010 were analyzed.
Results: The AIDS rate was 22.5%. AIDS patients were older (40.4 years) than non-AIDS patients (35.0 years), and a smaller proportion were men who have sex with men (MSM) in AIDS patients (81.7%) than in non-AIDS patients (89.9%). The AIDS rate was persistently lower (≤14.3%) in ≤29-year-old than in ≥30-year-old MSM. The rate of recent infection was 24.4%. Individuals with recent infection (33.0 years old) were younger than the others (37.2 years). The rate of recent infection was lower (≤18.5%) in MSM aged ≥40 years than in those aged ≤39 years during the study period, except for 2007 and 2008.
Conclusions: Younger MSM Tokyoites appear to be aware of the risk of their sexual behavior, sufficient to take voluntary HIV testing repeatedly, resulting in early diagnosis. Older MSM did not take HIV testing frequently enough and may be a good target for campaigns promoting testing.

1. Introduction

The overall growth of the global AIDS epidemic appears to have stabilized. The annual number of new cases of HIV infection has been in steady decline since the late 1990s.[1] In Japan, however, the annual number of newly diagnosed cases has almost doubled during the most recent decade (791 cases in 2000 and 1544 cases in 2010), although the prevalence of HIV in the adult population remains <0.1%.[2] The distribution of these cases is heavily concentrated in large cities, and approximately 35% of the newly diagnosed cases have been identified in Tokyo.[3]

Early diagnosis of HIV infection is critically important because some AIDS-defining diseases are fatal, even in the era of combination antiretroviral treatment (ART); also the introduction of ART after the development of AIDS is often complicated with immune reconstitution inflammatory syndrome (IRIS).[4,5] In this regard, the introduction of ART at the early stages seems to significantly reduce the sexual transmission of HIV-1.[6,7] Thus, it is important to identify newly infected individuals and provide early ART to reduce the incidence of AIDS and transmission of HIV. Knowledge about the proportion of patients diagnosed at the early stage of an HIV infection in the newly diagnosed cases is also useful for planning and evaluation of any prevention program and for resource allocation.[8,9] However, it is usually difficult to distinguish recent from long-standing HIV infections except for acute symptomatic infections.[10] Simple prediction of the infection time from CD4 cell counts appears inaccurate because the disease progression rate varies enormously among infected individuals.[11] The BED HIV-1 capture enzyme immunoassay (BED assay) uses the branched peptide to detect HIV-1 IgG antibodies from all subtypes (i.e., HIV-1 B, E, and D gp41 immunodominant sequences are included on a branched peptide used in the assay) and measures levels of anti-HIV-1 IgG relative to total IgG.[12] Since the ratio of anti-HIV-1 IgG to total IgG increases with time shortly after HIV-1 infection, the HIV-1-infected patient is considered to have recently acquired the infection when the normalized optical density (ODn) is less than 0.8 on the BED assay (ODn reaches 0.8 on average 197 days after seroconversion.).[13]

The present study was an attempt to delineate the trends in early diagnosis of HIV-1 infection in Tokyo from 2002 to 2010 by using the BED assay. The aim of this analysis was to enhance our understanding of the status of HIV-1 spread in Tokyo and to help in the design of strategies to control the HIV-1 epidemic in Japan.

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2. Materials and methods

2.1. Newly diagnosed patients

This study included all ART-naïve HIV-1-infected individuals who met the following criteria: (1) those who visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, between 2002 and 2010 within 30 days of their diagnosis with an HIV-1 infection and (2) availability of plasma samples taken at the first visit under signed informed consent for use in viral, immunological, and epidemiological studies. Participant information including CD4 count, HIV-1 load, age at the first visit, gender, nationality, probable HIV-1 transmission route, and history of HIV testing, were collected from the medical records. According to the Japanese law for infection control, physicians are obliged to report newly diagnosed HIV/AIDS cases to the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government). A total of 11 673 HIV/AIDS cases nationally, including 4048 cases diagnosed in Tokyo (Tokyo cases), which were entered into the registry of this committee from 2002 to 2010, were used as the control populations to evaluate the representativeness of the patients enrolled in the present study (AIDS Clinical Center cases).[2,3] Plasma samples obtained from the participants were stored at −80 °C. The viral subtype in each case was determined from the HIV-1 protease–reverse transcriptase sequence (which was analyzed for drug resistance genotyping) by the neighbor-joining method using the Genetic-Win system (Software Development, Tokyo).[14]

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the ethics committee of the National Center for Global Health and Medicine.

2.2. BED assay

The BED HIV-1 capture enzyme immunoassay (BED assay; Calypte Biomedical Corp., Portland, OR, USA) was used to estimate the time of HIV-1 infection.[12] In accordance with the manufacturer's instructions, 5 μl of plasma was diluted with 500 μl of the diluent in the kit, and the proportion of anti-HIV-1-specific IgG to the total IgG in the sample was measured by optical density (OD). The OD values of the test specimens were normalized (ODn) relative to the value of a calibrator (specimen OD/calibrator OD) to minimize inter-run variation. Samples with ODn ≤0.8 were considered to be from individuals who had seroconverted within 197 days and were defined as BED-positive.[13] BED-positive non-AIDS cases with CD4 cell counts ≥200/μl were defined as individuals with recent infection. The others were defined as chronic infection.

2.3. Statistical analysis

Differences in demographic data including age, gender, risk behavior, nationality, and AIDS development among the AIDS Clinical Center cases, national cases, and Tokyo cases, were examined for significance using one-way analysis of variance (ANOVA) and the Tukey test, or Pearson's Chi-square test. Differences in demographic data including age, CD4 count, logarithmic HIV-1 viral load, nationality, transmission category, HIV-1 subtype, cue for HIV diagnosis, and history of HIV testing, between AIDS and non-AIDS patients and between recent and chronic infection, were examined for significance using the *t*-test or Pearson's Chi-square test. To estimate the correlation with the development of AIDS, binominal logistic regression analysis including age, nationality (Japanese or not), and transmission category (men having sex with men (MSM) or not) was performed. A *p*-value of less than 5% denoted statistical significance. Statistical analyses were performed with SPSS Statistics 17.0 (IBM Japan Inc., Tokyo, Japan) and Stat Mate II (NANKODO, Tokyo).

3. Results

3.1. Newly diagnosed cases of HIV-1 infection

The study subjects were 809 ART-naïve HIV-1-infected patients. All of them had visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, within 30 days of the diagnosis of HIV-1 infection (median 8 days) between 2002 and 2010. They included 741 Japanese, 35 Asians other than Japanese, and 33 from other countries. They represented 20.0% of the total number of newly diagnosed Tokyoite cases during the same period (Table 1). There were no significant differences in the proportion of AIDS (22.5% vs. 21.9%), percentage of males (96.2% vs. 94.3%), or proportion of Japanese (91.6% vs. 90.7%) between our study patients and those of the Tokyo registry, although our patients included a significantly smaller proportion of AIDS cases (22.5% vs. 30.4%) and significantly larger population of male patients (96.2% vs. 91.8%) and Japanese patients (91.6% vs. 88.5%) compared with the patients of the national registry. Furthermore, our patients were significantly younger than the patients of the Tokyo and national registries (36.2 vs. 37.7 and 38.0 years), and the proportion of MSM among male patients was significantly higher than in the Tokyo and national registries (88.0% vs. 72.8% and 59.8%).

Subtype analysis successfully determined the HIV-1 subtype in 807 patients (99.8%); the majority were infected with HIV-1 subtype B (742 patients, 91.9%), while 5.7% were infected with HIV-1 subtype AE, which is comparable to previously published subtype data in Japan.[14] The HIV-1 subtype could not be determined in two patients because the viral load was below the detection limit (<40 copies/ml), although they were not being treated with anti-HIV drugs.

3.2. Features of AIDS patients

Among the 809 cases, 182 (22.5%, 95% confidence interval (95% CI) 19.6–25.4) had already developed AIDS at the first visit, while the other 627 were non-AIDS cases (Table 2). AIDS cases were significantly older (40.4 years, 95% CI 38.8–41.9 vs. 35.0 years, 95% CI 34.2–35.9), and as expected, had lower CD4 counts (61.7/μl, 95% CI 50.6–72.8 vs. 318.0/μl, 95% CI 303.0–333.0) and higher viral loads (5.22 log VL/ml, 95% CI 5.13–5.31 vs. 4.63 log VL/ml, 95% CI 4.56–4.70) than non-AIDS patients. There were no significant differences in nationality (Japanese 91.8%, 95% CI 87.8–95.8 vs. 91.5%, 95% CI 89.4–93.7) or HIV-1 subtype (subtype B 89.0%, 95% CI 84.5–93.6 vs. 92.5%, 95% CI 90.4–94.6) between AIDS and non-AIDS

Table 1
New cases of HIV-1-infected patients diagnosed between 2002 and 2010

	Japan[a]	Tokyo[b]	This study
Number of cases	11 673	4048	809
Age, years (mean ± SD)	38.0 ± 11.8[c]	37.7 ± 11.9[d]	36.2 ± 11.0
Males	10 721 (91.8%)[c]	3819 (94.3%)	778 (96.2%)
Men having sex with men	6408 (59.8%)[c]	2780 (72.8%)[c]	685 (88.0%)
Japanese	10 335 (88.5%)[d]	3673 (90.7%)	741 (91.6%)
AIDS cases	3551 (30.4%)[c]	885 (21.9%)	182 (22.5%)

Statistical analyses were performed by one-way ANOVA and Tukey test, or Chi-square test.
[a] Provided by the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government).
[b] Provided by the Bureau of Social Welfare and Public Health, Tokyo.
[c] $p < 0.001$, compared with the study participants.
[d] $p < 0.01$ compared with the study participants.

Table 2
Demographics of participants with and without AIDS

	AIDS (n = 182)		Non-AIDS (n = 627)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	40.4	(38.8–41.9)	35.0	(34.2–35.9)	<0.001
CD4 count /μl	61.7	(50.6–72.8)	318.0	(303.0–333.0)	<0.001
Log viral load/ml	5.22	(5.13–5.31)	4.63	(4.56–4.70)	<0.001
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.424
Japan	167	91.8 (87.8–95.8)	574	91.5 (89.4–93.7)	
Asia other than Japan	11	6.0 (3.3–10.8)	24	3.8 (2.6–5.7)	
North and South America	2	1.1 (0.2–4.0)	17	2.7 (1.7–4.3)	
Africa	2	1.1 (0.2–4.0)	6	1.0 (0.4–2.1)	
East and West Europe	0	0 (0–2.0)	4	0.6 (0.2–1.6)	
Oceania	0	0 (0–2.0)	2	0.3 (0–1.1)	
Transmission category					
Male	175	96.2 (93.4–98.9)	603	96.2 (94.7–97.7)	0.024
MSM	143	81.7 (76.0–87.4)	542	89.9 (87.5–92.3)	
Heterosexual	21	12.0 (7.2–16.8)	43	7.1 (5.4–9.6)	
IDU	1	0.6 (0–3.2)	2	0.3 (0.1–1.2)	
Unknown	10	5.7 (3.0–10.5)	16	2.7 (1.6–4.3)	
Female	7	3.8 (1.7–7.9)	24	3.8 (2.6–5.7)	-
Heterosexual	7	100 (46.8–100)	24	100 (100–100)	
Subtype					0.351
B	162	89.0 (84.5–93.6)	580	92.5 (90.4–94.6)	
AE	16	8.8 (5.4–14.3)	30	4.8 (3.4–6.8)	
C	1	0.5 (0–3.0)	7	1.1 (0.5–2.3)	
G	2	1.1 (0.2–4.0)	3	0.5 (0.1–1.4)	
AG	1	0.5 (0–3.0)	3	0.5 (0.1–1.4)	
A	0	0 (0–2.0)	2	0.3 (0–1.1)	
Unknown	0	0 (0–2.0)	2	0.3 (0–1.1)	
Cue for HIV diagnosis					<0.001
Voluntary testing	12	6.6 (3.7–11.5)	283	45.1 (41.2–49.0)	
Provider-initiated testing	167	91.8 (87.8–95.8)	338	53.9 (50.0–57.8)	
Unknown	3	1.6 (0.4–4.8)	6	1.0 (0.4–2.1)	
Previous testing					<0.001
Yes	29	15.9 (10.6–21.3)	282	45.0 (41.1–48.9)	
No	65	35.7 (28.8–42.7)	254	40.5 (36.7–44.4)	
Unknown	88	48.4 (41.1–55.6)	91	14.5 (11.8–17.3)	
BED assay					<0.001
Recent (ODn ≤0.8)	47	25.8 (19.5–32.2)	255	40.7 (36.8–44.5)	
Chronic (ODn >0.8)	135	74.2 (67.8–80.5)	372	59.3 (55.5–63.2)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user; ODn, normalized optical density.
[a] By t-test or Pearson's Chi-square test.

cases (Pearson's Chi-square test). MSM activity was the most frequent transmission route in both groups, and still more frequent in non-AIDS cases (89.9%, 95% CI 87.5–92.3) than in AIDS cases (81.7%, 95% CI 76.0–87.4). A larger proportion of patients in the non-AIDS group than in the AIDS group had undertaken previous HIV testing (45.0%, 95% CI 41.1–48.9 vs. 15.9%, 95% CI 10.6–21.3) and had been diagnosed with HIV-1 infection by voluntary testing (45.1%, 95% CI 41.2–49.0 vs. 6.6%, 95% CI 3.7–11.5), suggesting that repeated voluntary testing may prevent disease progression to AIDS in the high-risk groups.

Binominal logistic regression analysis of age, nationality (Japanese or not), and transmission category (MSM or not) identified age as the most significant factor associated with the development of AIDS (per 1-year increment, (hazard ratio) HR 1.041, 95% CI 1.026–1.057; $p < 0.001$).

To delineate the trends in late diagnosis of HIV-1 infection, the annual rates of AIDS cases in newly-diagnosed HIV-1-infected patients were plotted through the study period. The rate of AIDS cases remained around 30% between 2002 and 2004. It decreased to 15.0% in 2005, but then showed a gradual increase annually, reaching 24.8% in 2010 (Figure 1). To identify the population that influenced the increase in the rate of AIDS cases in the most recent years, we selected and categorized the study participants based on their features. Specifically, we focused on MSM patients, because 85% of our patients were MSM. Based on the above results of the significance of age in the binominal logistic regression analysis in the development of AIDS, we examined the effect of age in more detail by dividing the MSM patients into three age groups: those aged ≤29 years (217 patients, 31.7%), 30–39 years (273 patients, 39.9%), and ≥40 years (195 patients, 28.5%). In the ≥40 years MSM group, the rate was higher than 50% between 2002 and 2004, but decreased to 21.4% in 2005 and further decreased to 14.3% in 2006, but gradually increased and reached ~30% in 2009 and 2010 (Figure 1). On the other hand, in the ≤29 years MSM group, the AIDS rate was steadily lower than 20%, indicating that most young HIV-1-infected MSM were diagnosed before the development of AIDS throughout the study period. The AIDS rate in the 30–39 years MSM group was between those of the other two groups during most of the study period. A significantly larger proportion of patients in the ≤29 years MSM group had undergone voluntary HIV testing (43.8%, $p = 0.002$, Pearson's Chi-square test) and diagnosis with HIV (48.8%, $p < 0.001$, Pearson's Chi-square test), compared with the 30–39 years MSM group (43.6% and 36.6%, respectively) and the ≥40 years MSM group (34.9% and 32.3%, respectively). These results suggest that repeated voluntary testing may have prevented disease progression to AIDS in the younger MSM groups. The high rate of AIDS in all the study participants observed in 2002–2004 seemed mainly due to the ≥40-year-old MSM. Furthermore, the gradual increase in the AIDS rate in the ≥40-year-old MSM since 2006 also seemed to have contributed to

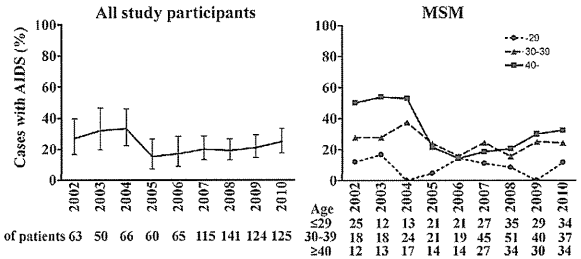

Figure 1. Annual rate of AIDS in newly diagnosed HIV-1-infected individuals. The annual AIDS rate for all study participants (809 patients; left panel), and men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S1).

the rising AIDS rate in all, suggesting that older MSM should be the main target for interventions aimed at promoting HIV testing for early diagnosis and prevention of the development of AIDS.

3.3. Trends in early HIV diagnosis

To identify individuals with recent HIV-1 infection, we performed a BED assay for the 809 study participants. Before analysis of the results, we dealt with the problem of potential misclassification. Previous studies reported small levels of anti-HIV-1-specific IgG relative to the total IgG in cases with both recent HIV-1 infection and long-standing chronic cases with severe immunodeficiency, which could result in false classification of chronic cases as recent infection.[12,15,16] To tackle this problem, previous studies classified AIDS cases and cases with CD4 cell counts <200/μl as chronic infection cases, in accordance with the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) guidelines.[17–21] We applied

Table 3
Demographics of participants with recent and chronic infection

	Recent (n = 197)		Chronic (n = 612)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	33.0	(31.7–34.3)	37.2	(36.3–38.1)	<0.001
CD4 count /μl	423.2	(399.2–447.3)	207.9	(193.3–222.4)	<0.001
Log viral load/ml	4.61	(4.46–4.76)	4.81	(4.74–4.87)	0.005
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.101
Japan	189	95.9 (93.2–98.7)	552	90.2 (87.8–92.6)	
Asia other than Japan	2	1.0 (0.2–3.7)	33	5.4 (3.9–7.6)	
North and South America	3	1.5 (0.4–4.4)	16	2.6 (1.6–4.2)	
Africa	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
East and West Europe	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
Oceania	1	0.5 (0–2.8)	1	0.2 (0–0.9)	
Transmission category					
Male	192	97.5 (95.3–99.7)	586	95.8 (94.2–97.3)	0.314
MSM	177	92.2 (88.4–96.0)	508	86.7 (83.9–89.4)	
Heterosexual	11	5.7 (3.1–10.2)	53	9.0 (7.0–11.8)	
IDU	0	0 (0–1.9)	3	0.5 (0.1–1.5)	
Unknown	4	2.1 (0.7–5.3)	22	3.8 (2.5–5.7)	
Female	5	2.5 (1.0–5.9)	26	4.2 (2.9–6.2)	-
Heterosexual	5	100 (34.4–100)	26	100 (81.5–100)	
Subtype					0.029
B	188	95.4 (92.5–98.3)	554	90.5 (88.2–92.8)	
AE	4	2.0 (0.7–5.2)	42	6.9 (5.2–9.3)	
C	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
G	1	0.5 (0–2.8)	4	0.7 (0.2–1.7)	
AG	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
A	0	0 (0–1.9)	2	0.3 (0–1.2)	
Unknown	2	1.0 (0.2–3.7)	0	0 (0–0.6)	
Cue for HIV diagnosis					<0.001
Voluntary testing	102	51.8 (44.8–58.8)	193	31.5 (27.9–35.2)	
Provider-initiated testing	94	47.7 (40.7–54.7)	411	67.2 (63.4–70.9)	
Unknown	1	0.5 (0–2.8)	8	1.3 (0.6–2.6)	
Previous testing					<0.001
Yes	116	58.9 (52.0–65.8)	195	31.9 (28.2–35.6)	
No	57	28.9 (22.6–35.3)	262	42.8 (38.9–46.7)	
Unknown	24	12.2 (7.6–16.8)	155	25.3 (21.9–28.8)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user.
[a] By t-test or Pearson's Chi-square test.

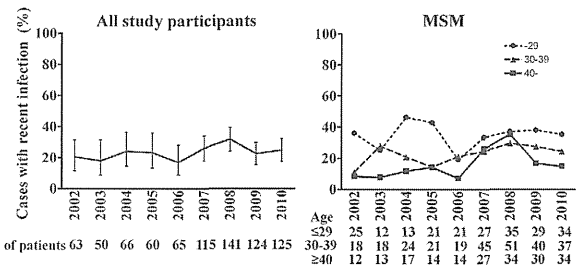

Figure 2. Annual rate of recent infection in newly diagnosed HIV-1-infected cases. The annual rate of recent infection in all study participants (809 patients; left panel), and in men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S2).

the same strategy in this study and thus defined only BED-positive non-AIDS cases with CD4 cell counts ≥200/μl as recent infection.

In the 456 non-AIDS cases with CD4 cell counts ≥200/μl, 197 cases were BED-positive and classified as recent infection (43.2%; 24.4% of the total cases) (Table 3). BED-negative cases, AIDS cases, and cases with CD4 cell counts <200/μl were classified as chronic infection. Patients with recent infection were younger (33.0 years, 95% CI 31.7–34.3 vs. 37.2 years, 95% CI 36.3–38.1) and had higher CD4 counts (423.2/μl, 95% CI 399.2–447.3 vs. 207.9/μl, 95% CI 193.3–222.4), as expected, and lower viral load (4.61 log VL/ml, 95% CI 4.46–4.76 vs. 4.81 log VL/ml, 95% CI 4.74–4.87), compared to patients with chronic infection. A larger proportion of recent infection (95.4%, 95% CI 92.5–98.3) was caused by HIV-1 subtype B than in those with chronic infection (90.5%, 95% CI 88.2–92.8). There were no significant differences in the nationality and transmission category between recent and chronic infection cases (Pearson's Chi-square test), although the proportion of Japanese patients was higher in recent infection (95.9%, 95% CI 93.2–98.7) than in chronic infection (90.2%, 95% CI 87.8–92.6) (p = 0.012, Chi-square test). A significantly larger proportion of patients underwent previous HIV testing (58.9%, 95% CI 52.0–65.8 vs. 31.9%, 95% CI 28.2–35.6) and were diagnosed with HIV-1 infection by voluntary testing (51.8%, 95% CI 44.8–58.8 vs. 31.5%, 95% CI 27.9–35.2) among recent infection cases than chronic infection cases ($p < 0.001$ in both, Pearson's Chi-square test).

To delineate the trends in early diagnosis of HIV-1 infection, the annual rate of recent infection in all 809 study participants was plotted over the study period (Figure 2). The rate was stable at ~20% between 2002 and 2010, except for 2007 (26.1%) and 2008 (31.9%), when a slight increase was evident. In order to identify the population that influenced the annual trends of early diagnosis, we focused on MSM patients and again divided them into three age groups: ≤29 years, 30–39 years, and ≥40 years. The rates of recent infection in the ≤29 and ≥40 years MSM groups were the highest and the lowest, respectively, in most years of the study period. The rate in the ≤29 years MSM group was high, ranging from 25.0% to 46.2% between 2002 and 2005, but it decreased to 19.0% in 2006, and increased again in 2007 and remained around 35% between 2007 and 2010. The rate of recent infection in the ≥40-year-old MSM group was steadily low at ~10% between 2002 and 2006, but increased in 2007 to 25.9% and 2008 to 35.3%, then decreased to around 15% in 2009 and 2010. The rate in the 30–39-year-old MSM ranged between those of the other two groups during most part of the study period. These results suggest that younger MSM tend to be diagnosed persistently earlier, whereas older MSM are usually diagnosed at a later stage of the HIV disease.

4. Discussion

The present study analyzed the trends in the proportion of AIDS patients and patients with recent infection among 809 new cases of HIV-1-infection diagnosed between 2002 and 2010. This group recruited from our AIDS Clinical Center represents 20.0% of the total number of newly diagnosed Tokyoites during the same period. We found that MSM, especially younger MSM, tend to be diagnosed at an earlier stage before the development of AIDS, probably because of frequent voluntary HIV testing. The proportion of AIDS cases remained at a steady low level and the rate of recent infection remained at a high level in younger MSM patients, indicating that younger MSM are aware of the risk of their sexual behavior sufficient to take HIV testing repeatedly. On the other hand, in the older MSM, the rate of AIDS was relatively high and the rate of recent infection comparatively low, but transiently increased in 2007 and 2008, suggesting that older MSM with a high-risk of HIV infection usually do not take HIV testing frequently and may respond to campaigns that promote such tests. Interestingly, the Japan Foundation for AIDS Prevention conducted several campaigns to promote voluntary HIV-1 testing in 2007. A popular male Japanese singer took part in one such campaign in July 2007, which was a great surprise among the Japanese in general, and this was followed by an increase in the number of voluntary HIV tests performed in 2007 and 2008.[2] The event may have prompted older MSM at high risk to take voluntary HIV testing, resulting in the transient increase in the rate of early diagnosis for 2007 and 2008. The sharp decline in the rate of early diagnosis observed in 2009 and 2010 in the older MSM group coincided with reductions in the number of voluntary tests,[2] and could be an omen of future increases in the number of AIDS patients in this population. Early diagnosis followed by early introduction of ART may reduce the spread of HIV-1 among MSM, which could help to prevent an HIV epidemic in this population.[6,7,22] A strategy based on the promotion of voluntary testing needs to be formulated, similar to the 2007 campaigns that resulted in significant increases in the rate of early diagnosis in older MSM.

Discordant shifts were observed between the rates of AIDS and recent infection. The reasons may be that AIDS usually develops several years after HIV infection and that disease progression varies enormously among infected individuals. Therefore, the variable length of time during which HIV infection was ignored resulted in the development of AIDS, the proportion of which does not always correlate with the rate of recent infection in the same year.[11] Furthermore, disease progression has been suggested to have become faster in a significant portion of Japanese patients, probably because the prevailing HIV-1 strains in Japan have adapted to the Japanese population by acquiring escape mutations from immune pressure restricted by human leukocyte antigens (HLAs) popular among the Japanese.[23,24] Based on this point of view, early diagnosis is even more important due to the shorter asymptomatic period before the development of AIDS.

The majority of our study participants were infected with HIV-1 subtype B, and HIV-1 subtype B infection correlated significantly with MSM (crude odds ratio 37.9, $p < 0.001$; Chi-square test). The non-AIDS patients were more likely to be infected with subtype B than AIDS patients (crude odds ratio 1.59, p = 0.098). The same was true for recent infection than chronic infection (crude odds ratio 2.81, p = 0.009). A previous Japan-wide survey also showed a close relationship between subtype B and MSM in Japan; all cases diagnosed with primary HIV-1 infection (n = 45) were caused by subtype B, and such primary infections were significantly frequent among MSM.[14] Considered together, the results indicate that subtype B is the major currently prevalent strain in Japan, especially among MSM, and such strains are probably adapting to the Japanese population by repeated exposure to immune pressure of the Japanese.

This study used case reporting-based surveillance to estimate the number of new HIV-1 infections in Tokyoites between 2002 and 2010. The data were collected at a single center and thus may have included some institutional bias. The study participants were statistically younger and were more likely to be MSM than those of the Tokyo registry. The BED assay was used in this study to determine the rate of recent infection in the selection study group and not to determine the national incidence rate. However, the data from this study suggest the following target-specific differential strategies for controlling the HIV epidemic and for AIDS prevention in Tokyo: campaigns aimed at promoting testing should be directed at older MSM for early diagnosis to prevent/halt the progression of AIDS; commencement of ART for HIV-infected younger MSM at early stages of the disease may effectively reduce the number of new cases based on the control of current hot-spots of HIV transmission among this group.

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Conflict of interest: The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2011.11.003.

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Renal Function Declines More in Tenofovir- than Abacavir-Based Antiretroviral Therapy in Low-Body Weight Treatment-Naïve Patients with HIV Infection

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Abstract

Objective: To compare the rate of decline of renal function in tenofovir- and abacavir-based antiretroviral therapy (ART) in low-body weight treatment-naïve patients with HIV infection.

Design: We conducted a single-center retrospective cohort study of 503 Japanese patients who commenced on either tenofovir- or abacavir-based initial ART.

Methods: The incidence of renal dysfunction, defined as more than 25% fall in estimated glomerular filtration rate (eGFR) from the baseline, was determined in each group. The effect of tenofovir on renal dysfunction was estimated by univariate and multivariate Cox hazards models as the primary exposure. Changes in eGFR until 96 weeks were estimated in both groups with a repeated measures mixed model.

Results: The median body weight of the cohort was 64 kg. The estimated incidence of renal dysfunction in the tenofovir and the abacavir arm was 9.84 per 100 and 4.55 per 100 person-years, respectively. Tenofovir was significantly associated with renal dysfunction by univariate and multivariate analysis (HR = 1.747; 95% CI, 1.152–2.648; p = 0.009) (adjusted HR = 2.080; 95% CI, 1.339–3.232; p<0.001). In subgroup analysis of the patients stratified by intertertile baseline body weight, the effect of tenofovir on renal dysfunction was more evident in patients with lower baseline body weight by multivariate analysis (≤60 kg: adjusted HR = 2.771; 95%CI, 1.494–5.139; p = 0.001) (61–68 kg: adjusted HR = 1.908; 95%CI, 0.764–4.768; p = 0.167) (>68 kg: adjusted HR = 0.997; 95%CI, 0.318–3.121; p = 0.995). The fall in eGFR was significantly greater in the tenofovir arm than the abacavir arm after starting ART (p = 0.003).

Conclusion: The incidence of renal dysfunction in low body weight patients treated with tenofovir was twice as high as those treated with abacavir. Close monitoring of renal function is recommended for patients with small body weight especially those with baseline body weight <60 kg treated with tenofovir.

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Introduction

Tenofovir disoproxil fumarate (TDF) and abacavir sulfate (ABC) are widely used nucleot(s)ide reverse transcriptase inhibitors (NRTIs) as part of the initial antiretroviral therapy for patients with HIV infection in the developed countries (URL:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf) (URL: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf). TDF is generally preferred to ABC, since ABC is reported to cause serious hypersensitivity reaction in 5–8% of the patients and its efficacy in viral suppression is reported to be inferior to TDF among patients with baseline HIV viral load of >100,000 copies/ml [1,2]. On the other hand, renal proximal tubular damage and renal dysfunction are well-known adverse effects of TDF [3–9]. A meta-analysis study that compared TDF and other NRTIs concluded that the decline in renal function with TDF use is significant but modest, and the ASSERT study conducted in Europe compared randomly-selected treatment naïve patients who commenced treatment with either TDF or ABC with efavirenz and showed no difference in estimated glomerular filtration rate (eGFR) between the two groups at 48 weeks [9,10]. To date, the nephrotoxicity of TDF have been regarded as mild and tolerable [2,5–7,9–11].

However, the TDF-related nephrotoxicity has hardly been evaluated in patients with small body weight, who are potentially at higher risk for larger drug exposure and thus, more severe toxicity [12–15]. Indeed, some recent studies including ours reported a higher incidence of TDF-related renal dysfunction among Asian patients with low body weight compared with previous studies on mostly Whites and African Americans with larger body weight [13,16]. Thus, it is important to provide more evidence in support of TDF-associated nephrotoxicity in patients with low body weight since such data can elucidate whether TDF-related nephrotoxicity is as mild in low-body-weighted patients as previously reported in Europe and the USA. This is also important because there is increasing use of TDF in resource-limited settings, where patients are often of relatively small body weight, following the revised 2010 WHO guidelines that recommend TDF as one of the components of first line therapies (URL:http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf)[13,16–19]. To our knowledge, there are no studies that compared renal function in treatment naïve Asian patients who commenced treatment with TDF or ABC.

Based on the above background, the present study was designed to compare the incidence of renal dysfunction and change in eGFR between treatment-naïve Japanese patients with low body weight who started either TDF or ABC as part of the antiretroviral regimen.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine, Tokyo. All patients included in this study provided a written informed consent for their clinical and laboratory data to be used and published for research purposes. This study has been conducted according to the principles expressed in the Declaration of Helsinki.

Study Subjects

We performed a retrospective, single-center cohort study of HIV-infected Japanese patients using the medical records at the National Center for Global Health and Medicine, Tokyo, Japan. Our facility is one of the largest clinics for patients with HIV infection in Japan with more than 2,700 registered patients. The study population was treatment-naïve patients with HIV infection, aged >17 years, who commenced treatment with either the recommended 300 mg/day dose of TDF or 600 mg/day dose of ABC-containing antiretroviral regimen at our clinic between January 1, 2004 and March 31, 2009. During this inclusion period, all except two patients at our clinic started ART with either ABC or TDF. Patients with an eGFR of >60 ml/min/1.73 m^2 were enrolled. Patients were followed up until March 31, 2011. They were excluded if they started ART with both TDF and ABC, their follow-up period at our facility was less than 24 weeks after commencement of ART, or if they had started ART at other facilities. Only Japanese patients were included in order to examine a population with comparatively homogenous basic demographics and background. The attending physician selected either TDF or ABC at baseline, and the use of these two drugs was based on the Japanese guidelines, which place both ABC and TDF as the preferred NRTIs (http://www.haart-support.jp/guideline2011.pdf. in Japanese). The attending physician also selected the key drug [non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), or integrase inhibitor (INI)]. All patients received standard ART with two NRTIs combined with either PI, NNRTI, or INI.

Measurements

We defined renal dysfunction as more than 25% decrease in eGFR relative to the baseline [13,16,20,21]. The baseline eGFR was estimated for each patient from the average of two successive serum creatinine measurements made closest to and preceding the commencement of antiretroviral therapy by no more than 90 days. Changes in eGFR were plotted from the baseline measurement until the average value of two successive measurements diminished to less than 75% of the baseline, discontinuation of TDF or ABC, or at the end of the follow-up period. Discontinuation of TDF and ABC was the choice of the attending physician, and was based on virologic failure or ART-related side effects other than renal dysfunction. Before the initiation of ART and until suppression of HIV-1 viral load, patients visited our clinic every month. However, after viral load suppression, the visit interval was extended up to every three months. Serum creatinine and eGFR were measured in every visit, and the frequency of measurements was similar in patients on TDF and ABC. eGFR was calculated using the equation from the 4-variable Modification of Diet in Renal Disease (MDRD) study, $eGFR = 186\times [\text{serum creatinine}]^{-1.154}\times [\text{age}]^{-0.203}\times$ [0.742 if patient is female] × [1.212 if patient is African American] [22]. In this study, the primary exposure variable was TDF use over ABC as part of the initial ART.

The potential risk factors for renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical records [15,23–25]. They included age, sex, body weight, body mass index, (BMI) = {body weight (kg) / [(height (m)]^2}, baseline laboratory data (CD4 cell count, HIV viral load, and serum creatinine), and presence or absence of other medical conditions (concurrent use of ritonavir-boosted protease inhibitors, concurrent nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, and non-steroidal anti-inflammatory agents, diabetes mellitus defined by using anti-diabetic agents or fasting plasma glucose >126 mg/dl or plasma glucose >200 mg/dl on two different days, co-infection with hepatitis B defined by positive hepatitis B surface antigen, co-infection with hepatitis C defined by positive HCV viral load, hypertension defined by current treatment with antihypertensive agents or two successive measurements of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg at the clinic, dyslipidemia defined by current treatment with lipid-lowering agents, and current smoking). At our clinic, weight and blood pressure were measured on every visit whereas other variables were measured in the first visit and at least once annually. We used the data on or closest to and preceding the day of starting ART by no more than 90 days.

Statistical analysis

The time to 25% decline in eGFR from the baseline was calculated from the date of commencement of treatment to the date of diagnosis of the above-defined renal dysfunction. Censored cases represented those who discontinued ABC or TDF, dropped out, were referred to other facilities, or at the end of follow-up period. The time from the start of ART to >25% decrease in eGFR was analyzed by the Kaplan Meier method for patients who started TDF (TDF arm) and ABC (ABC arm), 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 TDF use over ABC on the incidence of more than 25% decrease in eGFR relative to the baseline. The impact of each basic demographics, 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 TDF use over ABC for renal dysfunction, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for TDF use over ABC. Model 2 included age and weight plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with P values <0.05 in univariate analysis for adjustment (these included age per 1 year, weight per 1 kg decrement, CD4 count per 1 /μl decrement, HIV viral load per log10/ml, serum creatinine per 1 mg/dl, concurrent use of nephrotoxic drug(s), hepatitis B infection, and diabetes mellitus). The eGFR and the BMI were excluded from multivariate analysis because of their multicollinearity with age and serum creatinine, and weight, respectively, since eGFR and BMI are gained by the equation of those variables [22,26]. We chose to add weight instead of BMI because our previous work showed that weight was more useful and handy information to estimate the risk for TDF-related nephrotoxicity than BMI [16].

As a sensitivity analysis, creatinine clearance was similarly calculated with Cockcroft-Gault equation for each patient, creatinine clearance = [(140 - age) × weight (kg)] / (serum creatinine×72)(×0.85 for females) [27]. Actual body weight was used for the calculation. The impact of TDF use over ABC for >25% decrement of creatinine clearance from the baseline was estimated in univariate analysis and multivariate analysis adjusted with the before mentioned variables with Cox proportional hazards model.

To estimate the impact of weight on TDF-related nephrotoxicity, we did subgroup analysis for intertertile baseline body weight categories: ≤60, 61–68, and >68 kg. Then, the abovementioned multivariate analysis with eGFR was conducted for each subgroup.

We also used a repeated measures mixed model to estimate and compare changes in eGFR between ABC and TDF from baseline to 2 years after initiation of ART by 6-month intervals adjusted for baseline eGFR and weight [10]. For each patient, the eGFR values at closest to and preceding 24, 48, 72 and 96 weeks after commencement of ART were collected. In this analysis, censoring occurred at discontinuation of TDF or ABC, leaving care, or reaching the end of the observation period before 96 weeks. Sensitivity analysis with creatinine clearance calculated by Cockcroft-Gault equation was similarly conducted.

Statistical significance was defined at two-sided p values <0.05. We used hazard ratios (HRs) and 95% confidence intervals (95% CIs) to estimate the impact of each variable on renal dysfunction. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

The study subjects were 199 patients in the TDF arm and 304 patients in the ABC arm who fulfilled the abovementioned criteria. Table 1 shows the demographics, laboratory data, and medical conditions of the study population at baseline. The majority of the study population was males, comparatively young and had a small stature (median weight, 64 kg, median BMI, 22.2 kg/m^2). More than 80% of the patients in the two arms had ritonavir-boosted PI. In the ABC arm, patients had significantly lower CD4 count (p = 0.006), were significantly more likely to have hypertension (p<0.001), and tended to use more nephrotoxic drugs (p = 0.109). On the other hand, in the TDF arm, patients had marginally higher baseline eGFR (p = 0.098) and were significantly more likely to have hepatitis B virus infection (P<0.001). However, all other major background parameters were similar in the two groups (Table 1).

More than 25% decrement in eGFR from baseline occurred in 44 patients (22.1%) in the TDF arm and 41 (13.5%) in the ABC arm, with an estimated incidence of 9.84 and 4.55 per 100 person-years, respectively. Figure 1 shows the time from ART initiation to >25% decrease in eGFR by the Kaplan Meier method in the two groups. Patients who started TDF-containing ART were significantly more likely to develop renal dysfunction, compared to the ABC group (p = 0.001, Log-rank test). The median time from commencement of ART to occurrence of >25% decrement in eGFR was 246 days (range, 1–1,339 days) for the TDF arm and 501 days (range, 7–2,022) for ABC arm. The total observation period was 447.2 patient-years [median, 839 days, interquartile range (IQR), 357–1137 days] for the TDF arm and 901.7 patient-years (median, 1,119 days, IQR, 660.5–1509 days) for the ABC arm.

Univariate analysis showed a significant relationship between TDF use and >25% decrement in eGFR (HR = 1.747; 95%CI, 1.152–2.648; p = 0.009) (Table 2). Furthermore, old age, small body weight, low baseline CD4 count, high HIV viral load, high eGFR, low serum creatinine, concurrent use of nephrotoxic drugs, hepatitis B infection, and diabetes mellitus were associated with renal dysfunction. On the other hand, concurrent use of ritonavir boosted PIs was not associated with renal dysfunction (HR = 1.220; 95%CI, 0.663–2.244; p = 0.523). Multivariate analysis identified TDF use as a significant risk for >25% decrement in eGFR after adjustment for age and weight (adjusted HR = 1.893; 95%CI, 1.243–2.881; p<0.003) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR = 2.080; 95%CI, 1.339–3.232; p<0.001) (Table 3, Model 3). We also conducted a sensitivity analysis using BMI decrement instead of weight as a variable in Table 3, Model 3. The results were almost identical; TDF use over ABC use was a risk for renal dysfunction (adjusted HR 1.957, 95% CI 1.262–3.036, p = 0.003).

Sensitivity analysis with creatinine clearance confirmed the abovementioned findings: both univariate and multivariate analyses showed that TDF use was significantly associated with >25% decrement in eGFR (univariate analysis: HR = 2.212; 95%CI, 1.340–3.653; p = 0.002) (multivariate analysis: adjusted HR = 2.544; 95%CI, 1.493–4.335; p = 0.001).

Subgroup analysis of the patients stratified by intertertile baseline body weight showed that the lower the baseline body weight, the more evident the impact of TDF on renal dysfunction (≤60 kg: adjusted HR = 2.771; 95%CI, 1.494–5.139; p = 0.001) (61–68 kg: adjusted HR = 1.908; 95%CI, 0.764–4.768; p = 0.167) (>68 kg: adjusted HR = 0.997; 95%CI, 0.318–3.121; p = 0.995) (Table 4). These findings suggest that there is the effect modification by baseline body weight on TDF-associated renal dysfunction.

Data analysis by repeated measures mixed models showed a significant decrease in adjusted mean eGFR from the baseline to 96 weeks in both groups (TDF: -9.984 ml/min/1.73m^2, 95%CI -12.05 to -7.914 ml/min/1.73m^2, p<0.001; ABC: -5.393 ml/min/1.73m^2, 95%CI -7.087 to -3.699 ml/min/1.73m^2, p<0.001) (Figure 2). There was a statistically significant interaction between the two arms over time (p = 0.003), indicating that adjusted mean eGFR decreased more significantly in the TDF group than in the ABC group after initiation of ART. Analysis of eGFR in each group demonstrated a rapid decrease during the first 24 weeks,

Table 1. Baseline demographics and laboratory data of patients who received tenofovir- and abacavir-based antiretroviral therapy (n = 503).

	TDF (n = 199)	ABC (n = 304)	P value
Sex (male), n (%)	196 (98.5)	296 (97.4)	0.539
Median (IQR) age	36 (31–44)	37 (31–43)	0.436
Median (IQR) weight (kg)	64 (58–69)	64 (58.0–70.9)	0.426
Median (IQR) BMI (kg/m^2)	22.1 (20.4–23.9)	22.2 (20.3–24.6)	0.321
Median (IQR) eGFR (ml/min/1.73m^2)	119.4 (103.0–135.0)	115.6 (102.4–132.2)	0.098
Median (IQR) serum creatinine (mg/dl)	0.74 (0.67–0.84)	0.75 (0.68–0.83)	0.250
Median (IQR) CD4 count (/μl)	199 (109–272)	178.5 (75.3–234.8)	0.006
Median (IQR) HIV RNA viral load (log10/ml)	4.63 (4.20–5.20)	4.74 (4.23–5.20)	0.731
Ritonavir-boosted protease inhibitors, n (%)	173 (86.9)	256 (84.2)	0.441
Protease inhibitors (unboosted), n (%)	5 (2.5)	20 (6.6)	0.038
NNRTIs, n (%)	16 (8.0)	26 (8.6)	0.848
INIs, n (%)	5 (2.5)	2 (0.7)	0.119
Hypertension, n (%)	5 (2.5)	53 (17.4)	<0.001
Dyslipidemia, n (%)	4 (2.0)	4 (1.3)	0.718
Diabetes mellitus, n (%)	8 (4.0)	12 (3.9)	1.000
Concurrent use of nephrotoxic drugs, n (%)	65 (32.7)	121 (39.8)	0.109
Hepatitis B, n (%)	35 (17.6)	9 (3.0)	<0.001
Hepatitis C, n (%)	7 (3.5)	7 (2.3)	0.421
Current smoker, n (%)	93 (46.7)	149 (49.3)	0.585

TDF: tenofovir, ABC: abacavir, IQR: interquartile range, BMI: body mass index, eGFR: estimated glomerular filtration rate, NNRTI: non- nucleoside reverse transcriptase inhibitor, INI: integrase inhibitor.
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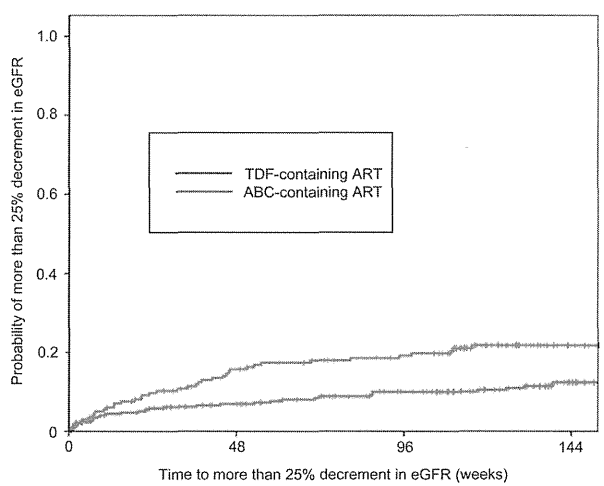

Figure 1. Kaplan-Meier curve showing the time to renal dysfunction in patients treated with TDF or ABC. Compared to treatment-naïve patients who commenced treatment with ABC, those on TDF were more likely to develop >25% fall in eGFR (p = 0.001, Log-rank test). TDF: tenofovir, ABC: abacavir, ART: antiretroviral therapy, eGFR: estimated glomerular filtration rate.
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Table 2. Univariate analysis to estimate the risk of various factors in inducing more than 25% fall in eGFR.

	Hazard ratio	95% CI	P value
TDF vs. ABC use	1.747	1.152–2.648	0.009
Female gender	0.048	0.000–16.93	0.310
Age per 1 year	1.031	1.011–1.051	0.002
Weight per 1 kg decrement	1.047	1.023–1.072	<0.001
BMI per 1 kg/m^2 decrement	1.152	1.066–1.244	<0.001
CD4 count per 1 /μl decrement	1.006	1.004–1.008	<0.001
HIV viral load per log10/ml	1.562	1.179–2.071	0.002
Ritonavir-boosted protease inhibitors	1.220	0.663–2.244	0.523
Baseline eGFR per 1 ml/min/1.73m^2	1.009	1.005–1.014	<0.001
Baseline serum creatinine per 1mg/dl	0.016	0.003–0.086	<0.001
Concurrent nephrotoxic drug	2.134	1.417–3.214	<0.001
Hepatitis B	1.866	1.038–3.356	0.037
Hepatitis C	1.721	0.631–4.695	0.289
Diabetes mellitus	2.558	1.181–5.540	0.017
Hypertension	0.865	0.448–1.669	0.664
Current smoking	0.989	0.657–1.489	0.958

eGFR: estimated glomerular filtration rate, CI: confidence interval, TDF: tenofovir, ABC: abacavir, BMI: body mass index.
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followed by a plateau until 96 weeks. In sensitivity analysis with creatinine clearance calculated by Cockcroft-Gault equation, the result was the same; a significant decrease from the baseline to 96 weeks in both groups (TDF: -10.62 ml/min, 95%CI -13.78 to -7.458 ml/min; ABC: -4.325 ml/min, 95%CI -6.893 to -1.756 ml/min) and significantly more eGFR decrement in the TDF group (p = 0.019).

Discussion

In this observational Japanese cohort, treatment-naïve patients who started TDF-containing ART experienced eGFR decline of >25% approximately twice as likely compared to those treated with ABC-containing regimen. Univariate and multivariate analyses identified TDF use as an independent risk factor for renal dysfunction. Subgroup analysis showed that the effect of TDF on renal dysfunction was more evident in patients with lower body weight. Furthermore, eGFR decrement was significantly larger in the TDF group than in ABC group over the 2-year observation period.

Table 4. Multivariate analysis to estimate the risk of TDF- over ABC-based antiretroviral therapy in the induction of more than 25% fall in eGFR according to baseline body weight.

	Adjusted HR	95% CI	P value
Baseline body weight ≤60 kg (n = 171)			
TDF vs. ABC use	2.771	1.494–5.139	0.001
Baseline body weight 61–68 kg (n = 167)			
TDF vs. ABC use	1.908	0.764–4.768	0.168
Baseline body weight >68 kg (n = 165)			
TDF vs. ABC use	0.997	0.318–3.121	0.995

TDF use was adjusted with the same variables indicated in Model 3, Table 3: age per 1 year, weight per 1 kg decrement, CD4 count per 1 /μl decrement, HIV viral load per log10/ml, serum creatinine per 1 mg/dl, concurrent use of nephrotoxic drugs, hepatitis B infection, and diabetes mellitus.
doi:10.1371/journal.pone.0029977.t004

In our previous study, we demonstrated a high incidence of TDF-associated nephrotoxicity in patients with low body weight, and the use of a robust statistical model indicated a greater decline in renal function in patients of low body weight treated with TDF [16]. The results of the present study further emphasize the importance of low body weight as a risk factor for TDF-related nephrotoxicity by showing that in a cohort of patients with low body weight, the incidence of renal dysfunction was twice higher with TDF use than with ABC use.

Among the studies designed to compare renal function after the commencement of TDF and ABC-containing ART for treatment-naïve patients, our cohort had the lowest median body weight (64 kg). This is lower than the median body weight of patients of the ASSERT study conducted in European countries (72 kg) [10]. The

Table 3. Multivariate analysis to estimate the risk of TDF- over ABC-based antiretroviral therapy in inducing more than 25% fall in eGFR.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95% CI	HR	95%CI	HR	95%CI
TDF vs. ABC use*	1.747	1.152–2.648	1.893	1.243–2.881	2.080	1.339–3.232
Age per 1 year			1.029	1.010–1.048	1.020	1.000–1.040
Weight per 1 kg decrement*			1.046	1.022–1.071	1.028	1.005–1.052
CD4 count per 1 /μl decrement*					1.004	1.002–1.007
HIV viral load per log10/ml					1.048	0.749–1.466
Serum creatinine per 1 mg/dl*					0.053	0.009–0.304
Use of nephrotoxic drug					1.309	0.825–2.077
Hepatitis B					1.070	0.573–2.000
Diabetes mellitus					1.565	0.684–3.582

*P<0.05 in Model 3.
TDF: tenofovir, ABC: abacavir, eGFR: estimated glomerular filtration rate, HR: hazard ratio, CI: confidence interval.
doi:10.1371/journal.pone.0029977.t003

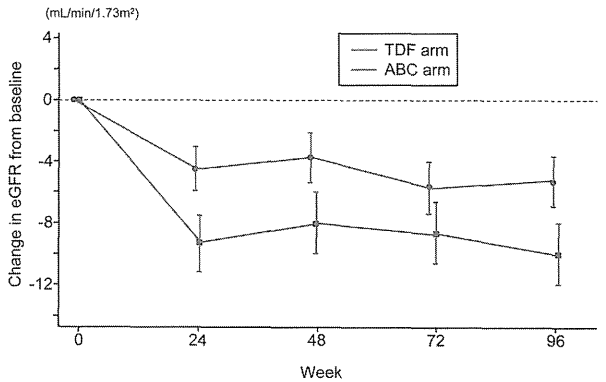

Figure 2. Changes in eGFR in patients treated with TDF or ABC between baseline and 96 weeks. The fall in eGFR was significantly greater in the TDF group than the ABC group (p = 0.003). Data are adjusted mean ±95% confidence interval. eGFR: estimated glomerular filtration rate, TDF: tenofovir, ABC: abacavir.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

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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.

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Minor contribution of HLA class I-associated selective pressure to the variability of HIV-1 accessory protein Vpu

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ABSTRACT

Host HLA class I (HLA-I) allele-associated immune responses are major forces driving the evolution of HIV-1 proteins such as Gag and Nef. The viral protein U (Vpu) is an HIV-1 accessory protein responsible for CD4 degradation and enhancement of virion release by antagonizing tetherin/CD317. Although Vpu represents one of the most variable proteins in the HIV-1 proteome, it is still not clear to what extent HLA-I influence its evolution. To examine this issue, we enrolled 240 HLA-I-typed, treatment naïve, chronically HIV-infected subjects in Japan, and analyzed plasma HIV RNA nucleotide sequences of the vpu region. Using a phylogenetically-informed method incorporating corrections for HIV codon covariation and linkage disequilibrium among HLA alleles, we investigated HLA-associated amino acid mutations in the Vpu protein as well as in the translational products encoded by alternative reading frames. Despite substantial amino acid variability in Vpu, we identified only 4 HLA-associations in all possible translational products encoded in this region, suggesting that HLA-associated immune responses had *minor effects on Vpu variability in this cohort. Rather, despite its size* (81 amino acids), Vpu showed 103 codon–codon covariation associations, suggesting that Vpu conformation and function are preserved through many possible combinations of primary and secondary polymorphisms. Taken together, our study suggests that Vpu has been comparably less influenced by HLA-I-associated immune-driven evolution at the population level compared to other highly variable HIV-1 accessory proteins.

1. Introduction

Immune-mediated adaptation occurs during an HIV-1 infection. The HLA class I (HLA-I)-restricted CD8$^+$ cytotoxic T lymphocyte (CTL) response is one of the major forces driving HIV evolution, resulting in the selection of CTL escape mutants [1,2]. Despite the extensive genetic diversity of both HIV-1 and HLA-I alleles, escape pathways are reproducible and broadly predictable based on host HLA-I alleles [3–6]. Moreover, analysis of linked HLA-I and HIV datasets from large cohorts of HIV-infected subjects has facilitated our ability to map the landscape of immune escape mutations across HIV-1, identify immunogenic regions, and identify novel CTL epitopes [3,7].

Viral protein U (Vpu) is an accessory protein that is unique to HIV-1 and a subset of related simian immunodeficiency viruses. The HIV-1 Vpu protein has two major functions: degradation of newly synthesized CD4 molecules in the endoplasmic reticulum and enhancement of the release of progeny virions from infected cells by antagonizing tetherin/CD317, a host restriction factor that directly binds and retains viral particles on the surface of infected cells (reviewed in [8,9]). As such, Vpu is thought to play a role in virus spread and pathogenesis *in vivo*. Interestingly, Vpu is the most variable protein among all HIV proteins as evidenced by a cross-sectional comparison of HIV-1 sequences isolated from HIV-infected individuals [10], raising the possibility that Vpu undergoes adaptation in response to host immune responses. However, Vpu has been shown to be a minor target for CTLs as revealed by IFN-γ Elispot assays with overlapping peptides based on the subtype B consensus sequence [11]. Considering the highly variable nature of Vpu, it is possible to miss responses if the autologous virus sequence is markedly different from the peptide sequence when using this Elispot assay system [12].

In the present study, we sought to identify HLA-associated polymorphisms in Vpu and alternate reading frames and examine to what extent they are involved in Vpu amino acid variability at the population level. We utilize a published phylogenetic dependency network model [13], a comprehensive evolutionary model that considers all important confounding effects such as HIV phylogeny, HIV codon covariation, and linkage disequilibrium of HLA alleles.

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2. Materials and methods

2.1. Patient samples

A total of 240 chronically HIV-1-infected, treatment-naïve subjects (CD4, median 237; IQR, 160-397; viral load, median 33,200; IQR, 222,000-55,400) followed at the AIDS Clinical Center, International Medical Center of Japan were enrolled in this study. All participants provided written informed consent. HLA-I typing was performed as previously described [14]. The most frequently observed *HLA-A, B,* and *C* alleles in this cohort were *HLA-A*24:02, HLA-B*52:01*, and *HLA-C*01:02*, respectively, consistent with HLA class I allelic frequencies of the Japanese people [14].

2.2. Sequence analysis of vpu

HIV-1 particles were precipitated by ultracentrifugation (50,000 rpm, 15 min) of patients' plasma, after which the viral RNA was extracted using standard methods. Following reverse transcription, DNA fragments encoding Vpu proteins were amplified by nested PCR, and gel purified as previously described [15,16]. The primers used were as follows: the primers for the first round of amplification were VVVa-F (5′-TTAAAAGAAAAGGGGG GATTGGGGG-3′) and VVVb-R (5′-ATTCCATGTGTACATT GTACTGT-3′); and those for the second round, VVVc-F (5′-AGATAATAGTGAC ATAAAAGTAGTGCCAAGAAG-3′) and VVVd-R (5′-CCATAATAGACT GTGACCCACAA-3′). The *vpu* sequence was then directly analyzed with an automated sequencer (Applied Biosystems 3500xL) and aligned to the *vpu* sequence of the HIV-1 subtype B reference strain HXB2 (Accession No. K03455). More than 90% of the subjects were infected with subtype B, as determined by phylogenetic analysis of concatenated sequences of *vif, vpr,* and *vpu* reading frames.

2.3. Analysis of amino acid sequence variability

A Shannon entropy score for each position in the Vpu protein was calculated and used to analyze amino-acid sequence variability, as described previously [10]. Entropy is a measure of the amino acid variability at a given position that takes into account both the number of possible amino acid residues allowed and their frequency.

2.4. Analysis of association between Vpu sequence polymorphisms and host HLA class I alleles

To identify HIV-HLA polymorphism associations, we employed a phylogenetically dependency network model [13], which comprehensively includes all confounding effects of the analysis, such as HIV founder effects, HIV codon co-variation, and linkage disequilibrium of HLA-I alleles. Multiple comparisons are addressed using q-values (refer the detailed methods given in refs. [4,5,13]); in the present study, a cutoff of $q < 0.2$ was used to denote statistically significant associations. HLA-associated polymorphisms were classified into two categories. "Nonadapted" amino acids are enriched in the absence of the restricting HLA of interest. Usually, "nonadapted" forms represent the consensus amino acid at that position, and they can be thought of as the "wild-type" or "susceptible" form particular to that allele. Conversely, "adapted" amino acids are those enriched in the presence of the HLA allele; these can be thought of as the escape variant particular to that allele.

3. Results and discussion

3.1. Genetic variability of the vpu gene

We successfully amplified DNAs encompassing the *vpu* region from 216 of 240 samples (90%). Firstly, we analyzed the amino acid variability at each codon of Vpu by determining its Shannon entropy score. Two amino acid residues, Trp23 and Arg49, showed highly conserved (>98%) among individual sequences. Instead, most codons displayed substantial variability, with the average of the entropy score reaching 0.58 (Fig. 1A), confirming the findings by Yusim et al., which showed that Vpu is a highly variable protein [10]. Also, the amino acid variability of each codon in the present study correlated strongly with that of published subtype B sequence data from the Los Alamos database (Fig. 1B), suggesting that our observed pattern of amino acid variation in Vpu was generally representative of the variation observed in HIV-1 subtype B. In fact, the consensus amino acid sequences of subtype B and the present dataset were identical except for 3 amino-acid residues: positions 3, 5, and 24 (Fig. 1C). These amino acid residues were highly variable (Fig. 1A) and not directly associated with known Vpu functions (Fig. 1C).

3.2. HLA-associated polymorphisms in Vpu

As HLA-I-mediated selective contributes to HIV-1 sequence variability, especially the accessory protein Nef [4], we sought to examine whether HLA-I-mediated selective pressure substantially influenced the evolution of Vpu, another accessory protein. We applied a phylogenetic dependency network model [13], which adjusts for the confounding effects of HIV phylogeny, HIV codon covariation and linkage disequilibrium of HLA-I alleles.

In our dataset of 216 individuals, we identified only three HIV-HLA associations in Vpu: a nonadapted association between C*03 and Glu-5, a nonadapted association between A*33:03 and Arg-37, and an adapted association between A*33:03 and Lys-37. The presence of both nonadapted and adapted A*33:03-associated polymorphisms at Vpu codon 37 is consistent with an Arginine-to-Lysine escape mutation occurring at the C-terminus of the immunodominant HLA-A*33:03-restricted epitope in Vpu, ^{29}EYRKILRQR^{37} [11]. However, there was no HLA-restricted T cell epitopes around Vpu position 5 have been reported. Although we might have missed some polymorphisms due to the limited sample size in this study, these data suggest that HLA-I-mediated selective pressure toward Vpu does not substantially drive Vpu variability at the population level in this cohort.

3.3. HLA-associated polymorphisms in alternating reading frames

CTLs can recognize epitopes encoded by alternate reading frames including the antisense-strand sequences of HIV-1 *gag, pol,* and *nef* [17,18]. Therefore, we also investigated HIV-HLA polymorphism associations in peptide sequences encoded by alternative reading frames of the *vpu* gene. We observed no statistically significant HLA-associated polymorphisms in alternate or antisense reading frames, except for a single HLA-B*40:01 associated "adapted" lysine polymorphism at codon 2 of the overlapping Envelope reading frame which is initiated in the middle of the *vpu* gene (ORF + 2; Table 1, Fig. 2). Although this association was between Lys-2 of Env and *HLA-B*40:01*, no CTL epitopes have been reported in the context of HLA-B*40:01 in this region. Using bioinformatic prediction programs Epipred [19] and BIMAS [20] we

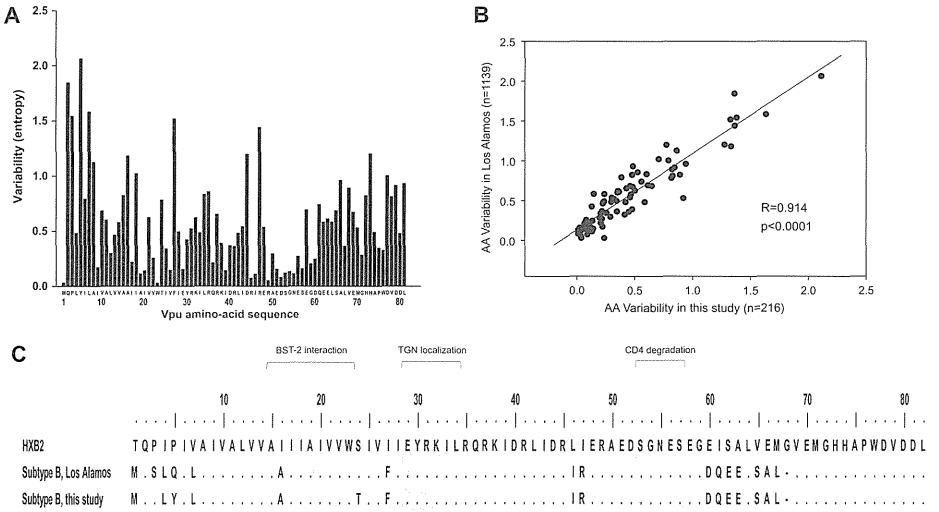

Fig. 1. Variability of the amino acid residues of HIV-1 Vpu. The amino acid sequence of Vpu was analyzed based on the cross-sectional studies on 216 HIV-infected subjects. The amino acid variability at each position of Vpu was analyzed by determining its Shannon entropy score (panel A). The Vpu sequence (subtype B, $n = 1139$) was retrieved from the Los Alamos HIV sequence database, analyzed for its amino acid variability, and compared with subtype B obtained from this study using Spearman Rank Order Correlation (panel B). The consensus sequences of Vpu obtained from Los Alamos database and this study were aligned with reference strain HXB2 and regions responsible for some key Vpu functions highlighted (panel C).

Table 1
Summary of HIV-HLA associations in the Vpu-encoded region.

RF	Protein	Pos HXB2	aa	HLA	Association	*p*-Value	*q*-Value	Known epitope	
								Sequence	Reference
+1	Vpu	5	E	C*03	Nonadapted	2.13×10^{-5}	1.52×10^{-1}	none	–
		37	R	A*22:03	Nonadapted	3.40×10^{-6}	5.50×10^{-2}	^{29}EYRKILRQR^{37}	[11]
		37	K	A*33:03	Adapted	2.80×10^{-5}	1.52×10^{-1}	^{29}EYRKILRQR^{37}	[11]
+2	Env	2	K	B*40:01	Adapted	1.63×10^{-5}	1.67×10^{-1}	none	–

RF, reading frame; Pos HXB2, amino acid position when aligned to HXB2 sequence.

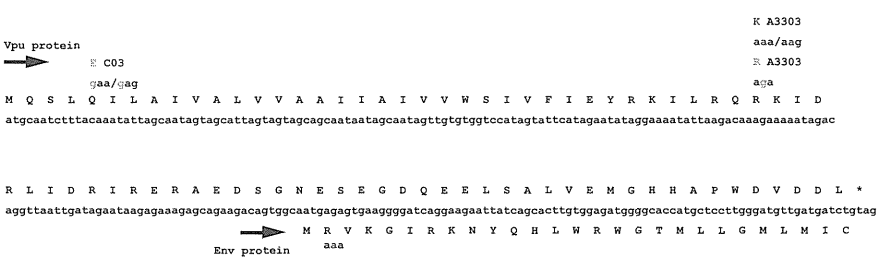

Fig. 2. The Vpu and a part of Env proteins and their associations with host HLA class I alleles. The nucleotide sequence and its deduced amino acid sequence of Vpu and of an overlapping part of Env with reference to the subtype B consensus sequence of Los Alamos database is shown. The amino acid residues associated with the indicated HLA class I alleles ($p < 0.05$, $q < 0.2$) are shown with adapted (red) and nonadapted (blue) residues. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

attempted to predict B*40:01-restricted CTL epitopes, but found none (data not shown). This failure is most likely due to the presence of several basic amino acids, such as Arg and Lys, in this region of Env (Fig. 2), as it has been shown that HLA-B*40:01

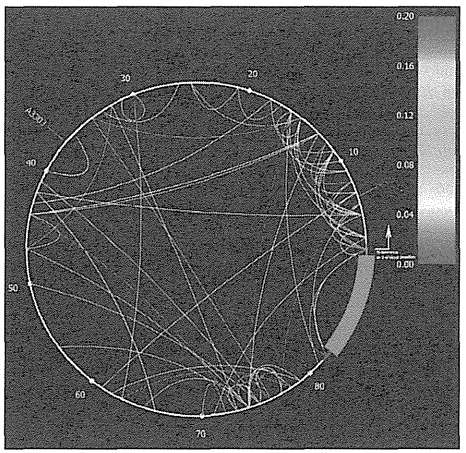

Fig. 3. Amino acid codon–codon covariation in Vpu. The circular map, generated by the PhyloDv software [13], shows Vpu codon–codon covariation associations as inner arcs connecting the association sites, with the HLA associations as tags pointing to their corresponding sites. Q values of individual codon pairs are represented as a heat map shown at the right.

preferentially binds peptides with acidic residues at their anchors [21]. This issue needs to be clarified in further studies using immunologic assays. Taken together, our results suggest that HLA-I-mediated selective pressure do not contribute to a large extent to population-level sequence variation in HIV-1 Vpu.

3.4. Codon–codon covariation of Vpu

Given that Vpu is functionally important in viral replication *in vivo*, the highly variable nature of Vpu amino acid sequences could be explained by complex networks of codon–codon covariation and/or secondary/compensatory mutation pathways. We therefore examined the codon–codon covariation of Vpu by using the phylogenetic dependency network model. Although Vpu consists of only 81 amino acids, we identified 103 covarying codon pairs in Vpu, displayed in Fig. 3. The covariation network in Vpu showed an uneven distribution, with a large number of codon–codon covariation networks at the N-terminal membrane-spanning region, a region responsible for BST-2 interaction [22]. Interestingly, the 3 HIV-HLA associations (Table 1, Fig. 2) were not significantly linked to any other amino acid residues. These data suggest that the conformation and function of Vpu may be preserved through many possible combinations of primary and secondary polymorphisms and that the HLA-I-associated immune-mediated selective pressure may have only a minor effect on such Vpu polymorphisms.

3.5. Association between Vpu polymorphisms and clinical parameters

Finally, we explored associations between Vpu polymorphisms and clinical parameters of HIV-infected patients (i.e., CD4 counts and plasma viral load). We observed no significant associations between Vpu polymorphisms and CD4 counts. However we identified a statistically significant association between amino acid residues at position 5 and viral load (Fig. 4). The patients harboring Val at Vpu-5 had significantly higher viral loads compared to those with amino acid residues other than Val at this position. Thus, amino acid residues at position 5 of Vpu showed several interesting features, i.e., the highest variability of all Vpu amino acids (Fig. 1A), nonadapted association of Glu-5 with *HLA-Cw*03*, and association of Val-5 with the increased viral load. Considering that the amino acid residue at this position is located in close proximity to the membrane-spanning region and that this region is functionally important for BST-2 binding, it would be interesting to examine functional effects of amino acid polymorphisms at position 5, whether they are mediated by host immune responses or otherwise.

In summary, we report here comparably fewer HLA-associated mutations in Vpu in this cohort although host HLA class I allele-associated immune responses are major forces driving the evolution of HIV-1 accessory proteins, such as Nef. Taken together, we conclude that the influence of immune selection on evolution of Vpu

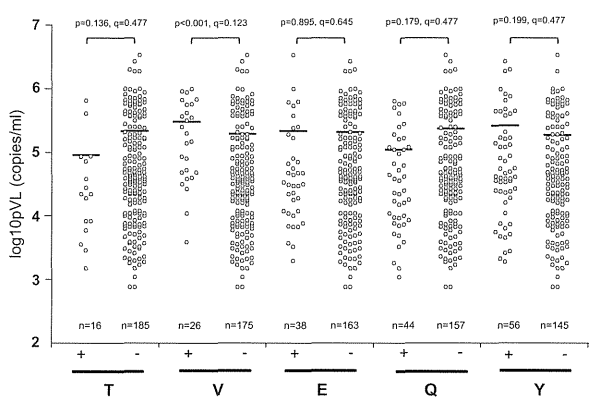

Fig. 4. Association between plasma viral load and amino acid polymorphism at position 5 of Vpu. HIV plasma viral loads, stratified by amino acid expression at Vpu codon 5, are shown. Vpu codon 5 exhibited 11 different amino acids positioning in our dataset; only those observed in >10 patients are shown here. Horizontal bars indicate medians. Statistical analysis was performed using the Mann-Whitney U-test.

at the population level may be reduced compared to other highly variable HIV-1 proteins, providing us with additional insight into differential evolutional pathways among viral accessory proteins.

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HLA Class I-Mediated Control of HIV-1 in the Japanese Population, in Which the Protective HLA-B*57 and HLA-B*27 Alleles Are Absent

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We investigated the effect of HLA class I alleles on clinical parameters for HIV-1 disease progression in the Japanese population, where two strongly protective alleles, HLA-B*57 and HLA-B*27, are virtually nonexistent. HLA-B alleles showed a dominant role, primarily through HLA-B*67:01 and the HLA-B*52:01-C*12:02 haplotype. Neither a rare-allele nor a heterozygote advantage was found, suggesting that the effect of HLA alleles in the Japanese population is either different from those observed in Africans and Caucasians or undetectable due to limited power.

The presence of particular HLA class I alleles is associated with the rate of progression to AIDS and/or with clinical markers of disease progression, such as viral load (VL) and CD4 T cell count (2, 3, 7, 11, 13). HLA-B*57 and HLA-B*27 are well-known to associate with successful control of HIV-1 or slow progression to disease in Caucasians and Africans (1, 4, 5, 9–11) but not in Asians, since the frequencies of these alleles are very low (<1%) in this population. There is growing evidence that HIV-1-specific CTL responses restricted by HLA-B allotypes play a key role in determining disease outcomes relative to HLA-A and -C (7, 9), but it remains possible that the association between HLA-B and HIV-1 control is biased by the extremely strong effects of only a few HLA-B alleles, particularly the protective HLA-B*57 and -B*27 alleles and the detrimental HLA-B*58:02 and -B*35:02/B*35:03 alleles (6–8). The exceptional protection afforded by HLA-B*57 and -B*27 may mask weaker effects of other HLA alleles, in part due to the potential for inappropriate correction for the effects of these two alleles. Since both of these alleles are virtually nonexistent (i.e., present in 0% and 0.5% of the population, respectively) in Japan (12), studies of Japanese cohorts provide an unbiased means of determining the effects of other HLA alleles on HIV control. Therefore, we analyzed the association of HLA alleles with markers of disease progression, VL and CD4 count, in Japanese individuals chronically infected with HIV-1.

We recruited 504 chronically HIV-1-infected, antiretroviral therapy (ART)-naïve Japanese individuals (464 men and 40 women) who visited our hospital during 2000-2010 and who did not meet any criteria for clinical AIDS. The median (interquartile ranges) VL and CD4 count are 35,000 (9,175/100,000) and 288 (183/402), respectively. HLA alleles of these individuals were determined at the 4-digit level. Associations between HLA alleles and VL or CD4 count obtained at the first visit were determined among these individuals. We considered 48 HLA class I alleles occurring at a frequency of greater than 1% (11 HLA-A, 22 HLA-B, and 15 HLA-C), which excluded both B*57 (0 identified) and B*27:05 (1 identified) (see Table S1 in the supplemental material). The differential contribution of HLA-A, -B, and -C alleles on VL in the cohort as a whole was determined using the Kruskal-Wallis statistic H, which is a nonparametric measure of variation between data groups (i.e., among alleles at a given locus). The range of effects (protective to susceptible) across alleles at each given class I locus on VL was largest for HLA-B ($H = 81$, $P = 0.0005$) but was also significant for both HLA-A ($H = 37$, $P = 0.006$) and HLA-C ($H = 52$, $P = 0.0001$) (Table 1). HLA-B also showed the greatest range of effects on CD4 counts, and for this outcome, it was the only locus that showed significance ($H = 71$, $P = 0.004$ for HLA-B; $H = 18$, $P = 0.43$ for HLA-A; $H = 29$, $P = 0.084$ for HLA-C). These results indicate that the HLA-B locus has the greatest effect on HIV-1 control in Japanese and confirmed previous findings in Caucasians and Africans.

We found that 36% (4 of 11) of HLA-A alleles, 41% (9 of 22) of HLA-B alleles, and 27% (4 of 15) of HLA-C alleles were associated significantly with VL before correction for multiple tests (Fig. 1). Associations with CD4 count were observed for a more limited number of HLA class I alleles than those with VL: 18% (2 of 11) for HLA-A, 18% (4 of 22) for HLA-B, and 13% (2 of 15) for HLA-C (Fig. 1). Three alleles, HLA-B*67:01, -B*52:01, and -C*12:02, were significantly associated with both low VL and high CD4 count and two alleles, HLA-A*02:07 and -B*35:01, associated with both higher VL and lower CD4 count. After local false discovery rate estimation, the numbers of HLA alleles associated with VL and CD4 count were 14 and 5, respectively (see Table S2 in the supplemental material). HLA-B*52:01 and -C*12:02 form a known haplotype, which reaches a frequency of approximately 20% of the Japanese population (12), and given the protection associated with the individual alleles, it is possible that CTL or NK cell responses restricted by this haplotype play an important role in control of HIV-1 in Japanese. HLA-B*35:01 associated with high VL and low CD4 counts, which is consistent with data from Cauca-

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TABLE 1 Kruskal-Wallis test for associations of viral load and absolute CD4 count with alleles at three HLA class I loci in Japanese individuals chronically infected with HIV-1[a]

Allele (n)	VL		CD4 cell count	
	P	H	P	H
HLA-A (504)	**0.006**	37	0.43	18
HLA-B (504)	**0.0005**	**81**	**0.004**	**71**
HLA-C (503)	**0.0001**	**52**	0.084	29

[a] Values in bold are statistically significant.

sians, in which this allele was more commonly observed among HIV noncontrollers (5). On the other hand, HLA-B*35:02 and -B*35:03 associate with rapid disease progression in Caucasians, whereas HLA-B*35:01 does not (6), highlighting distinctions across allelic associations depending on the clinical outcome being considered. HLA-A*02:07 (frequency = 0.073), an allele that is

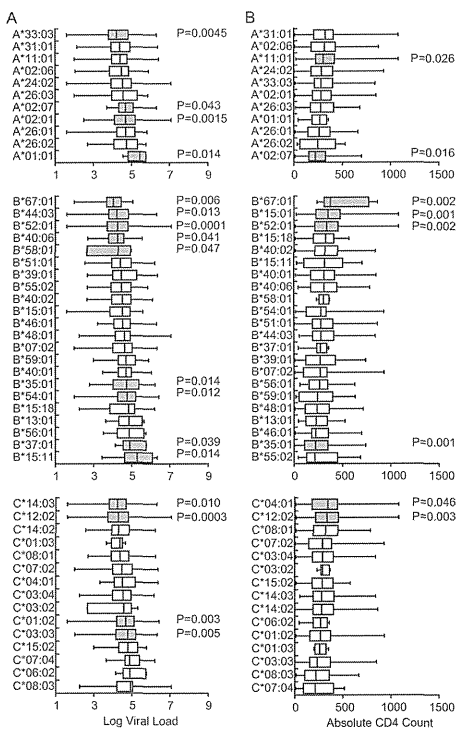

FIG 1 Association of HLA allele expression with viral load and absolute CD4 count in Japanese individuals chronically infected with HIV-1. All HLA alleles occurring at a phenotypic frequency greater than 1% were examined for their associations with viral load (A) and absolute CD4 count (B) in a cohort of 504 chronically HIV-1-infected Japanese individuals recruited from 2000 to 2010. Associations with a P value of <0.05 are highlighted in gray.

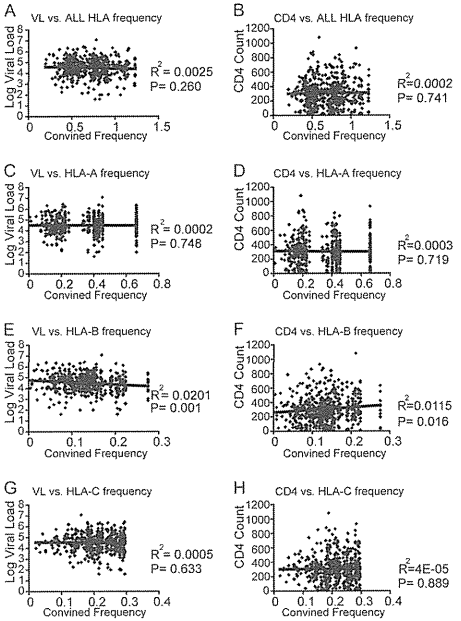

FIG 2 HLA frequency-dependent effects on viral load and CD4 counts. The correlations between VL or CD4 count with combined frequencies for all subjects (n = 504) are shown. HLA frequencies used to calculate the combined frequencies for each individual were derived from the overall allele frequencies in the entire cohort of 504 subjects. Linear regression lines are included in the plots. The distribution of HLA class I frequencies among 504 chronically HIV-1-infected Japanese individuals used in this analysis is shown in Table S4 in the supplemental material.

missing in Caucasians and Africans, also associated with susceptibility.

An advantage of rare HLA alleles in HIV-1 disease progression has been reported previously (13). Therefore, we investigated the effect of HLA frequency on VL or CD4 count in our cohort by comparing these clinical measurements first to the sum of the frequencies of the two alleles at each HLA locus individually (HLA-A, -B, and -C separately) and second to the sum of the frequencies of all HLA class I alleles (HLA-A, -B, and -C combined) for each subject. No significant correlation was observed between the overall HLA allele frequency and VL or CD4 count (Fig. 2A and B), nor was there a correlation between HLA-A allele frequencies or HLA-C allele frequencies with these clinical measurements (Fig. 2C, D, G, and H). In contrast to previous observations, the frequencies of HLA-B alleles were positively and negatively associated with CD4 count and VL, respectively (Fig. 2E and F). We also analyzed the effect of HLA supertype frequency on VL and found no effect (see Fig. S1 in the supplemental material). These results indicate no advantage of rare alleles on VL and CD4 count in the Japanese cohort. Further, we detected no significant

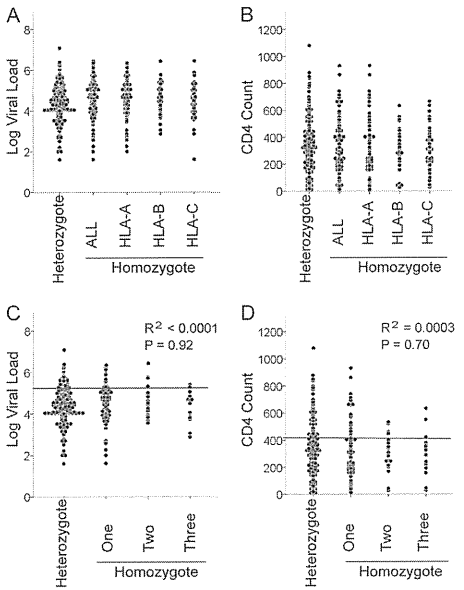

FIG 3 Effect of HLA class I homozygosity on VL and CD4. The comparison between heterozygotes at all three class I loci (n = 349) and homozygotes (n = 155) for at least one HLA allele is shown (A and B). (A and B) Homozygotes are grouped according to homozygosity for the HLA-A (n = 106), -B (n = 42), or -C (n = 64) locus. No significant difference was observed between these groups. Median VLs and CD4 counts, respectively, are 32,500 and 289 (fully heterozygous), 44,000 and 280 (HLA-A homozygous), 35,500 and 290 (HLA-B homozygous), 29,500 and 305 (HLA-C homozygous), and 42,000 and 288 (homozygous for at least one HLA locus). (C and D) Homozygotes were grouped according to homozygosity for one (n = 115), two (n = 23), or three (n = 17) HLA class I loci. Median VLs and CD4 counts, respectively, are 32,500 and 289 (fully heterozygous), 47,000 and 284 (homozygous at a single locus), 28,000 and 335 (homozygous at two loci), and 31,000 and 288 (homozygous at all three loci). No significant difference was observed between these groups. The lines in panels C and D are linear regression lines.

differences in either VL or CD4 count between heterozygotes and homozygotes at any individual HLA locus (Fig. 3A and B) or homozygosity at one, two, or all three class I loci (Fig. 3C and D). Thus, a heterozygote advantage of HLA class I was not observed in this cohort.

We also analyzed 147 ART-naïve Japanese individuals with clinical AIDS. There were no strong associations of HLA alleles with either VL or CD4 count (see Table S3 in the supplemental material). We excluded these individuals in the present study for the following reasons: (i) There were radical differences in VL and CD4 count between AIDS and non-AIDS groups, and (ii) since AIDS represents an effective breakdown of the immune response, a putative association observed in the non-AIDS group would not be comparable to that in the AIDS group even if HLA class I alleles had an effect on VL and CD4 count.

In summary, the HLA-B locus appears to have the strongest allelic effects on VL and CD4 counts in this Japanese cohort, with the HLA-B*52:01-C*12:02 haplotype showing the greatest significance. These findings in a Japanese cohort highlight the differences of the effects of HLA class I alleles on HIV-1 control between Japanese and Africans/Caucasians.

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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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(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.

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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 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

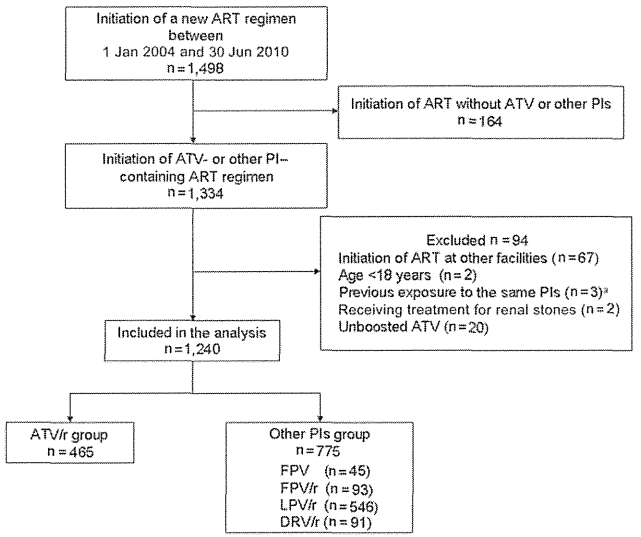

Figure 1. Flow diagram of patient selection. [a]Three patients were excluded for past lopinavir/ritonavir (LPV/r) exposure and commencement of LPV/r in the study. Abbreviations: ART, antiretroviral treatment; ATV, atazanavir; ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; FPV,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

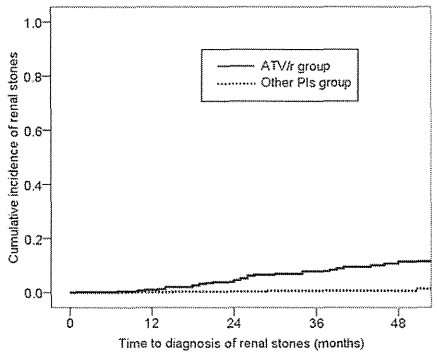

Figure 2. Kaplan-Meier curve showing time to diagnosis of renal stones. Abbreviations: ATV/r, ritonavir-boosted atazanavir; PIs, protease inhibitors.

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,

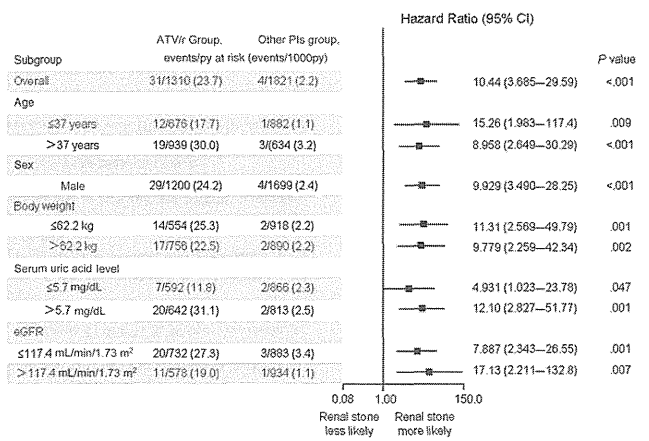

Figure 3. Estimated effect of ritonavir-boosted atazanavir, compared with other protease inhibitors on the hazard of renal stone formation, according to baseline characteristics. Abbreviations: ATV/r, ritonavir-boosted atazanavir; CI, confidence interval; eGFR, estimated glomerular filtration rate; PI,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

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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.

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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]. 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).

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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.

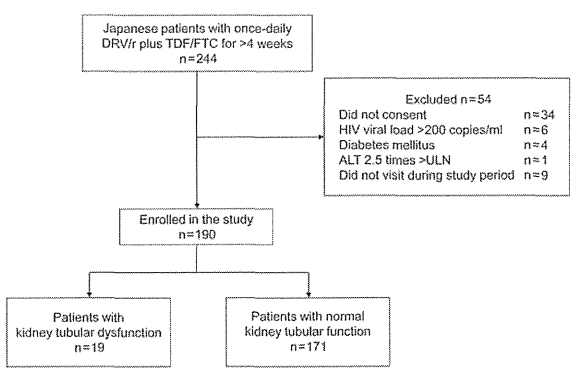

Figure 1. Patient enrollment. Abbreviations: ALT, alanine transaminase; DRV/r, ritonavir-boosted darunavir; HIV, human immunodeficiency virus; TDF/FTC, tenofovir/emtricitabine; ULN, upper limit of normal.

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-o-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)	

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