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

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

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References

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

29. Lowther SA, Dworkin MS, Hanson DL. *Entamoeba histolytica/Entamoeba dispar* infections in human immunodeficiency virus–infected patients in the United States. Clin Infect Dis. 2000;30:955–9. http://dx.doi.org/10.1086/313811
30. HIV/AIDS in Japan in 2009. Infectious Agents Surveillance Report. 2010;31:226–7.
31. Joint United Nations Program on HIV/AIDS. UNAIDS report on the global AIDS epidemic changes in the incidence rate of HIV infection, 2001 to 2009, selected countries [cited 2011 Apr 15]. http://www.unaids.org/globalreport/Global_report.htm

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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

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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.

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

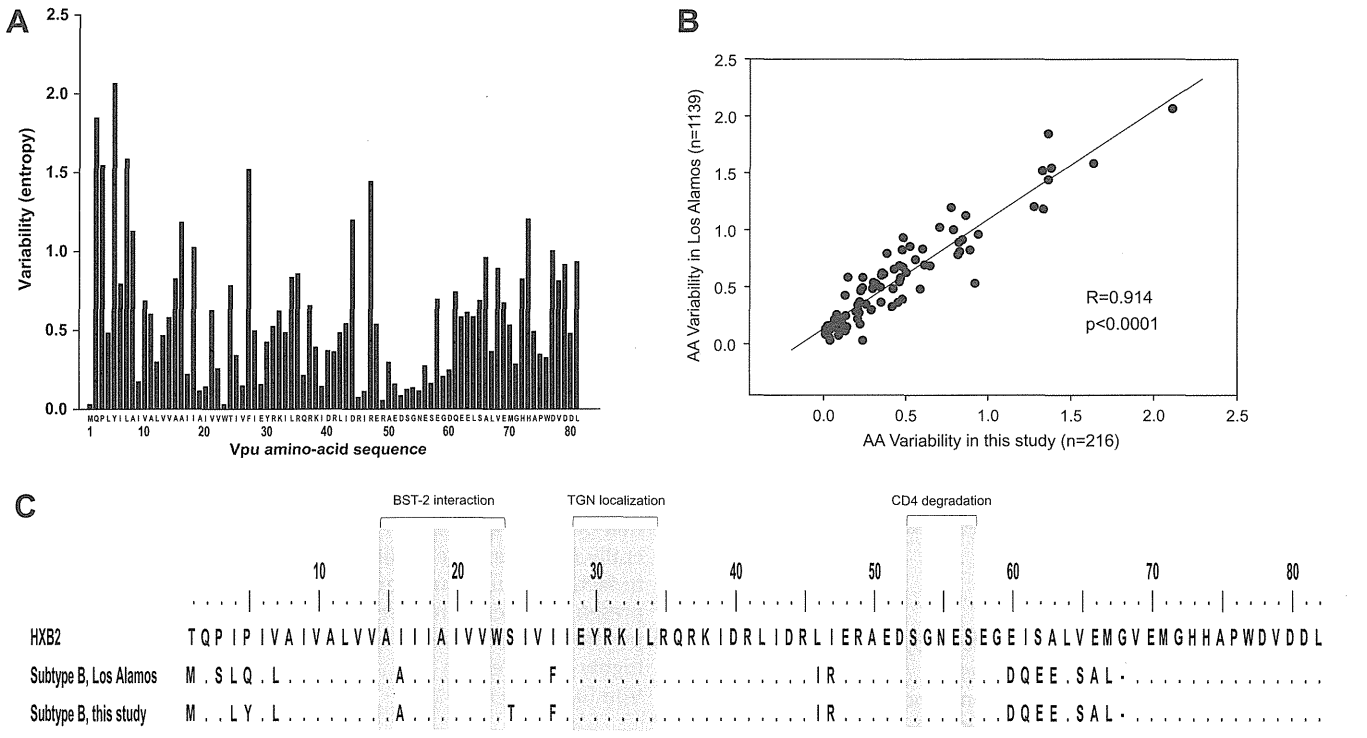

Fig. 1. Variability of the amino acid residues of HIV-1 Vpu. The amino acid sequence of Vpu was analyzed based on the cross-sectional studies on 216 HIV-infected subjects. The amino acid variability at each position of Vpu was analyzed by determining its Shannon entropy score (panel A). The Vpu sequence (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.

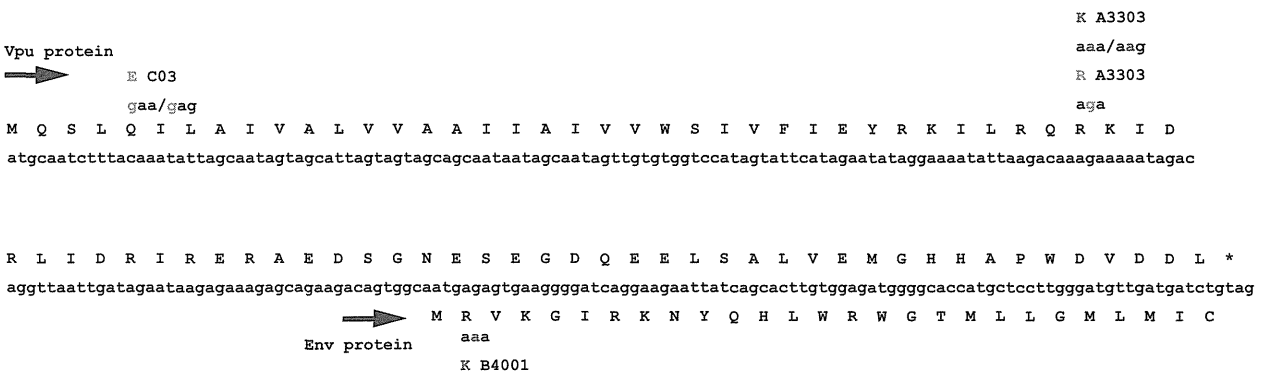

Fig. 2. The Vpu and a part of Env proteins and their associations with host HLA class I alleles. The nucleotide sequence and its deduced amino acid sequence of Vpu and of an overlapping part of Env with reference to the subtype B consensus sequence of Los Alamos database is shown. The amino acid residues 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

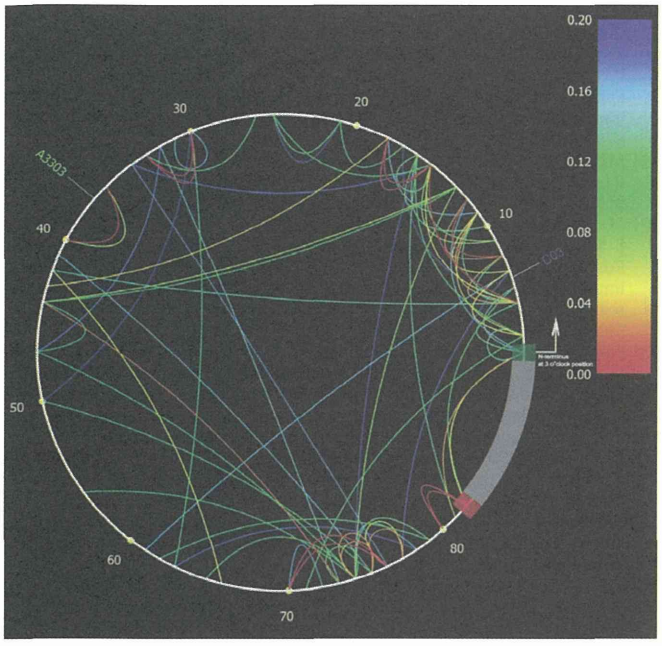

Fig. 3. Amino acid codon–codon covariation in Vpu. The circular map, generated by the PhyloDv software [13], shows Vpu codon–codon covariation associations as inner arcs connecting the association sites, with the HLA associations as tags pointing to their corresponding sites. Q values of individual codon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

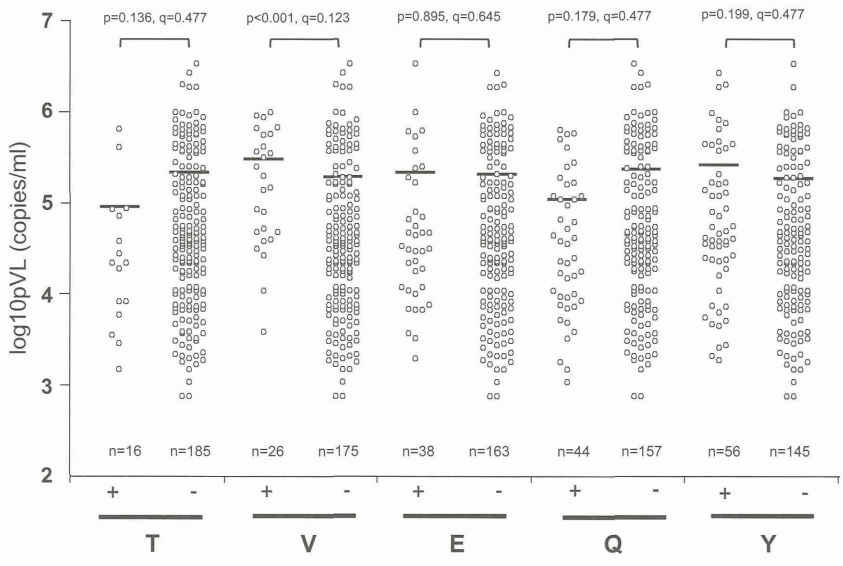

Fig. 4. Association between plasma viral load and amino acid polymorphism at position 5 of Vpu. HIV plasma viral loads, stratified by amino acid expression at Vpu codon 5, are shown. Vpu codon 5 exhibited 11 different amino acids positioning in our dataset; only those observed in >10 patients are shown 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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References

[1] P.J.R. Goulder, D.I. Watkins, HIV and SIV CTL escape: implications for vaccine design, Nat. Rev. Immunol. 4 (2004) 630–640.
[2] C. Motozono, P. Mwimanzi, T. Ueno, Dynamic interplay between viral adaptation and immune recognition during HIV-1 infection, Protein Cell 1 (2010) 514–519.
[3] T. Bhattacharya, M. Daniels, D. Heckerman, B. Foley, N. Frahm, C. Kadie, J. Carlson, K. Yusim, B. McMahon, B. Gaschen, S. Mallal, J.I. Mullins, D.C. Nickle, J. Herbeck, C. Rousseau, G.H. Learn, T. Miura, C. Brander, B. Walker, B. Korber, Founder effects in the assessment of HIV polymorphisms and HLA allele associations, Science 315 (2007) 1583–1586.
[4] Z.L. Brumme, C.J. Brumme, D. Heckerman, B.T. Korber, M. Daniels, J. Carlson, C. Kadie, T. Bhattacharya, C. Chui, J. Szinger, T. Mo, R.S. Hogg, J.S. Montaner, N. Frahm, C. Brander, B.D. Walker, P.R. Harrigan, Evidence of differential HLA class I-mediated viral evolution in functional and accessory/regulatory genes of HIV-1, PLoS Pathog. 3 (2007) e94.
[5] Z.L. Brumme, M. John, J.M. Carlson, C.J. Brumme, D. Chan, M.A. Brockman, L.C. Swenson, I. Tao, S. Szeto, P. Rosato, J. Sela, C.M. Kadie, N. Frahm, C. Brander, D.W. Haas, S.A. Riddler, R. Haubrich, B.D. Walker, P.R. Harrigan, D. Heckerman, S. Mallal, HLA-associated immune escape pathways in HIV-1 subtype B Gag Pol and Nef proteins, PLoS One 4 (2009) e6687.
[6] C.B. Moore, M. John, I.R. James, F.T. Christiansen, C.S. Witt, S.A. Mallal, Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level, Science 296 (2002) 1439–1443.
[7] C.-A.M. Almeida, S.G. Roberts, R. Laird, E. McKinnon, I. Ahmad, N.M. Keane, A. Chopra, C. Kadie, D. Heckerman, S. Mallal, M. John, Exploiting knowledge of immune selection in HIV-1 to detect HIV-specific CD8 T-cell responses, Vaccine 28 (2010) 6052–6057.
[8] M. Dube, M.G. Bego, C. Paquay, E.A. Cohen, Modulation of HIV-1-host interaction: role of the Vpu accessory protein, Retrovirology 7 (2010) 114.
[9] M. Nomaguchi, M. Fujita, A. Adachi, Role of HIV-1 Vpu protein for virus spread and pathogenesis, Microbes Infect. 10 (2008) 960–967.
[10] K. Yusim, C. Kesmir, B. Gaschen, M.M. Addo, M. Altfeld, S. Brunak, A. Chigaev, V. Detours, B.T. Korber, Clustering patterns of cytotoxic T-lymphocyte epitopes in human immunodeficiency virus type 1 (HIV-1) proteins reveal imprints of immune evasion on HIV-1 global variation, J. Virol. 76 (2002) 8757–8768.
[11] M.M. Addo, M. Altfeld, A. Rathod, M. Yu, X.G. Yu, P.J. Goulder, E.S. Rosenberg, B.D. Walker, HIV-1 Vpu represents a minor target for cytotoxic T lymphocytes in HIV-1-infection, AIDS 16 (2002) 1071–1073.
[12] M. Altfeld, M.M. Addo, R. Shankarappa, P.K. Lee, T.M. Allen, X.G. Yu, A. Rathod, J. Harlow, K. O'Sullivan, M.N. Johnston, P.J.R. Goulder, J.I. Mullins, E.S. Rosenberg, C. Brander, B. Korber, B.D. Walker, Enhanced detection of human immunodeficiency virus type 1-specific T-cell responses to highly variable regions by using peptides based on autologous virus sequences, J. Virol. 77 (2003) 7330–7340.
[13] J.M. Carlson, Z.L. Brumme, C.M. Rousseau, C.J. Brumme, P. Matthews, C. Kadie, J.I. Mullins, B.D. Walker, P.R. Harrigan, P.J. Goulder, D. Heckerman, Phylogenetic dependency networks: inferring patterns of CTL escape and codon covariation in HIV-1 Gag, PLoS Comput. Biol. 4 (2008) e1000225.
[14] Y. Itoh, N. Mizuki, T. Shimada, F. Azuma, M. Itakura, K. Kashiwase, E. Kikkawa, J.K. Kulski, M. Satake, H. Inoko, High-throughput DNA typing of HLA-A, -B, -C, and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population, Immunogenetics 57 (2005) 717–729.
[15] T. Ueno, Y. Idegami, C. Motozono, S. Oka, M. Takiguchi, Altering effects of antigenic variations in HIV-1 on antiviral effectiveness of HIV-specific CTLs, J. Immunol. 178 (2007) 5513–5523.
[16] T. Ueno, C. Motozono, S. Dohki, P. Mwimanzi, S. Rauch, O.T. Fackler, S. Oka, M. Takiguchi, CTL-mediated selective pressure influences dynamic evolution and pathogenic functions of HIV-1 Nef, J. Immunol. 180 (2008) 1107–1116.
[17] A. Bansal, J. Carlson, J. Yan, O.T. Akinsiku, M. Schaefer, S. Sabbaj, A. Bet, D.N. Levy, S. Heath, J. Tang, R.A. Kaslow, B.D. Walker, T. Ndung'u, P.J. Goulder, D. Heckerman, E. Hunter, P.A. Goepfert, CD8 T cell response and evolutionary pressure to HIV-1 cryptic epitopes derived from antisense transcription, J. Exp. Med. 207 (2010) 51–59.
[18] C.T. Berger, J.M. Carlson, C.J. Brumme, K.L. Hartman, Z.L. Brumme, L.M. Henry, P.C. Rosato, A. Piechocka-Trocha, M.A. Brockman, P.R. Harrigan, D. Heckerman, D.E. Kaufmann, C. Brander, Viral adaptation to immune selection pressure by HLA class I–restricted CTL responses targeting epitopes in HIV frameshift sequences, J. Exp. Med. 207 (2010) 61–75.
[19] D. Heckerman, C. Kadie, J. Listgarten, Leveraging information across HLA alleles/supertypes improves epitope prediction, J. Comput. Biol. 14 (2007) 736–746.
[20] K.C. Parker, M.A. Bednarek, J.E. Coligan, Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains, J. Immunol. 152 (1994) 163–175.
[21] K. Falk, O. Rotzschke, M. Takiguchi, V. Gnau, S. Stevanovic, G. Jung, H.G. Rammensee, Peptide motifs of HLA-B58, B60, B61, and B62 molecules, Immunogenetics 41 (1995) 165–168.
[22] R. Vigan, S.J. Neil, Determinants of tetherin antagonism in the transmembrane domain of the human immunodeficiency virus type 1 Vpu protein, J. Virol. 84 (2010) 12958–12970.

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

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

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

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

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

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

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

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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

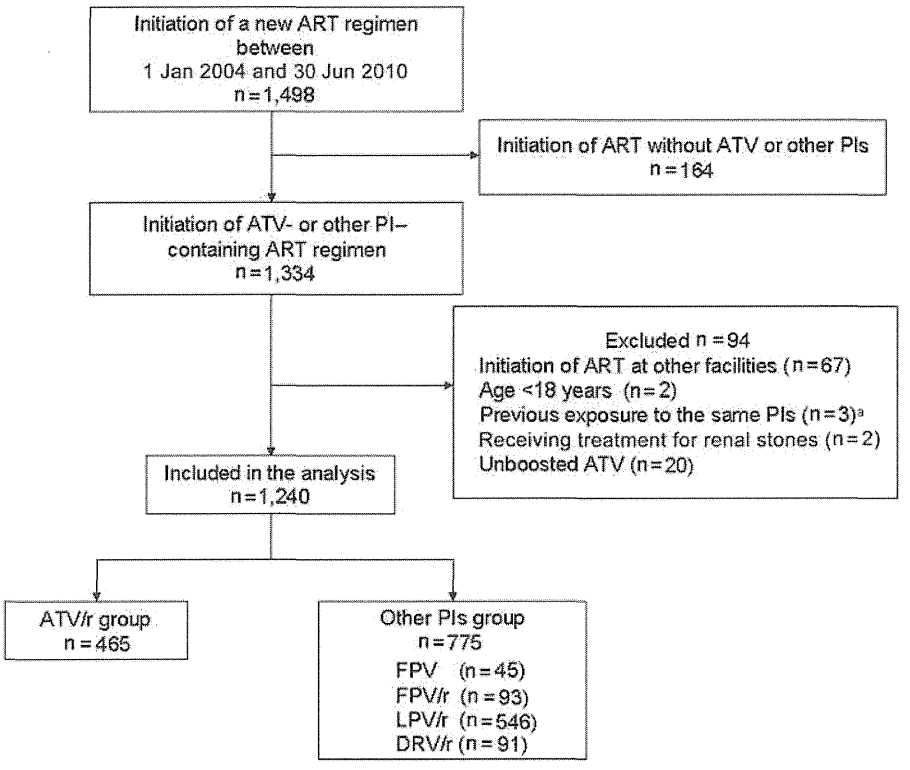

Figure 1. Flow diagram of patient selection. [a]Three patients were excluded for past lopinavir/ritonavir (LPV/r) exposure and commencement of LPV/r in the study. Abbreviations: ART, antiretroviral treatment; ATV, atazanavir; ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; FPV,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

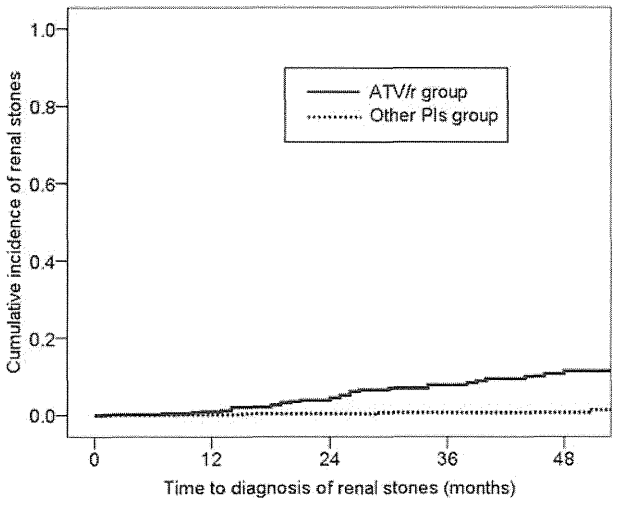

Figure 2. Kaplan-Meier curve showing time to diagnosis of renal stones. Abbreviations: ATV/r, ritonavir-boosted atazanavir; PIs, protease inhibitors.

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,

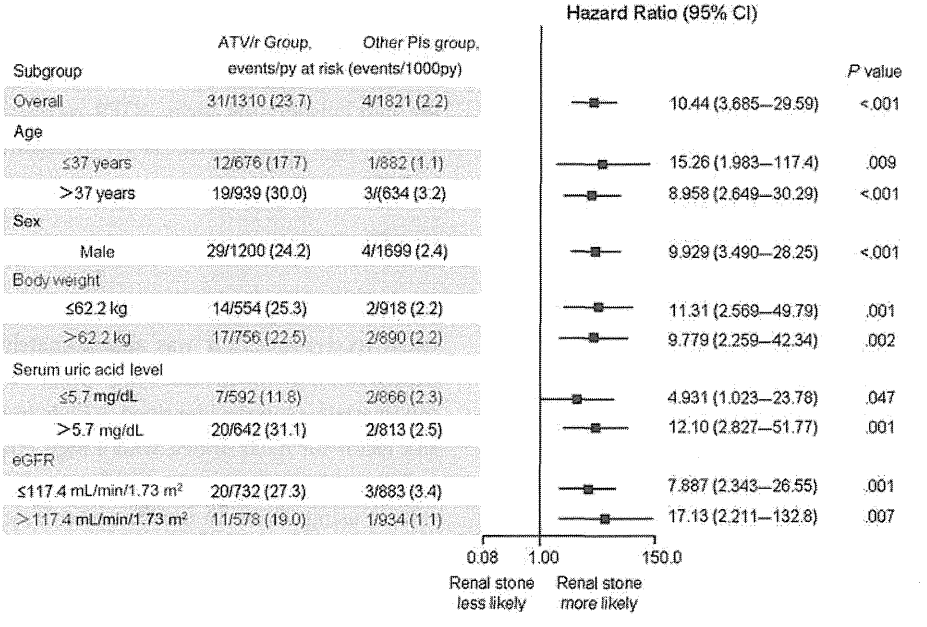

Figure 3. Estimated effect of ritonavir-boosted atazanavir, compared with other protease inhibitors on the hazard of renal stone formation, according to baseline characteristics. Abbreviations: ATV/r, ritonavir-boosted atazanavir; CI, confidence interval; eGFR, estimated glomerular filtration rate; PI, 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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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

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

5. Chang HR, Pella PM. Atazanavir urolithiasis. N Engl J Med **2006**; 355:2158–9.
6. Anderson PL, Lichtenstein KA, Gerig NE, Kiser JJ, Bushman LR. Atazanavir-containing renal calculi in an HIV-infected patient. AIDS **2007**; 21:1060–2.
7. Nadler RB, Rubenstein JN, Eggener SE, Loor MM, Smith ND. The etiology of urolithiasis in HIV infected patients. J Urol **2003**; 169:475–7.
8. Chan-Tack KM, Truffa MM, Struble KA, Birnkrant DB. Atazanavir-associated nephrolithiasis: cases from the US Food and drug administration's adverse event reporting system. AIDS **2007**; 21:1215–8.
9. Couzigou C, Daudon M, Meynard JL, et al. Urolithiasis in HIV-positive patients treated with atazanavir. Clin Infect Dis **2007**; 45:e105–8.
10. Rule AD, Bergstralh EJ, Melton LJ 3rd, Li X, Weaver AL, Lieske JC. Kidney stones and the risk for chronic kidney disease. Clin J Am Soc Nephrol **2009**; 4:804–11.
11. Jungers P, Joly D, Barbey F, Choukroun G, Daudon M. ESRD caused by nephrolithiasis: prevalence, mechanisms, and prevention. Am J Kidney Dis **2004**; 44:799–805.
12. Gupta SK, Eustace JA, Winston JA, et al. Guidelines for the management of chronic kidney disease in HIV-infected patients: recommendations of the HIV medicine association of the infectious diseases society of America. Clin Infect Dis **2005**; 40:1559–85.
13. The Japanese Ministry of Health, Labour, and Welfare. Guidelines for antiretroviral treatment for patients with HIV infection [in Japanese]. Available at: http://www.haart-support.jp/guideline2011.pdf. Accessed 3 April 2012.
14. Parmar MS. Kidney stones. BMJ **2004**; 328:1420–4.
15. Levey AS, Coresh J, Greene T, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med **2006**; 145:247–54.
16. Rockwood N, Mandalia S, Bower M, Gazzard B, Nelson M. Ritonavir-boosted atazanavir exposure is associated with an increased rate of renal stones compared with efavirenz, ritonavir-boosted lopinavir and ritonavir-boosted darunavir. AIDS **2011**; 25:1671–3.
17. Mocroft A, Kirk O, Reiss P, et al. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. AIDS **2010**; 24:1667–78.
18. Albini L, Cesana BM, Motta D, et al. A randomized, pilot trial to evaluate glomerular filtration rate by creatinine or cystatin C in naive HIV-infected patients after tenofovir/emtricitabine in combination with atazanavir/ritonavir or efavirenz. J Acquir Immune Defic Syndr **2012**; 59:18–30.
19. Young J, Schafer J, Fux CA, et al. Renal function in patients with HIV starting therapy with tenofovir and either efavirenz, lopinavir or atazanavir. AIDS **2012**; 26:567–75.
20. Engeler DS, John H, Rentsch KM, Ruef C, Oertle D, Suter S. Nelfinavir urinary stones. J Urol **2002**; 167:1384–5.
21. Feicke A, Rentsch KM, Oertle D, Strebel RT. Same patient, new stone composition: amprenavir urinary stone. Antivir Ther **2008**; 13:733–4.
22. Doco-Lecompte T, Garrec A, Thomas L, Trechot P, May T, Rabaud C. Lopinavir-ritonavir (Kaletra) and lithiasis: seven cases. AIDS **2004**; 18:705–6.
23. Kaletra (lopinavir/ritonavir): full prescription information [package insert]. Barcelona, PR: Abbott Pharmaceuticals PR, **2012**.
24. Reyataz (atazanavir sulfate): full prescription information [package insert]. Princeton, NJ: Bristol-Myers Squibb, **2012**.
25. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. JAMA **1997**; 277:145–53.
26. Rodriguez Novoa S, Barreiro P, Rendon A, et al. Plasma levels of atazanavir and the risk of hyperbilirubinemia are predicted by the 3435C → T polymorphism at the multidrug resistance gene 1. Clin Infect Dis **2006**; 42:291–5.

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].

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

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

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

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

METHODS

Ethics Statement

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

Study Design

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

Study Subjects

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

Measurements

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

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

Definition of Renal Proximal Tubular Dysfunction

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

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

Genetic Polymorphisms

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

Pharmacogenetic Analyses

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

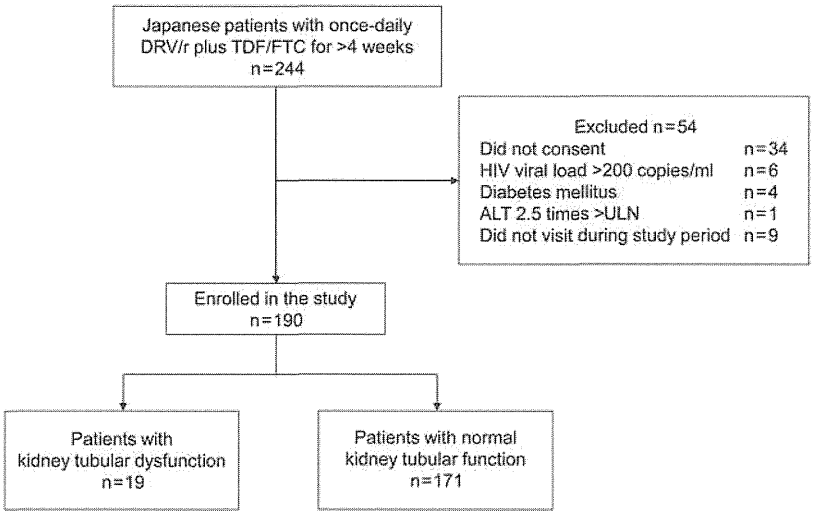

Figure 1. Patient enrollment. Abbreviations: ALT, alanine transaminase; DRV/r, ritonavir-boosted darunavir; HIV, human immunodeficiency virus; TDF/FTC, tenofovir/emtricitabine; ULN, upper limit of normal.

Statistical Analysis

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

RESULTS

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

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

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

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

[a] Median (interquartile range).

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

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