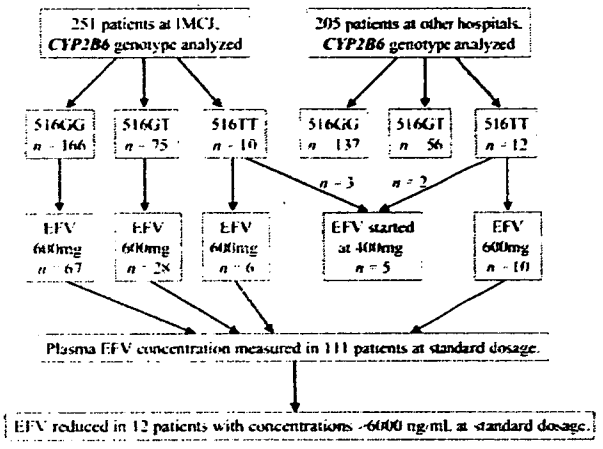

Figure 2. Flow diagram of study participants. The *CYP2B6* genotype was analyzed in 251 patients at the International Medical Center of Japan (IMCJ) and in 205 patients at other hospitals. Standard dosage of EFV was administered in 101 patients at IMCJ, including sixty-seven *CYP2B6* 516GG, twenty-eight 516GT, and six 516TT holders, whose EFV concentrations were measured. Ten 516TT holders at the other hospitals were administered standard dosages of EFV, and their EFV concentrations were also measured. A reduced-dose (400 mg) regimen of EFV was initiated in 5 other 516TT holders.

the EFV dose was reduced from 600 to 400 mg in 11 subjects and was further reduced to 200 mg in 7 of them who consented to further reduction. The plasma EFV concentrations decreased by approximately one-third (36%–46%), to 3720–6160 ng/mL, with dose reduction from 600 to 400 mg in 10 of 11 subjects, and further decreased by approximately one-half (51%–59%), to 1620–2960 ng/mL, with reduction from 400 to 200 mg in 6 of 7 subjects (figure 4). In one patient who had a markedly high EFV concentration (14,690 ng/mL) at the standard 600-mg dose, however, the concentration decreased unexpectedly by 69%, to 4500 ng/mL, with the reduction to 400 mg and further decreased by 82%, to 790 ng/mL, lower than the recommended range (>1000 ng/mL) [1], with the reduction from 400 to 200 mg. Therefore, the dose was increased in this patient back to 400 mg. In another patient who had reported severe dizziness during treatment with the standard dose (600 mg), the dose was reduced immediately to 200 mg at the patient's request. The plasma EFV concentration was also markedly high in this patient (14,360 ng/mL) during treatment with the standard dosage. However, it decreased by 83%, to 2410 ng/mL, with the dose reduction to 200 mg. Consequently, the final EFV dose was 400 mg in 5 subjects and 200 mg in 7 subjects. The determined dosage for each patient was continued for >6 months (the longest was 26 months for a patient who received the 200-mg dose), and the plasma HIV-1 load was continuously undetectable in all patients.

EFV initiation at 400-mg dose. Our analysis showed that *CYP2B6* **6/*6* and **6/*26* carriers had extremely high EFV concentrations, without exception (figure 3), and that dose reduction was possible in patients with high EFV concentration with retention of therapeutically effective anti–HIV-1 activity (figure 4). In the next phase of our study, we used an antiretroviral regimen containing a reduced dose (400 mg) of EFV in 5 EFV-naive patients (four **6/*6* homozygotes and one **6/*26* heterozygote). Before the introduction of low-dose EFV-containing regimen, the plasma HIV-1 loads had been undetectable during receipt of the previous protease inhibitor–containing regimen in all 5 patients. Their EFV concentrations were 4080–9450 ng/mL, and all such concentrations (95% CI, 99.5%–100%) were therapeutically adequate (>1000 ng/mL) at the 400-mg dose (figure 5). One **6/*6* homozygote developed severe dizziness, necessitating discontinuation of EFV-treatment at day 16. His EFV concentration was 5430 ng/mL. In one **6/*26* heterozygote, severe thrombocytopenia emerged, probably because of overdosage of rifabutin prescribed for the treatment of coinfection with *Mycobacterium intracellulare*, and EFV treatment was stopped at day 15. The EFV concentration was 5770 ng/mL. Two of the remaining 3 patients still had extremely high EFV concentrations (6760 and 9450 ng/mL) at the 400-mg dose, and their dose was subsequently reduced to 200 mg. The plasma EFV concentrations decreased to 2690 and 3660 ng/mL (i.e., by 60% and 61%, respectively). Consequently, 2 subjects

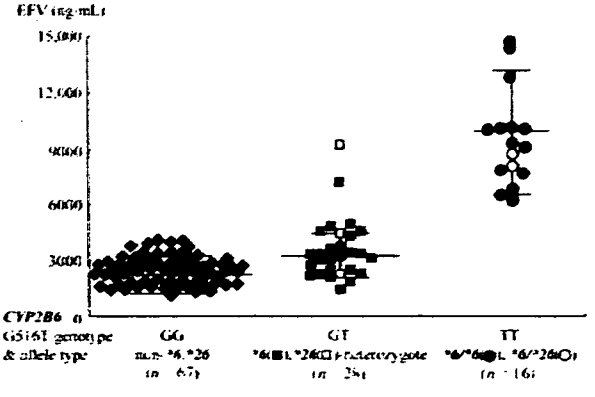

Figure 3. Plasma efavirenz (EFV) concentrations measured during EFV treatment with standard dose (600 mg). A total of 111 HIV-1–infected patients treated with EFV-containing regimens were divided into 3 groups on the basis of nucleotide genotype at *CYP2B6* position 516 (GG, GT, or TT), and their plasma EFV concentrations were compared. Blackened squares, **6* heterozygote with allele other than **26*; unblackened squares, *CYP2B6* 499C→G carriers (**26* heterozygote with allele other than **6*); blackened circles, **6* homozygote (**6/*6*); unblackened circles, *CYP2B6* 499C→G carriers (**6/*26* heterozygotes); blackened diamonds, other genotype carriers. Horizontal lines represent the mean (± SD) plasma EFV concentrations for each group.

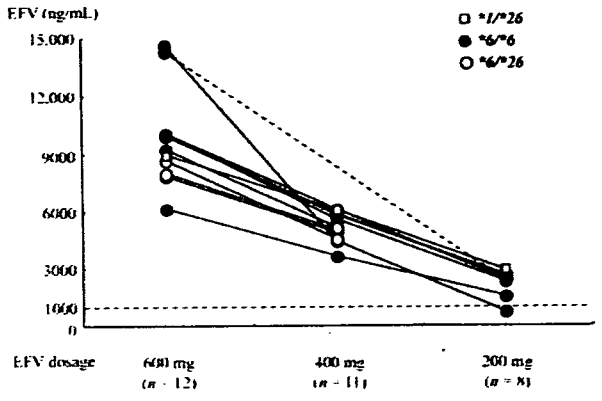

Figure 4. Dose reduction of efavirenz (EFV) in 12 patients whose concentrations were extremely high while receiving treatment with standard dose (600 mg). EFV dose was reduced from 600 to 400 mg in 11 patients and was further reduced, to 200 mg, in 7 patients. In one patient who had severe CNS symptoms while receiving treatment with standard dose, EFV dose was directly reduced to 200 mg (concentrations connected with a dotted line). The suggested minimum target concentration (1000 ng/mL) is indicated by the thin line.

discontinued the EFV-containing regimen, and 3 subjects continued low-dose EFV-containing regimen (400 mg for 1 patient and 200 mg for 2 patients). The low-dose regimen was continued for >6 months, and the plasma HIV-1 load was persistently undetectable in all 3 patients.

Improvement of CNS symptoms. As described above, the EFV dose was reduced from 600 to 400 and 200 mg as the final dose in 5 and 7 subjects, respectively (figure 4), and it was decreased from 400 mg as the initial dose to 200 mg for 2 other subjects (figure 5). To delineate the changes in CNS symptoms associated with the decrease in EFV concentration, a questionnaire survey of these 14 patients was conducted regarding 6 items: dizziness, strange dreams, depression, irritability, concentration problems, and sleep difficulty. More than 1 month after the dose had been reduced to the lowest dose, the patients were asked to judge the 6 CNS symptoms above at initial and final doses, with use of a 5-grade system ("none," "slight," "sometimes," "often," and "always"). Ten (71%) of the 14 patients had some of the aforementioned CNS symptoms during treatment with the initial dose (table 2). The most common symptom was dizziness (57%), followed by strange dreams (50%). Interestingly, all the symptoms improved after dose reduction in the 10 patients. Furthermore, dizziness and concentration problems disappeared during treatment with the final dose in one-half of the patients, although strange dreams and sleep difficulty were still reported by all the patients who had those difficulties at the initial dose. Finally, when the patients were asked whether they wanted to reincrease EFV to the previous dose, all 10 patients with CNS symptoms at the initial dose answered "no" (9 answered "absolutely no").

DISCUSSION

In this study, we identified a novel *CYP2B6* allele, **26*, which includes 499C→G, 516G→T, and 785A→G in 12 Japanese patients, and we showed that, without exception, all **6/*6* and **6/*26* carriers, all holding 516TT, had extremely high plasma EFV concentrations while receiving the standard dose (600 mg) [4]. In other reports, however, there were some exceptional subjects with 516TT who had normal concentrations of EFV, and the discreteness of the EFV concentration with the position 516 genotype was not as clear as it was in our patients [5–8]. This difference may be because some of the 516TT carriers had other *CYP2B6* alleles, such as **7* (containing 516G→T, 785A→G, and 1459C→T), **9* (containing 516G→T only), and **13* (containing 415A→G, 516G→T, and 785A→G). Those alleles could not be found in our subjects, and their effects on EFV concentration were not well described. Because numerous additional *CYP2B6* variants with impact on expression and/or function were recently reported [12–18], correct determination of *CYP2B6* haplotype seems indispensable for prediction of EFV plasma levels.

We reduced the EFV dose in 12 patients whose plasma EFV concentrations were extremely high while receiving the standard dose, and we initiated EFV treatment at a 400-mg dose in 5 EFV-naive **6/*6* and **6/*26* carriers. In most patients, the plasma EFV concentration decreased proportionally with the dose-reduction ratio. In 2 subjects, however, the concentrations decreased much more than expected, given the dose reduction

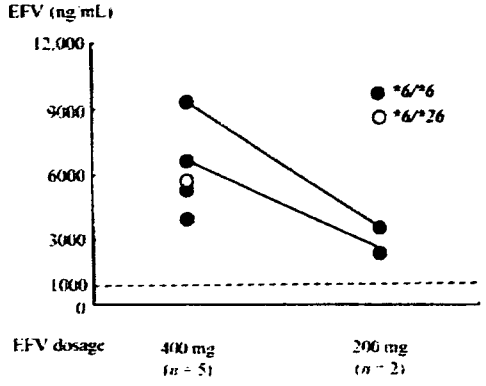

Figure 5. Introduction of low-dose efavirenz (EFV)-containing antiretroviral regimen to *CYP2B6 *6/*6* and **6/*26* carriers. Treatment was started in 4 EFV-naive carriers *CYP2B6 *6/*6* and one **6/*26* carrier, with 400-mg EFV-containing regimens. EFV dose was further reduced, to 200 mg, in 2 patients whose EFV concentrations were >6000 ng/mL while receiving treatment with the 400-mg dose.

Table 2. Changes in CNS-related symptoms after reduction of efavirenz dosage.

Symptom	No. (%) of subjects who reported symptom status during efavirenz treatment		
	Present[a] ($n=14$)	Improved[b]	Disappeared[b]
Dizziness	8 (57)	8 (100)	4 (50)
Strange dreams	7 (50)[c]	7 (100)[c]	0 (0)
Depression	5 (36)	5 (100)	1 (20)
Irritability	5 (36)	5 (100)	1 (20)
Concentration problem	4 (29)	4 (100)	2 (50)
Sleep difficulty	3 (21)	3 (100)	0 (0)
Any of the above	10 (71)[c]	10 (100)[c]	4 (40)

[a] Including the 4 grades "slight," "sometimes," "often," and "always" at the initial dosage. Includes 2 patients whose efavirenz treatment was originally 400 mg and was reduced to 200 mg.
[b] Percentage of those who initially reported "present."
[c] Including 1 patient whose efavirenz dose was originally 400 mg and was reduced to 200 mg.

ratio. Both of these patients had markedly high concentrations at standard dosage. Hasse et al. [19] reported a patient with excessively high plasma EFV concentration at standard dose, which decreased to one-thirtieth following dose reduction from 600 to 200 mg. Long-term exposure to such excessively high concentrations may induce CYP2B6 enzymatic expression in the liver, which could result in an unexpectedly large decrease in plasma EFV concentration by dose reduction if deinduction of the enzyme takes several weeks. At the 400-mg dose, the plasma concentrations of EFV were therapeutically adequate in all the treated **6/*6* and **6/*26* carriers in this study. Regarding the reduced dose, it is noteworthy that a phase II study during EFV development supported the use of a lower dose [20]. The same study indicated that the 600-mg dose of EFV is associated with a high rate of adverse events that could lead to discontinuation, which suggests that the lower dose of 400 mg may be almost as effective without the high discontinuation rate. In the present study, associated with the dose-reduction regimen, a significant number of patients experienced improvement of CNS symptoms, which was unexpected on the basis of previous reports [5, 21, 22]. Interestingly, some of these patients did not appreciate their clinical state and considered themselves to have no CNS-related symptoms during the standard-dose treatment. However, after the dose reduction, they reassessed the status and evaluated symptoms during the treatment with the standard dose as associated with CNS symptoms and indicated that the reduced dose of EFV relieved them of such symptoms. Because EFV-treated patients often stick to the regimen, previous reports of symptom questionnaires conducted during the standard treatment might have underestimated the EFV-associated CNS symptoms [5, 21, 22]. However, this finding might be confounded by placebo effect, because the patients were told that their EFV levels were high while receiving the initial dose and decreased throughout the dose-reduction protocol. Because of this possible placebo effect, a double-blind, placebo-controlled study would best address this question.

EFV dose reduction and initiation of EFV treatment at reduced dose is possible with therapeutic anti–HIV-1 potency retained in *CYP2B6 *6/*6* homozygotes and **6/*26* heterozygotes, which could relieve the patients of the EFV-associated CNS symptoms. It may also decrease the risk of development of EFV-resistant HIV-1 after mandatory treatment discontinuation, such as abdominal surgery [23], and reduce the treatment cost, an important issue in developing countries [24]. After dose reduction, however, careful monitoring is necessary until larger studies confirm the safety of reduced dose in such specific genotype carriers.

Acknowledgments

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Potential conflicts of interest. All authors: no conflicts.

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Drug-resistant HIV-1 prevalence in patients newly diagnosed with HIV/AIDS in Japan☆

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Abstract

The increasing prevalence of drug-resistant HIV transmission has become a critical epidemic in the world today. Studies in developed countries reported 8–27% of newly diagnosed HIV/AIDS patients are infected by drug-resistant strains. To determine the prevalence of drug-resistant HIV-1 among newly diagnosed cases in Japan, eight HIV/AIDS clinical centers, three public health laboratories and the National Institute of Infectious Diseases conducted a nationwide survey. Between January 2003 and December 2004, 575 newly diagnosed HIV/AIDS patients with both acute and chronic infections were enrolled in the study. Twenty-three cases, including three recently infected patients, were infected with HIV-1 having major drug-resistance mutations, including M41L, D67N, L100I, K103N, V106A, M184I, M184V, L210W, and revertant mutations at the 215 codon in reverse transcriptase and M461 in protease encoding regions. In this newly diagnosed population, we also clarified the prevalence of hepatitis virus coinfection, which was 8.8% for HBV and 4.3% for HCV. In conclusion, the drug-resistant transmission rate was 4.0% in Japan. Although this rate is significantly lower than that of other developed countries, this rate almost reaches the threshold at which baseline genotypic resistance testing would be cost-effective for all infected persons before initiating therapy.

Keywords: HIV-1; Drug resistance; Newly infected; Japan

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1. Introduction

The prognosis for patients infected with HIV/AIDS has improved dramatically in the last decade due to the introduction of highly active antiretroviral therapy (HAART). However, the active use of antiretroviral agents has opened the door for HIV-1 to escape and evolve resistance to these agents (Richman, 2001). Patients who develop drug resistance have limited treatment alternatives and usually have poor therapeutic responses. Therefore, successful treatment of these patients requires preventing resistance mutations and suppressing the replication of drug-resistant viral populations. Despite considerable effort to overcome drug resistance to HIV-1, the prevalence of infected patients that cannot be treated because of drug resistance is still quite high (Richman et al., 2004). The increasing number of drug-resistant cases in patients exposed to antiretroviral drugs has raised the risk of new infections by drug-resistant viral strains. Indeed, studies from the US and European countries have reported that 8 to 27% of newly diagnosed HIV/AIDS patients are infected by drug-resistant strains (Barbour et al., 2004; Boden et al., 1999; Chaix et al., 2003; Descamps et al., 2005; Jayaraman et al., 2006; Little et al., 2002; Novak et al., 2005; Perno et al., 2002; Romano et al., 2000; Simon et al., 2002; UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance, 2001; Weinstock et al., 2004). This situation must be monitored and controlled, as patients infected with drug-resistant HIV-1 have weaker responses to the initial antiretroviral treatment and significantly shorter times to the first virological failure than patients infected with wild-type HIV-1 (Little et al., 2002). Therefore, evaluation of drug resistance before initiating antiretroviral treatment has become beneficial to successful treatment (Vandamme et al., 2004).

In Japan, the choice of available antiretroviral drugs is mostly equal to that of the USA and EU countries, except that T20 and tipranavir have not been currently approved. Furthermore, the prevalence of drug resistance in Japan is estimated to be 30–50% in populations exposed to antiretroviral drugs or unsuccessful treatment (Sugiura, 2001). In the population of newly diagnosed cases of HIV/AIDS in Japan, the prevalence of drug resistance has been reported as 17% (Ibe et al., 2003). However, the data in that study were based on a limited sample from one hospital and may not represent the overall status of drug resistance transmission in the country. To monitor the nationwide prevalence of drug resistance in newly diagnosed patients, we have established a multi-center network to surveil drug-resistant HIV-1. Here, we report our summary of prevalence results for 2003 and 2004.

2. Materials and methods

2.1. Study design and patient sample

Eight AIDS clinical centers, three public health laboratories and the National Institute of Infectious Diseases (NIID) were involved in surveillance of newly diagnosed HIV/AIDS cases. HIV/AIDS patients with both acute and chronic infections, newly diagnosed at these centers from January 2003 to December 2004, were enrolled in the study. Among those enrolled, cases with an obvious record or Western blotting evidence of seroconversion within 1 year were grouped as a recently infected sub-sample (Hachiya et al., 2004). Patient information collected included age, sex, risk behavior, date of seropositivity, estimated time of infection, viral load, CD4 positive cell count, and complications.

According to Japanese law for infection control, doctors are obligated to report newly diagnosed HIV/AIDS cases to the Committee on HIV/AIDS Trends (the Ministry of Health, Labor, and Welfare of the Japanese government). The 1375 HIV/AIDS cases registered by this committee in 2003 and 2004 were used as a control population to evaluate the representativeness of the patients enrolled in our study. The demographics of both patient groups were compared. Statistical analyses were performed using StatView software (SAS Institute).

A multiple logistic regression model was used to determine the demographic and disease-related factors associated with drug resistance. Age, sex, race (Japanese versus others), risk behavior for HIV-1 transmission (men who have sex with men [MSM] versus heterosexual), CD4 cell count (as a continuous variable), HIV-1 load (as a continuous variable, log-transformed), recent infection or not, hepatitis B virus (HBV) coinfection, hepatitis C virus (HCV) coinfection, and HIV-1 subtype (B versus non-B) were included in the multiple logistic regression model.

2.2. Analysis of drug-resistance genotype and determination of drug-resistance mutations

Drug resistance genotyping was carried out by in-house genotypic protocols. In brief, viral RNA extracted from 200 μl plasma was reverse transcribed, and whole HIV-1 protease (99 amino acids) and the N-terminal half of HIV-1 reverse transcriptase (RT, 240 amino acids) were amplified by nested PCR. Subsequently, cycle sequence reactions were performed by Big-dye terminator (Applied BioSystem), and the products were analyzed in a direct sequencing manner by an auto-sequencer apparatus. To capture the maximum possible number of cases in which resistance was transmitted, in cases where wild-type and resistance mutations were mixed at drug resistance mutation loci, resistance mutations were preferentially counted. In addition, when a mixture of multiple resistance mutations was suspected, the most predominant mutation (as judged from the peak height of the electropherogram) was counted. Major drug-resistance mutations were defined as those which meet both the criteria of the International AIDS Society (IAS)-USA (Johnson et al., 2006) and Stanford HIV Drug Resistance Database (Shafer et al., 2006). According to both criteria, cysteine (C), aspartic acid (D), glutamic acid (E), isoleucine (I), asparagine (N), serine (S) and valine (V) substitutions at codon 215 in RT were considered revertants of F or Y and recognized as signatures of previous resistance; cases with these mutations were counted as having transmitted major drug resistance (Garcia-Lerma et al., 2001; Violin et al., 2004). Therefore, the following mutations were counted as major resistance mutations: M41L, K65R, D67N, T69insert, K70R, L74V, F77L, L100I, K103N, V106A/M, Y115F, F116Y, Q151M, Y181C/I, M184I/V, Y188C/H/L,

G190A/S, L210W, T215F/Y/C/D/E/I/N/S/V, K219E/Q, P225H, P236L in RT, and D30N, V32I, M46I, I47A/V, G48V, I50L/V, I54M/L, V82A/F/L/T/S, I84A/C/V, L90M in protease. Minor resistance mutations in protease listed in the 2005 version of the ISA-USA table (L10F/I/R/V, K20I/L/M/R/T, L24I, L33F/I, M36I/L/V, M46L, F53L, I54A/S/T/V, L63P, A71T/V, V77I, N88D/S) (Johnson et al., 2005) were counted as minor resistance mutations, because this version was the latest when the data were collected from each center.

The viral sub-type for each case was determined from the HIV-1 protease-RT sequence by the neighbor-joining method using the Genetic-Mac system (Software Development, Tokyo).

3. Results

3.1. Demographics of newly diagnosed HIV/AIDS cases in 2003 and 2004

During the study period, 575 newly diagnosed HIV/AIDS cases (267 in 2003 and 308 in 2004) were enrolled in the study (the study sample). This sample had the following demographic characteristics: median age was 34 years old (quartile range = 29–43), 521 males and 54 females, and 508 Japanese and 67 others (Table 1). To evaluate the representativeness of our sample, it was compared with the population of 1375 patients registered with the Committee on HIV/AIDS Trends in Japan (the registered population). Differences were examined for significance using Fisher's exact test and the Mann–Whitney U-test. A p value <0.05 denoted statistical significance. As shown in Table 1, significant differences were observed only in risk behaviors, and the proportion of MSM was larger in our sample than in the registered population. However, these differences may be due to the different definitions and classifications of the category "Other" used by the Committee on HIV/AIDS Trends in Japan and our study. In the registered population, cases with more than one suspected risk behavior were classified as "Other", whereas, in our study those cases were classified by the most likely transmission route, MSM. Thus, we conclude that our study sample well represented the registered patient population (Table 1).

Among the 575 cases in our sample, 45 patients (7.8%) had evidence of recent seroconversion and were classified as recently infected cases. These cases were significantly different from other cases in age, risk behavior, viral load and CD4-positive cell count (Table 2). Recently infected cases were younger, included more MSM, and had higher viral loads and CD4 cell counts. The higher viral load in these recently infected cases suggests that they were still in the acute phase of infection. The greater prevalence of MSM and their younger age indicates that HIV-1 infection is spreading mainly in the younger MSM population in Japan.

The study sample had 477 sub-type B cases and 97 non-B sub-types. Among the sub-type B-infected patients, significantly more were male, Japanese, MSM (for men with identified risk), and their CD4 cell count was significantly higher than for the non-B sub-type-infected patients. All recently infected patients were infected with sub-type B.

Coinfection with hepatitis viruses is a critical complication of HIV infection. Therefore, we also determined the status of HBV or HCV coinfection in our study sample. The HBs antigen was positive in 8.8% of 353 patients, and HCV antibody was detected in 4.3% of 352 patients. Interestingly, HBs antigen-positive patients had significantly lower CD4-positive cell counts than HBs antigen-negative patients (173.2 ± 30.6 versus 271.5 ± 12.9, $p < 0.05$). In HCV-coinfected cases, no significant difference was found in CD4-positive cell counts between HCV antibody-positive and -negative patients.

To understand possible risk factors for transmission of HIV-1 drug resistance, multiple logistic regression model analyses were performed. Because our sample included few patients infected by drug injection ($n = 1$) or mother-to-child transmission ($n = 2$), these cases were excluded from the multiple logistic regression analysis. The prevalence of major resistance muta-

Table 1
Demographics of the study sample and registered population

Characteristic	Study sample ($N = 575$)	Registered population[a] ($N = 1375$)	p
Age in years, median (quartile range)	34 (29–43)	30–39[b]	
Male (%)	521 (90.6)	1231 (89.5)	0.51
Race (%)			
Japanese	508 (88.3)	1198 (87.1)	0.50
Other	67 (11.7)	177 (12.9)	
Risk behavior[c] (%)			
MSM[d]	383 (78.5)	795 (72.3)	0.01
Heterosexual	149 (27.7)	372 (30.7)	0.23
Injection drug	1 (0.19)	6 (0.50)	0.68
MTCT[e]	2 (0.37)	2 (0.17)	0.59
Other[f]	2 (0.37)	37 (3.1)	0.001

[a] Patients registered with the Committee on HIV/AIDS Trends in Japan.
[b] Age is given only as a 10-year range by the Committee on HIV/AIDS Trends in Japan. Median range is shown.
[c] Risk behaviors were identified in 537 study patients and in 1212 registered patients.
[d] Men who have sex with men. Percentage is for men with identified risks only.
[e] Mother-to-child transmission.
[f] Includes cases infected by transfusion of HIV-1-contaminated blood products and cases with more than one suspected route.

Table 2
Demographics of the study sample by infection status and HIV-1 subtype

Characteristics	All patients ($N=575$)	Infection status		p	HIV-1 sub-type[b]		p
		Recent[a] ($n=45$)	Other ($n=530$)		B ($n=477$)	Non-B[c] ($n=97$)	
Age (years)[d]	34 (29–43)	32 (28.5–37.5)	35 (29–44)	0.02	34 (29–43)	37 (29–47.75)	0.07
Male (%)	521 (90.6)	43 (95.6)	478 (90.2)	0.29	460 (96.4)	61 (62.9)	$<10^{-4}$
Japanese (%)	508 (88.3)	42 (93.3)	466 (87.9)	0.34	439 (92.0)	68 (70.1)	$<10^{-4}$
MSM[e]	383 (78.5)	38 (88.4)	345 (72.2)	0.01	370 (80.4)	13 (21.3)	$<10^{-4}$
CD4 (cells/μl)	217 (62–401)	370 (242–511.75)	195.5 (53–390.5)	$<10^{-4}$	239 (67.75–401.25)	145 (14.5–379.25)	0.009
HIV load[f]	4.82 (4.30–5.38)	5.32 (4.58–5.73)	4.81 (4.28–5.34)	0.001	4.81 (4.28–5.41)	4.85 (4.40–5.32)	0.62
Coinfection							
HBV[g]	31 (8.8%)	1 (4.8%)	30 (9.0%)	>0.99	27 (8.9)	4 (8.5)	>0.99
HCV[h]	15 (4.3%)	0 (0%)	15 (4.5%)	>0.99	12 (3.9)	3 (6.4)	0.43

[a] Infected within 1 year as determined by recent seroconversion or Western blot analysis.
[b] In one patient, HIV-1 could not be sub-typed because of negative PCR for both RT and protease encoding regions.
[c] Includes 71 patients with sub-type AE, 11 patients with sub-type C, 8 patients with sub-type A, 4 patients with sub-type G, 1 patient with sub-type AG, 1 patient with sub-type D, and 1 patient with sub-type F.
[d] Median (quartile range) is shown.
[e] Men who have sex with men. Percentage for men with identified risk only.
[f] Logarithmic median (quartile range) is shown.
[g] Hepatitis B virus S antigen was analyzed in 21 recently infected patients and 332 others (305 sub-type B-infected, 47 non-B sub-type-infected, and 1 unsub-typed-HIV-1-infected patients).
[h] Hepatitis C virus antibody was analyzed in 21 recently infected patients and 331 others (304 sub-type B-infected, 47 non-B sub-type-infected, and 1 unsub-typed-HIV-1-infected patients).

tions did not differ by age, sex, race, risk behavior, CD4 cell count, HIV-1 RNA viral load, HBV infection, HCV infection, or HIV-1 sub-type.

3.2. Prevalence of mutations for drug resistance in newly diagnosed HIV/AIDS cases in 2003 and 2004

Among all 575 cases, HIV-1 protease and RT regions were successfully sequenced in 570 and 572 patients, respectively. In the analyses summarized in Table 3, 23 cases (4.0%) had at least one major resistance mutation. Of these, 22 cases were infected with sub-type B, and one case harboring T215S in RT was found to be sub-type A. When the prevalence of transmitted resistance was categorized by drug class, 16 (2.8%) patients had major resistance mutations to nucleoside RT inhibitors (NRTI), 4 (0.7%) had resistance mutations to non-nucleoside RT inhibitors (NNRTsI), and 4 (0.7%) had major resistance mutations to protease inhibitors (PIs).

A more detailed examination of the study sample's patterns of major resistance mutations (Table 3) shows that for NRTI resistance, mutations at codon 215 were the most frequently observed (12 patients, 2.1%). However, these mutations did not include phenylalanine (F) or tyrosine (Y), known to be due to AZT resistance, but were aspartic acid (D), glutamic acid (E), and serine (S), which are suspected reverted mutations of F or Y.

Regarding the lamivudine resistance mutations, M184V/I, five cases possessed these mutations. However, two patients were coinfected with HBV and had been exposed to lamivudine before the study. Therefore, these cases were excluded from the final determination of prevalence of transmitted drug resistance even though no evidence indicated that M184V/I in these two cases had not been transmitted but selected by HBV treatment.

Table 3
Prevalence of major resistance mutations in newly diagnosed HIV/AIDS patients from 2003 to 2004 ($N=575$)

Mutation	n	%
Any (NRTI, NNRTI, PI)[a]	23[b]	4.0
NRTI		
Any	16[c]	2.8
M41L	4	0.7
D67N	1	0.2
M184I	1[d]	0.2
M184V	2[d]	0.3
L210W	2	0.3
T215D	9[c]	1.6
T215E	1[c]	0.2
T215S	2	0.3
NNRTI		
Any	4	0.7
L100I	1	0.2
K103N	2[c,f]	0.3
V106A	1	0.2
PI		
M46I	4	0.7

Only observed mutations are shown.
[a] NRTI = nucleoside RT inhibitor, NNRTI = non-nucleoside RT inhibitor, PI = protease inhibitor.
[b] Includes one patient infected with HIV-1 sub-type A harboring T215S in RT and 22 patients infected with HIV-1 sub-type B.
[c] Includes two patients with multiple NRTI resistance mutations (M41L, D67N, M184V, L210W, T215D, and M41L, L210W, T215D).
[d] Five cases had an M184I/V mutation, but two were excluded from this table, because, the patients had been treated with lamivudine for HBV infections.
[e] Includes one recently infected patient.
[f] Both were reported from the same hospital.

Table 4
HIV-1 sub-types and prevalence of minor mutations in protease in newly diagnosed HIV/AIDS cases from 2003 to 2004

Mutation	All patients[a] ($N=570$)	Sub-type B ($n=475$)	Non-B ($n=95$)	p
Any minor mutation	426 (74.7)	332 (69.9)	94 (98.9)	$<10^{-4}$
L10F	2 (0.4)	2 (0.4)	0 (0)	>0.99
L10I	49 (8.6)	37 (7.8)	12 (12.6)	0.16
L10V	12 (2.1)	8 (1.7)	4 (4.2)	0.12
K20I	13 (2.3)	2 (0.4)	11 (11.6)	$<10^{-4}$
K20R	19 (3.3)	7 (1.5)	12 (12.6)	$<10^{-4}$
L24I	1 (0.2)	1 (0.2)	0 (0)	>0.99
L33F	2 (0.4)	1 (0.2)	1 (1.1)	0.31
L33I	3 (0.5)	3 (0.6)	0 (0)	>0.99
M36I	160 (28.1)	76 (16.0)	84 (88.4)	$<10^{-4}$
M36L	1 (0.2)	0 (0)	1 (1.1)	>0.99
M36V	1 (0.2)	0 (0)	1 (1.1)	>0.99
M46L	1 (0.2)	1 (0.2)	0 (0)	>0.99
L63P	244 (42.8)	212 (44.6)	32 (33.7)	0.05
A71T	45 (7.9)	45 (9.5)	0 (0)	0.0003
A71V	39 (6.8)	38 (8.0)	1 (1.1)	0.012
V77I	170 (29.8)	161 (33.9)	9 (9.5)	$<10^{-4}$

Only observed mutations are shown.
[a] Five patients were excluded because of negative PCR for the protease gene.

If these two cases had been included in the analysis, the overall prevalence of transmitted drug-resistant cases would have been 4.3%.

NNRTI resistance and PI resistance were less frequently transmitted in the study sample. The most frequent NNRTI resistance mutation was K103N (0.3%), and the only PI resistance mutation found was M46I in four cases (0.7%).

Most of the cases analyzed in the study had only one resistance mutation, but three patients had multiple mutations. Two cases had multiple NRTI resistance (M41L, D67N, M184V, L210W, T215D, and M41L, L210W, T215D), and one case had NRTI (M184V) and NNRTI (L100I) resistance mutations. No multiple major NNRTI or PI resistance mutation holders were found in this study.

Three recently infected patients were carrying one resistance mutation in RT: T215D, T215E, or K103N. However, the frequency of major resistance mutations did not differ significantly between the 45 recently infected patients and the remaining 530 patients, and between patients enrolled in 2003 and in 2004, suggesting that transmission cases of resistant HIV-1 were not increasing during the study period.

3.3. Prevalence of minor PI resistance mutations and their significance in different sub-types

The prevalence of minor PI resistance mutations in our study sample is summarized in Table 4. Of 570 patients, 426 (74.7%) had at least one minor resistance mutations. Among the minor mutations found, the most frequently observed was L63P in protease (42.8%). Multiple minor PI mutations were observed in 247 patients (43.3%), most of which were probably natural polymorphisms. The major PI resistance mutation M46I seen in four patients was accompanied by at least one minor mutation, suggesting that these accompanying minors contributed to the PI resistance and increased viral fitness (Johnson et al., 2006).

Considering sub-type, non-B sub-type viruses had significantly more minor PI resistance mutations than sub-type B viruses (Table 4). The different sub-types also demonstrated significant differences in minor mutation patterns. Non-B sub-types had a higher prevalence of L10I/V, K20I/R, and M36I mutations, whereas, sub-type B had a higher prevalence of L63P, A71T/V, and V77I than non-B sub-types.

The frequency of minor PI resistance mutations did not differ significantly between years 2003 and 2004. Furthermore, no difference was observed between recently infected patients and other patients.

4. Discussion

This study provides the first nationwide description of the prevalence of drug-resistant HIV-1 among newly diagnosed HIV/AIDS patients in Japan. Between 2003 and 2004, the overall prevalence rate of infection with major drug-resistant HIV-1 mutations was 4.0% in Japan, which is significantly lower than in developed countries in Europe and North America (UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance, 2001; Weinstock et al., 2004). This low prevalence of drug-resistant HIV transmission is noteworthy, as Japan and other developed countries share a nearly identical history of antiretroviral treatment and number of available antiretrovirals for HIV/AIDS. In addition, the incidence of HIV/AIDS itself is significantly lower in Japan than in Europe and North America, but similar to rates in Korea and the Philippines. Although we cannot yet explain the low prevalence of drug-resistance transmission, we suspect that it may result from differences in sexual culture and risk behaviors, as frequency of injection drug user was low among HIV/AIDS patients in Japan (Table 1). Injection drug use is recognized as a risk factor of poor adherence to antiretroviral treatment (Ammassari et al., 2004; Moss et al., 2004; Palepu et al., 2004), resulting in the development of drug-resistant HIV-1. Another possible explanation is that a threshold

incidence of HIV/AIDS must be reached in a population before survey methods can detect transmission of drug resistance in newly infected cases.

Most major resistance mutations were found in sub-type B, probably because sub-type B prevails in developed countries where antiretroviral agents have been used for longer than 10 years. Individuals infected with sub-type B and non-B sub-type HIV-1 had significantly different demographic characteristics. Most sub-type B-infected patients were Japanese males and many had sex with men, whereas more than one-third of non-B sub-type-infected patients were female and around 30% were foreigners, including Africans and non-Japanese Asians (Table 2). A significant portion of non-B sub-type-infected patients in Japan may have difficulty accessing medical care, so that they do not visit hospitals until they have recognizable symptoms. Such a phenomenon would explain the lower CD4 cell count observed in this study in non-B sub-type-infected patients compared to that of sub-type B-infected patients. All recently infected patients were infected with sub-type B, which suggests that sub-type B infections may be actively occurring in Japan, while non-B sub-types may be carried by patients already infected from overseas rather than spreading domestically.

The most prevalent major resistance mutations in this study were in the NRTI class (Table 3). This finding is not surprising, since the median CD4-positive cell count of 217 cells/μl indicates that many patients had established HIV-1 infections approximately 7–8 years before their diagnosis (CASCADE Collaboration, 2003), when NNRTIs and PIs were not yet commercially available. NRTIs have been available since the late 1980s, and it would be expected that the longer exposure to these drugs would lead to a higher prevalence of resistance mutations.

We found significantly different patterns of minor PI-resistance mutations in individuals infected with sub-type B and non-B sub-type strains. K20I/R and M36I mutations were more frequently identified in non-B sub-type-infected individuals than in sub-type B-infected patients, consistent with previous reports (Ariyoshi et al., 2003; Snoeck et al., 2006). Considering that certain drug-resistance mutations found in one sub-type can often be detected as natural polymorphisms in other sub-types (Cornelissen et al., 1997; Quinones-Mateu et al., 1998), sub-type identification and polymorphism information are critical for accurately interpreting genotypic resistance assays.

Our study also revealed an epidemic of HIV and hepatitis virus coinfection in Japan. The frequency of HBV coinfection in our study sample (8.8%) was similar to that of the US and EU countries (6–14%) (Alter, 2006; Brook et al., 2003; Kellerman et al., 2003; Novak et al., 2005; Strader, 2005). HBV chronic infection has been prevalent in Asia, including Japan. The main route of HBV infection has been mother-to-child transmission, with the HBV genotype C as the most commonly observed genotype in Japan. Interestingly, the HBV sub-type found with HIV-1 infections was mainly genotype A (Shibayama et al., 2005), the type more common in the US and Europe, and thus, clearly distinct from the genotype traditionally found in Japan (Orito et al., 2001). In addition, the trend in HBV genotype is changing in Japan, with more HBV genotype A-infected cases being found, regardless of HIV-1 coinfection (Kobayashi et al., 2004). This trend indicates a recent increase in HBV transmission from foreign countries. In our study sample, HBs antigen-positive patients had lower CD4 cell counts than antigen-negative patients, suggesting that HIV-1-induced immunodeficiency may be a risk factor for developing chronicity after acute HBV infection (Gatanaga et al., 2000; Puoti et al., 2006).

In contrast to our findings with HBV, HCV coinfection was less frequent in our study sample (4.3%) than in the US and EU countries (25–30%). One explanation for the low HCV prevalence in our study sample may be that intravenous drug use known to be the main route of HCV infection (Alter, 2006; Strader, 2005), is less common in Japan (Table 1). In addition to clarifying the epidemic status of HBV coinfection, our study results highlight the importance of considering antiretroviral treatment when starting lamivudine treatment for HBV. It should be noted that two newly diagnosed patients with M184I/V were on lamivudine treatment for HBV infection not combined with other antiretroviral agents. This approach is not recommended, because, lamivudine easily induces M184I/V in HIV-1 RT and compromises subsequent anti-HIV-1 treatment (Brook et al., 2003; Puoti et al., 2006). To avoid this problem, HBV-infected patients should be screened for HIV infection (Aberg et al., 2004), which has not routinely been performed in Japan.

Although the 4% transmission rate is significantly lower than that of other developed countries, this rate almost reaches the threshold at which baseline genotypic resistance testing would be cost-effective for all infected persons before initiating therapy (Weinstein et al., 2001). In Japan, health insurance has recently started to cover genotypic resistance assays only to guide the treatment of patients experiencing virological treatment failure. This policy may be shortsighted, however, considering the possible increase in resistant HIV-1 transmission among treatment-naïve patients. Thus, we recommend that this population should be also covered by health insurance.

The prevalence of drug-resistant HIV-1 in Japan was reported to increase from 4.7–6.7% (1999–2001) to 17.1% in 2002 (Ibe et al., 2003), suggesting a rapid spread of drug-resistant HIV-1. However, that study counted as major resistance mutations the RT mutations E44D and V118I, which have been excluded from the latest version of the IAS-USA mutation table. These mutations were not counted in our study, because, they can be considered as natural polymorphisms (Romano et al., 2002; Walter et al., 2002; Weinstock et al., 2004). When these polymorphic mutations were excluded from the data of Ibe et al. the resistance mutation prevalence was 7.3% in 2002, suggesting a gradual increase in their local region rather than a rapid spread of drug-resistant HIV-1. In our study, we did not see clear regional outbreaks of certain drug-resistant HIV-1 infections, except two cases with K103N were reported from the same hospital.

The data and information provided by our study are valuable for understanding the latest epidemiological features and developing models of HIV/AIDS transmission. For these purposes, continued surveillance is needed to predict future outbreaks of transmitted drug resistance.

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