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REVIEW

Pharmacogenetic information derived from analysis of *HLA* alleles

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A large amount of pharmacogenetic information has, in particular, accumulated on the association between human leukocyte antigen (*HLA*) alleles and hypersensitivity to certain drugs. Prospective *HLA* typing has dramatically reduced the risk of abacavir hypersensitivity because of its strong association with *HLA-B*5701*. Significant predisposition to nevirapine hypersensitivity has been reported in Caucasian Australians harboring *HLA-DRB1*0101* with high $CD4^+$ T-cell counts, and Sardinians and Japanese harboring *HLA-Cw8*. A strong association between carbamazepine hypersensitivity and *HLA-B*1502* has been reported in Han Chinese. Most Han Chinese individuals with allopurinol-induced severe cutaneous adverse reactions are positive for *HLA-B*5801*. *HLA* typing can stratify risk of hypersensitivity to certain drugs and allow personalized treatment, although the patients should be monitored closely even if they are negative for *HLA* alleles associated with hypersensitivity.

Hypersensitivity reactions can occur with most drugs, although their frequency, severity and clinical manifestations vary. They commonly involve the skin and mucosal surfaces, and in severe cases can result in Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Other severe hypersensitivity reactions can affect other organs such as the liver (hepatitis), lung (pneumonitis) and digestive system (gastrointestinal bleeding), and show more generalized symptoms [1]. Rechallenge with the same drugs usually induces more severe reactions, even fatal reactions in some cases, suggesting that hypersensitivity reactions are immunological memory responses after sensitization. These reactions affect only a minority of patients taking the drug. However, hereditary forms of severe drug hypersensitivity and cases occurring in identical twins have been reported, implying the involvement of certain genetic factors in predisposing individuals to such hypersensitivity reactions [2,3]. Given the immunological basis of their mechanisms, it is not surprising that the associations between human leukocyte antigen (*HLA*) alleles and hypersensitivity to some drugs have been reported during the past decade. HLA is a key molecule in T-cell-mediated immune reactions. It presents antigens (usually eight or nine peptide residues) to T-cell receptors (TCRs), thereby selecting antigen-specific T cells and initiating immune responses. Such reactions usually occur in viral and bacterial infections, and microbe-derived peptides restricted by host HLA are targeted by antigen-specific immune responses [4]. Since drugs and their metabolites are small chemical compounds, they do not usually trigger immune reactions by themselves. However, they may conjugate or bind to intracellular proteins, where they are presented as antigens or haptens by MHC class I or class II molecules to $CD8^+$ or $CD4^+$ T cells, resulting in activation of drug-specific T cells [5,6].

We will review in this article the recent literature on the association between *HLA* allele and hypersensitivity reactions to abacavir, nevirapine, carbamazepine and allopurinol. We will also discuss the clinical implications of such associations, with a special focus on the association of *HLA-B*5701* with hypersensitivity to abacavir, an anti-HIV-1 agent, because it is the most well analyzed and reported. Widespread genetic screening of such association in HIV-1-infected individuals can be used to prevent hypersensitivity reactions.

Abacavir hypersensitivity & *HLA-B*5701*

The currently recommended anti-HIV-1 treatment is the use of a combination regimen. The initial regimen for treatment-naive infected individuals should contain two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) and either a non-nucleoside reverse transcriptase (NNRTI) or an HIV protease inhibitor [7,10]. The action of the NRTI drug class is to inhibit viral replication through competitive inhibition of viral RNA-dependent DNA polymerase (reverse transcriptase) that allows the creation of a nascent DNA sequence from its own RNA template, whereas NNRTI drugs function by direct binding and inactivation of the polymerase. HIV protease

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inhibitors prevent the cleavage of the Gag protein and Gag–Pol protein precursors, thus inhibiting viral replication at a later stage in the replication cycle [8]. NRTIs have been prescribed since the late 1980s, and their advantages and disadvantages are well recognized. A major adverse effect of NRTI is mitochondrial toxicity, which can result in life-threatening lactic acidosis [9–11]. Two recently developed NRTIs, tenofovir disoproxil fumarate (TDF) and abacavir, have low mitochondrial toxicity and both can be prescribed with once-daily dosing [12,13]. However, only TDF is listed as a preferred NRTI in the guideline of the Department of Health and Human Services. On the other hand, abacavir is listed as an alternative NRTI because of its potential for serious hypersensitivity reactions in 5–8% of Caucasians [14,101].

The safety data for abacavir are well described and based on approximately 200,000 patients who received abacavir in clinical trials. The most important limitation to continuous use of this drug is hypersensitivity reactions [15,16]. Such reactions are multi-organ clinical syndromes, which generally occur within the first 6 weeks of abacavir treatment, and typically present with fever, skin rash, malaise/fatigue, gastrointestinal symptoms (e.g., nausea, vomiting and diarrhea) and/or respiratory symptoms (e.g., dyspnea, cough and pharyngitis) [15]. It is important to make a correct diagnosis of abacavir-related hypersensitivity reactions, since a rechallenge with abacavir after an initial reaction can evoke a more rapid reappearance of more severe symptoms within hours of re-exposure, which could result in death in some cases [17–19]. Unfortunately, abacavir hypersensitivity reactions are sometimes difficult to distinguish from systemic viral illness or similar drug reactions caused by other concurrently administered antiretroviral drugs or antibiotics [20].

Meta-analysis of clinical trials indicating a low risk of abacavir hypersensitivity reactions in black people, as well as a case report of familial hypersensitivity, are strong indicators of a genetic basis of this idiosyncratic syndrome [21,22]. Two independent studies identified a strong association between abacavir hypersensitivity and *HLA-B*5701*, which can assist clinicians in predicting those individuals who could develop hypersensitivity reactions and to make a correct diagnosis of hypersensitivity reactions in abacavir-treated individuals, although the association was observed only in Caucasians but not in the black people originally (**Table 1**) [23,24]. In addition to *HLA-B*5701*, the possession of *HLA-DR7* and *HLA-DQ3*, which are markers of the 57.1 ancestral haplotype, is associated with an increase in the odds ratio of hypersensitivity risk, suggesting that another causative genetic region is linked to *HLA-B*5701* [23]. Fine recombinant genetic mapping has identified a significant linkage disequilibrium of the haplotypic M493T polymorphism of heat shock protein-Hom (Hsp70-Hom; Hsp1AL) and *HLA-B*5701* in abacavir hypersensitive cases, which simplified and enhanced the discrimination of hypersensitive subjects from tolerant controls when compared with the *HLA-B*5701* test alone (**Table 1**) [25]. The Hsp70-Hom M493T polymorphism may facilitate loading of abacavir- or its metabolite-haptenated endogenous peptides onto *HLA-B*5701* [26]. High intracellular and extracellular levels of TNF are

Table 1. Drug hypersensitivity and associated *HLA* alleles.

Study	Drug	HLA	Population	OR	Pc	Ref.
Mallal *et al.* (2002)	Abacavir	*B*5701*	Australian	117	$<10^{-4}$	[23]
Hetherington *et al.* (2002)	Abacavir	*B*5701*	British	24	$<10^{-4}$	[24]
Martin *et al.* (2004)	Abacavir	*B*5701*	Australian	960	$<10^{-4}$	[25]
Martin *et al.* (2005)	Nevirapine	*DRB1*0101* and high CD4	Caucasian Australian	18	0.0006	[58]
Littera *et al.* (2006)	Nevirapine	*Cw8-B14(65)*‡	Sardinian	15	0.05	[59]
Gatanaga *et al.* (2007)	Nevirapine	*Cw8*	Japanese	6.2	0.03	[60]
Chung *et al.* (2004)	Carbamazepine	*B*1502*	Han Chinese	2504	$<10^{-4}$	[68]
Hung *et al.* (2006)	Carbamazepine	*B*1502*	Han Chinese	1357	$<10^{-4}$	[69]
Hung *et al.* (2005)	Allopurinol	*B*5801*	Han Chinese	580	$<10^{-4}$	[75]

‡Cw*0802 *and* B*1402 *are in strong linkage equilibrium in Sardinians.*

present in abacavir-stimulated peripheral blood mononuclear cells (PBMCs) of abacavir-hypersensitive patients, relative to those of abacavir-tolerant individuals, and depletion of $CD8^+$ T cells results in reduction of TNF levels [25]. Considering that marked infiltration of $CD8^+$ T cells is observed in cutaneous abacavir patch testing of hypersensitive patients and that higher $CD8^+$ T-cell count is a risk factor of hypersensitivity reactions, *HLA-B*5701*-restricted $CD8^+$ T cells must play a major pathogenic role in abacavir hypersensitivity reactions [27–29].

Prospective *HLA-B*5701* genetic screening has been instituted in clinical practice in Western Australia, the UK and Paris for abacavir-naive patients, and this had markedly reduced the risk of developing abacavir hypersensitivity (Table 2) [30–32]. This strategy unexpectedly reduced the proportion of patients who stopped their treatment after the appearance of symptoms that were otherwise unrelated to hypersensitivity reactions, suggesting that genetic screening seems to prevent overestimation of hypersensitivity reactions with subsequent discontinuation of abacavir in *HLA-B*5701*-negative individuals [30,32]. The PREDICT-1 study randomized patients either to receive abacavir according to standard of care or to be prospectively screened for *HLA-B*5701* before starting abacavir (to exclude *HLA-B*5701* carriers) [33]. The incidence of hypersensitivity reactions was significantly lower in the prospective screening arm compared with the control arm. However, most of the screened patients described above were Caucasian, and the utility and cost–effectiveness of the genetic screening largely depends on the prevalence of *HLA-B*5701* in the targeted population [34]. The prevalence of *HLA-B*5701* among Hispanics and black people is lower than Caucasians, and the relationship between *HLA-B*5701* and abacavir hypersensitivity was described as weak in Hispanics and nonexistent in black patients [35,36]. The SHAPE study corroborated the low rate of abacavir hypersensitivity immunologically confirmed by skin patch testing in black patients, but it also reported high sensitivity of *HLA-B*5701* in immunologically validated cases in both whites and blacks, suggesting the importance of supplementing a clinical definition of abacavir hypersensitivity by immunological assessment [37]. In our study, none of the 669 Japanese HIV-1-infected patients had *HLA-B*5701*, yet hypersensitivity reactions occurred in seven (all *HLA-B*5701*-negative, not immunologically confirmed) of 536 Japanese patients exposed to abacavir [38]. Thus, genetic screening of *HLA-B*5701* does not seem cost-effective in Japanese populations. Close monitoring of patients after abacavir prescription without HLA typing may be a more reasonable approach in the populations that do not carry *HLA-B*5701*.

Interestingly, strong reponses of *HLA-B*57*-restricted cytotoxic T lymphocytes can ocur against multiple HIV-1 epitopes, which is considered to result in slow disease progression of *HLA-B*57*-positive HIV-1-infected individuals [39,40]. One of the major *HLA-B*57*-restricted epitopes is located in codons 244–252 of HIV-1 reverse transcriptase, which is routinely sequenced as a part of drug-resistance testing [7,41,101]. Furthermore, cytotoxic T lymphocytes escape mutations (wild-type V to E, M and L) are commonly observed at codon 245 in *HLA-B*57*-positive patients, which may serve as an indirect marker for the presence of *HLA-B*5701* [40,42]. In one study [43], the negative predictive value was over 99% (meaning that the presence of wild-type amino acid V at codon 245

Table 2. Reduced frequencies of abacavir hypersensitivity reactions after *HLA-B*5701* genetic screening.

Study	Country	n (%)‡		p-value	Ref.
		Before screening	*After screening*		
Rauch *et al.* (2006)	Australia	16/199 (8.0)	3§/151 (2.0)	0.01	[30]
Reeves *et al.* (2006)	UK	20/321 (6.2)	1¶/155 (0.6)	0.002	[31]
Zucman *et al.* (2007)	France	11#/49 (22.4)	0/128 (0)	$<10^{-4}$	[32]

‡*Number (%) of hypersensitive patients/abacavir-treated patients.*

§*All three individuals were* HLA-B*5701 *positive; two inadvertently exposed to abacavir because of a lack of review of HLA results, and one on the basis of his own content.*

¶HLA-B*5701 *negative; non-HIV-expert physician discontinued therapy because of possible hypersensitivity reactions.*

#*Included five* HLA-B*5701 *negative cases of possible hypersensitivity based on wide-range clinical criteria.*

excludes the possibility of *HLA-B*5701* in >99% of cases), while the positive predictive value was low (20%). These results suggest that abacavir can be safely prescribed to most HIV-1-infected patients harboring wild-type V at codon 245 in reverse transcriptase [43]. This method can save the cost of HLA typing by utilizing the HIV-1 sequence data, which are obtained from routine resistance testing approved by the public and private health insurance industries of many developed countries. However, it may result in inadequate withholding of abacavir in a significant number of *HLA-B*5701*-negative patients infected with escape HIV-1 variants, because these escape mutations are often observed and probably able to persist over long periods even in the absence of *HLA-B*5701*-restricted cytotoxic T lymphocyte pressure. Another problem is differences among HIV-1 subtypes. The wild-type amino acid at codon 245 in reverse transcriptase is V only in HIV-1 subtype B, which is most prevalent in developed countries, but is another amino acid such as Q or E in non-B subtypes. Therefore, this method is not suitable when the obtained HIV-1 sequence in phylogenetic analysis belongs to non-B subtypes, which decreases its utility in African and Asian countries where non-B subtypes are prevalent. Considering that practical and accurate HLA typing has already been implemented and is effectively identifying *HLA-B*5701* carriers [44], direct HLA typing is a more simple and better approach to stratify the risk of abacavir hypersensitivity than speculating HLA type from HIV-1 sequences.

Nevirapine hypersensitivity & associated *HLA* alleles

Nevirapine is also a well-tolerated anti-HIV-1 agent, which is listed as an alternative NNRTI in the HIV-1 treatment guideline of the Department of Health and Human Services [45,101]. The most common adverse event associated with the use of nevirapine is hypersensitive reactions (observed in 4.9% of recipients), which are characterized by a combination of rash, fever or hepatitis, and typically occurs within the first 6 weeks of initiation of treatment and can be more rapid and severe with re-challenge [46,47]. Woman with high $CD4^+$ T-cell counts appear to be at higher risk of hypersensitivity reactions [48,49]. The HIV-1 treatment guidelines do not recommend the use of nevirapine for female patients with $CD4^+$ T cell counts over 250 cells/mm^3 and male patients with $CD4^+$ T-cell counts over 400 cells/mm^3 [7,50–53,101]. A higher incidence of hypersensitivity reactions was reported in non-HIV-infected individuals who received nevirapine as part of post-exposure prophylactic treatment, probably associated with a high CD4 count [54]. Usually, cutaneous diseases, including drug hypersensitivity to sulfamethoxazole, dapsone and antituberculous agents, are extremely common in patients with HIV infection, and their incidence increases as immune function deteriorates [55]. However, conversely, in the case of nevirapine hypersensitivity, normal and relatively maintained immune function is a risk factor for unknown reasons [56].

The description of nevirapine-induced SJS in a Ugandan mother and her son suggests a genetic basis for nevirapine hypersensitivity [57]. The possession of *HLA-DRB1*0101* is associated with increased risk of nevirapine hypersensitivity involving multisystemic or hepatotoxic reactions, and which was abrogated by low $CD4^+$ T-cell counts, in the Western Australian HIV Cohort (Table 1) [58]. Littera *et al.* reported that the *HLA-Cw*0802-B*1402* haplotype is associated with nevirapine hypersensitivity in Sardinian patients [59]. We also reported a significant association between *HLA-Cw8* and nevirapine hypersensitivity in Japanese patients, suggesting that nevirapine or its metabolite coupled with *HLA-Cw8* antigen may be expressed on the cell surface and may induce hypersensitivity reactions (Table 1) [60]. In this regard, there was no significant association between *HLA-DRB1*0101* and hypersensitivity in the Sardinian and Japanese cohorts described above, implying that primarily determining HLA alleles may be different among populations. Isolated mild rash and simple hepatotoxicity often occur within 6 weeks of nevirapine treatment initiation. It is possible that this reaction is pathologically different from the severe hypersensitivity reactions, making the definition of hypersensitivity confusing and comparison of different studies difficult [58,61,62]. Establishment of a standardized definition and accurate diagnosis of hypersensitivity seems indispensable for further study of the linkage between HLA alleles and nevirapine hypersensitivity.

Carbamazepine-induced SJS/TEN & *HLA-B*1502*

Carbamazepine is one of the most widely used anticonvulsants, and is also used in bipolar depression and trigeminal neuralgia. Carbamazepine is generally well tolerated but can cause dose-dependent adverse reactions such as dizziness and nystagmus [63]. It is also associated with idiosyncratic hypersensitivity reactions, most

commonly skin rashes such as SJS and TEN, accompanied with fever, lymphadenopathy, and multiorgan-sysem abnormalities [64]. A high frequency of carbamazepine-related hypersensitivity reactions was reported in South-East Asian countries compared with 0.01–0.1% in Caucasians [64–67]. Furthermore, carbamazepine hypersensitivity was reported in identical twins [3]. These studies suggest that susceptibility to such reactions may be genetically determined.

A Taiwanese study reported a strong association between carbamazepine-induced SJS/TEN and the *HLA-B*1502* allele in Han Chinese [68]. The finding was confirmed later by the same group in another study that included patients who were Han Chinese or Chinese descendants from Taiwan, Hong Kong, China and the USA (**Table 1**) [69]. The allele frequency of *HLA-B*1502* is 3–12% in South-East Asians and less than 0.1% in Caucasians, which may explain the higher incidence of carbamazepine-induced SJS/TEN in South-East Asia. In one European study, 15 patients with carbamazepine-induced SJS/TEN were analyzed and five patients who had a parent of Asian origin were positive for the *HLA-B*1502* allele. The remaining ten patients, who were Caucasians, were *HLA-B*1502*-negative [70]. Another European study of Caucasians did not find any *HLA-B*1502*-positive patients who were hypersensitive to carbamazepine [71]. Considered together, *HLA-B*1502* does not seem to be associated with carbamazepine hypersensitivity in the Caucasian population and ethnicity seems important. While it seems conceivable that the causative genetic region of carbamazepine hypersensitivity is linked to *HLA-B*1502*, especially in the Han Chinese population, fine recombinant genetic mapping confirmed the susceptibility gene is *HLA-B*1502* itself [69].

Allopurinol-induced severe cutaneous adverse reactions & *HLA-B*5801*

Allopurinol is widely used for hyperuricemia and recurrent urate kidney stones [72]. However, it is also one of the most frequent causes of severe cutaneous adverse reactions including SJS and TEN [73]. Familial predisposition has been reported and susceptibility to such idiosyncratic reactions is thought to be genetically determined [74]. One Taiwanese study reported a strong association between allopurinol hypersensitivity and *HLA-B*5801* in a Han Chinese population and recombinant genetic mapping further identified *HLA-B*5801* itself as the major susceptibility gene (**Table 1**) [75]. In support of these results, a Japanese group reported three cases with different manifestations of allopurinol hypersensitivity and all of them were positive for *HLA-B*58* [76].

Conclusion

We reviewed here the HLA association with hypersensitivity to abacavir, nevirapine, carbamazepine and allopurinol. Considering that hypersensitivity reactions to abacavir can be life-threatening and even fatal, abacavir prescription to *HLA-B*5701* should be avoided. The following prescriptions should be followed by close monitoring of the patients: nevirapine to patients positive for *HLA-DRB1*0101* or *Cw8*, carbamazepine to *HLA-B*1502* holders and allopurinol to *HLA-B*5801*-positive patients, even if the patient is from a population with no described allele association, because one cannot exclude possible association. It is noteworthy that pharmacogenetic studies are more likely to yield negative results when conducted in populations with low frequencies of the possibly associated allele [77]. More importantly, patients treated with any of these drugs should be monitored closely even if they are negative for *HLA* alleles that are known to be associated with hypersensitivity. Hypersensitivity reactions can potentially occur in any patient as they may hold *HLA* alleles that have yet unreported associations with hypersensitivity. Application of genetic screening should not substitute appropriate clinical vigilance and patient management.

Before abacavir-containing treatment is introduced for HIV-infected patients, HLA analysis should be performed to exclude *HLA-B*5701*, unless the patient is from a population which does not carry *HLA-B*5701*. Such exclusion of *HLA-B*5701* would markedly reduce the possibility of hypersensitivity reactions and prevent overestimation of hypersensitive reaction that could otherwise result in excessive discontinuation of treatment [29–31].

HLA associations with nevirapine hypersensitivity have been reported, but the odds ratios are not high [58–60]. According to the HIV-1 treatment guidelines, avoiding nevirapine prescription is reasonable for female patients with $CD4^+$ T-cell counts over 250 cells/mm^3 and male patients with $CD4^+$ T-cell counts over 400 cells/mm^3, without HLA typing [7,53,101].

Strong associations between carbamazepine hypersensitivity and *HLA-B*1502*, and between allopurinol hypersensitivity and *HLA-B*5801* have been reported in Han Chinese population [68,69,75].

Analysis of these associations in different ethnic populations is urgently needed before it is widely applied in clinical practice.

Future perspective

Current pharmacogenetic information is limited in relation to the genes of HLA, metabolizing enzymes and drug transfer proteins. Considering that the technology to identify genetic variants across the whole genome is advancing rapidly, many more significant genetic factors for drug efficacy and adverse reactions are likely to be identified in the future. Identification of such factors is important not only to discover new pharmacological mechanisms, but also to improve the currently available drugs and to develop novel drugs. In such whole-genome analysis, drug-induced phenotypes should be carefully observed in genetically variable populations, which will be feasible only through international collaboration.

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Executive summary

- Human leukocyte antigen (HLA) information can help predict risk of some drug hypersensitivity.

***Abacavir hypersensitivity* & HLA-B*5701**

- Abacavir hypersensitivity is strongly associated with *HLA-B*5701*.
- Prospective HLA screening can markedly reduce the risk of abacavir hypersensitivity.

Nevirapine hypersensitivity & associated* HLA *alleles

- Significant predisposition to nevirapine hypersensitivity has been reported in Caucasian Australians harboring *HLA-DRB1*0101* with high $CD4^+$ T-cell counts, and Sardinians and Japanese harboring *HLA-Cw8*.

***Carbamazepine-induced SJS/TEN* & HLA-B*1502**

- Carbamazepine hypersensitivity is frequent in *HLA-B*1502*-positive Han Chinese.

***Allopurinol-induced severe cutaneous adverse reactions* & HLA-B*5801**

- Most Han Chinese individuals with allopurinol-induced severe cutaneous adverse reactions are positive for *HLA-B*5801*.

Conclusion

- Prospective HLA screening can stratify the risk of hypersensitivity to abacavir, nevirapine, carbamazepine and allopurinol, and allows personalized medicine.
- Application of genetic screening should not substitute appropriate clinical vigilance and patient management.

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Trends in transmitted drug-resistant HIV-1 and demographic characteristics of newly diagnosed patients: Nationwide surveillance from 2003 to 2008 in Japan

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ABSTRACT

The emergence and transmission of drug-resistant human immunodeficiency virus-1 (HIV-1) compromises antiretroviral treatment for HIV-1. Thus, testing for drug resistance is recommended at diagnosis and before initiating highly active antiretroviral treatment. We conducted an epidemiological study enrolling newly diagnosed patients between 2003 and 2008 in our nationwide surveillance network. In the 6-year study period, the prevalence of drug-resistant HIV-1 among 2573 patients, consisting mainly of Japanese men in their late-30s and infected through male-to-male sexual contacts, followed an increasing trend from 5.9% (16/273) in 2003 to 8.3% (50/605) in 2008. Nucleoside reverse transcriptase inhibitor-associated mutations predominated in each year, with T215 revertants being the most abundant. The predictive factor for drug-resistant HIV-1 transmission was subtype B (OR = 2.36; $p = 0.004$), and those for recent HIV-1 infection were male gender (OR = 3.79; $p = 0.009$), MSM behavior (OR = 1.67; $p = 0.01$), Japanese nationality (OR = 2.31; $p = 0.008$), and subtype B (OR = 5.64; $p < 0.05$). Continued activities are needed to raise awareness of the risks of HIV-1 infection and complications of drug-resistant strains. Continued surveillance is also needed to understand trends in the HIV-1 epidemic.

Abbreviations: HIV-1, human immunodeficiency virus type 1; HAART, highly active antiretroviral therapy; PI, protease inhibitor; HBV, hepatitis B virus; HCV, hepatitis C virus; PR, protease; RT, reverse transcriptase; RT-PCR, reverse transcription polymerase chain reaction; CRF, circulating recombinant form; NRTI, nucleoside RT inhibitor; NNRTI, non-nucleoside RT inhibitor; OR, odds ratio; CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user.

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1. Introduction

The emergence of drug-resistant human immunodeficiency virus type 1 (HIV-1) among patients under highly active antiretroviral therapy (HAART) limits the successful suppression of HIV-1 replication. Several years after the introduction of HAART, drug-resistant strains are being detected among newly diagnosed HAART-naïve patients, suggesting the transmission of drug-resistant HIV-1 from the treatment-exposed population. Thus, treatment-naïve patients have been recommended by the US Department of Health and Human Services, International AIDS Society-USA, and other drug-resistance testing guidelines to undergo drug resistance testing at diagnosis and before initiation of HAART (DHHS, 2009; Hirsch et al., 2000, 2008). Indeed, choosing effective antiretrovirals according to the results obtained from this testing has led to successful control of HIV-1 infection. Furthermore, the drug resistance testing at diagnosis helps to understand transmission of drug-resistant HIV-1 in HAART-naïve individuals which in turn may help prevent transmission events.

The prevalence of drug-resistant HIV-1 among treatment-naïve patients has been closely monitored and reported from many countries. Before and early in the HAART era, when only mono or dual therapy was available, the prevalence was as high as 10–20% (Boden et al., 1999; Gómez-Cano et al., 1998; Tambussi et al., 1998). However, after the introduction of antiretrovirals with better pharmacokinetics, such as ritonavir-boosted protease inhibitor (PI), the emergence of drug-resistant viruses seemed to decrease (Gallego et al., 2001; Maia Teixeira et al., 2006).

Furthermore, despite the great number of HIV-1-infected patients, the prevalence tended to be low in developing countries where patients had limited or no access to antiretroviral drugs, e.g., 0–4.2% in Africa (Bártolo et al., 2009; Mintsa-Ndong et al., 2009; Ndembi et al., 2008; Pillay et al., 2008), 1.5% in Cambodia (Nouhin et al., 2009), and 2.6% in Vietnam (Ishizaki et al., 2009). In contrast, in countries where antiretroviral drugs are more accessible, the prevalence has been higher, e.g., 5.2% in Thailand (Apisarnthanarak et al., 2008), 9.4% in Taiwan (Chang et al., 2008), 10.0% in India (Lall et al., 2008), 7.8% in Portugal (Palma et al., 2007), 9.0% in Germany (Sagir et al., 2007), 9.5% in Belgium (Vercauteren et al., 2008), 10.9% in France (Chaix et al., 2009), and 15.9% in the US (Eshleman et al., 2007).

In Japan, since the first HIV-1-infected case was identified in 1985, the annual number of reported cases has been increasing every year, reaching 15 451 by the end of 2008. With more people getting infected, larger numbers of patients are starting anti-HIV-1 treatment and the risk of emerging drug-resistant HIV-1 is increasing. To understand the trends in drug-resistant HIV-1 in Japan, a nationwide surveillance project has been in effect since 2003. In our previous report of surveillance results from 2003 to 2004, the prevalence of drug-resistant HIV-1 in newly diagnosed patients was 4.0% (Gatanaga et al., 2007). We have continued collecting and analyzing data from newly diagnosed HIV-1-infected patients at participating clinical and research facilities in Japan. We report here the prevalence of drug-resistant HIV-1 among newly diagnosed therapy-naïve patients between 2003 and 2008.

2. Materials and methods

2.1. Sample

The study population included all the HIV-1-infected patients newly diagnosed between January 2003 and December 2008 at any of the participating HIV/AIDS clinics. Drug resistance genotypic tests were performed at 12 laboratories including 8 clinical laboratories at HIV/AIDS clinics, 3 public health laboratories, and the National Institute of Infectious Diseases. After patients agreed to participate in our surveillance project and gave informed consent, peripheral blood was drawn with EDTA added, and their demographic and clinical information were collected. Demographic information included age, gender, nationality, and risk behavior. Clinical data included HIV-1 viral loads, $CD4^+$ T cell counts, status of hepatitis B and C virus (HBV, HCV) co-infection, baseline sequence data, and drug-resistant amino acid mutations.

This study was conducted according to the principles in the Declaration of Helsinki, and was approved by the ethical committee of the National Institute of Infectious Diseases, Japan. By Japanese law, HIV-1-infected patients must be reported to the Japanese Ministry of Health, Labour, and Welfare upon diagnosis. The numbers reported to the Ministry are considered the "official numbers" of newly diagnosed HIV/AIDS cases, and were used as comparison controls to evaluate our study population.

2.2. Drug resistance genotypic testing

Drug resistance genotypic testing was performed using in-house protocols. Briefly, viral RNA was extracted from patient plasma samples. HIV-1 protease (PR, 1–99 amino acids) and the N-terminal region of reverse transcriptase (RT, 1–240 amino acids) were amplified in reverse transcription polymerase chain reaction (RT-PCR) followed by nested PCR using in-house primer sets. Subsequently, the amplified PCR products were purified and their sequences were analyzed by direct sequencing method using an automated sequencer. The resulting electropherograms were analyzed using commercially available software. The quality of testing methods used at each participating facility was assessed and confirmed for detection of drug-resistant mutations (Fujisaki et al., 2007). Thus, detection of drug-resistant mutations was consistent among facilities.

2.3. Determination of HIV-1 subtypes and drug-resistant HIV-1

HIV-1 subtypes were determined using the sequences of HIV-1 PR and RT genes obtained in the drug resistance genotypic testing explained above. Each sequence was aligned with the reference sequences of HIV-1 subtypes A through K, and circulating recombinant forms (CRFs), all of which were obtained from the Los Alamos HIV Databases (Los Alamos, 2010), using ClustalW, and phylogenetic trees were constructed using the neighbor-joining method with bootstrap value of 1000.

The resulting sequences were compared to that of HXB2 to judge the presence of amino acid mutations. The drug-resistant mutations were determined according to criteria of the HIV Drug Resistance Database of Stanford University (Bennett et al., 2009). Thus, a sample was considered to harbor drug-resistant HIV-1 if it possessed any of the following mutations: in the PR gene, L23I, L24I, D30N, V32I, M46I/L, I47V/A, G48V/M, I50V/L, F53L/Y, I54V/L/M/A/T/S, G73S/T/C/A, L76V, V82A/T/F/S/C/M/L, N83D, I84V/A/C, I85V, N88D/S, and L90M (indicating PI resistance); in the RT gene, M41L, K65R, D67N/G/E, T69D/insertion, K70R/E, L74V/I, V75M/T/A/S, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F/I/S/C/D/V/E, K219Q/E/N/R (indicating nucleoside RT inhibitor [NRTI] resistance), and L100I, K101E/P, K103N/S, V106M/A, V179F, Y181C/I/V, Y188L/H/C, G190A/S/E, P225H, M230L (indicating non-nucleoside RT inhibitor [NNRTI] resistance).

2.4. BED assay

The time of HIV-1 seroconversion was estimated in randomly selected samples as recent (within 155 days) or not recent using the BED assay (Calypte HIV-1 BED Incidence EIA, BioRad) according to the Manufacturer's instruction. Briefly, 5 μL of plasma was diluted

with 500 μL of sample diluent in the kit, and the proportion of anti-HIV-1 IgG to a total IgG in the sample was measured by optical density.

2.5. Statistical analysis

Statistical analyses were performed using R software (SAS Institute). Chi-square or Fisher's exact probability tests were used to determine associations among patients' demographic characteristics, nationality, BED assay results, and transmission of drug resistance. The odds ratio (OR) and 95% confidence intervals (CI) were calculated for all the variables. Recent and not-recent seroconversion groups were examined for differences in HIV-1 viral loads by analysis of covariance (ANCOVA), with $CD4^+$ T cell count as the covariate.

3. Results

3.1. Majority of treatment-naïve patients are Japanese men who have sex with men (MSM) in mid-30s

The demographics of the 2573 newly diagnosed HIV-1-infected patients enrolled between 2003 and 2008 are summarized in Table 1. Male ($n = 2397$, 93.2%), Japanese (90.1%), and those infected through male-to-male sexual contact (68.9%) predominated, and the median age was 35. For the female cases ($n = 170$), high-risk heterosexual contact was the major risk factor ($n = 152$, 89.4%), and approximately half were non-Japanese ($n = 63$, 41.4%). Further analysis showed a significant association between the transmission route and nationality, i.e., most Japanese patients were infected through male-to-male sexual contact, while non-Japanese patients were infected by other routes (OR = 5.60; 95% CI 4.14–7.63; $p < 0.01$) (Table 2). It should be noted that sexual contacts (92.1%) are the major risk factor for HIV-1 infection in Japan. On the other hand, injecting drug usage, one of the high risk factors in other countries, accounts for only 0.4%.

HBV and/or HCV co-infection, an important clinical factor affecting prognosis and treatment of HIV infection (Ockenga et al., 1997; Piroth et al., 2000), was found to have a prevalence of 8.4% of 2101 patients, and 4.7% of 2071, respectively (Table 1). These prevalence rates did not change significantly throughout the study period (supplementary Table 1). HBV co-infection was found to be significantly associated with subtype B (OR = 2.04; $p < 0.05$) or infection through male-to-male sexual contact (OR = 1.66; $p < 0.05$).

3.2. Subtype B HIV-1 predominates in Japan

Of 2573 plasma samples collected during the study period, the sequences of PR and RT genes were successfully amplified and analyzed in 2536 (98.6%) and 2534 (98.5%) samples, respectively. Of these, we examined sequences of the PR-RT region from 2496 cases by phylogenetic tree analysis to determine the distribution of HIV-1 subtypes in Japan. Subtype B HIV-1 was found to predominate among the study population ($n = 2194$, 87.9%). The remaining non-B subtypes included 210 (8.4%) CRF01_AE, 30 (1.2%) C, 19 (0.8%) CRF02_AG, 18 (0.7%) A, 9 (0.4%) G, 7 (0.3%) F, 5 (0.2%) D, and 1 (0.04%) CRF08_BC (Table 1). In addition, 1 recombinant case of K/C, A/K, and D/B was detected in 2005, 2006, and 2007, respectively. These non-B subtype viruses were found mostly among the heterosexually infected population (223/302, 73.8%). In contrast, subtype B HIV-1 was found in the vast majority of MSM (1700/1773, 95.9%). In terms of nationality, Japanese patients, most of whom were MSM, were infected with subtype B HIV-1. On the other hand, only about a half of non-Japanese patients harbored subtype B HIV-1, and the remaining half were infected with non-B HIV-1, such as CRF01_AE (OR = 8.85; 95% CI 6.46–12.1; $p < 0.01$) (Table 2). This result is reasonable considering that the predominant HIV-1 subtype differs by country, and our study population included many Thais and Malaysians. In addition, this result suggests that subtype B HIV-1 is transmitted in a closed community of MSM, while non-B subtype strains are spread in wider areas among those infected through high-risk heterosexual contacts.

Table 1
Demographic characteristics of newly diagnosed HIV/AIDS patients.

	6-Year total (2573)	
Age		
Average	37.4	
Median	35	
Mode	35	
Quartile (Q1, Q3)	29, 43	
Nationality	*n*	(%)
Japanese	2319	(90.1)
Non-Japanese	225	(8.7)
Asian	83	(3.2)
Oceanian	4	(0.2)
North American	17	(0.7)
South American	58	(2.3)
European	10	(0.4)
African	26	(1.0)
Unspecified[a]	27	(1.0)
Unknown	29	(1.1)
Transmission category		
Male	2397	(93.2)
Male-to-male sexual contact	1773	(68.9)
High-risk heterosexual contact	369	(14.3)
Sexual contact	75	(2.9)
IDU	8	(0.3)
Other[b]	26	(1.0)
Unidentified	146	(5.7)
Female	170	(6.6)
High-risk heterosexual contact	152	(5.9)
IDU	3	(0.1)
Other[b]	5	(0.2)
Unidentified	11	(0.4)
Unknown	6	(0.2)
Unidentified	6	(0.2)
Hepatitis co-infection[c]		
HBV		
(+)	176	(8.4)
(−)	1925	(91.6)
Unknown	472	
HCV		
(+)	98	(4.7)
(−)	1973	(95.3)
Unknown	502	
HIV-1 subtype[c]		
B	2194	(87.9)
non-B	302	(12.1)
AE	210	(8.4)
C	30	(1.2)
AG	19	(0.8)
A	18	(0.7)
G	9	(0.4)
F	7	(0.3)
D	5	(0.2)
Other	4	(0.2)
Unidentified	77	

[a] Unspecified individuals in the nationality category were identified only as of non-Japanese origin.
[b] Other transmission categories include mother-to-child, blood products, transfusion, and needle stick.
[c] Prevalence of subtypes, HBV, and HCV was calculated after omitting the unidentified or unknown data. DU, intravenous drug user; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1.

3.3. Prevalence of drug-resistant HIV-1 is increasing in Japan

A total of 194 cases (7.7%) in the 6-year study period were found to harbor HIV-1 strains with at least one major drug-resistant muta-

Table 2
Characteristics of newly diagnosed Japanese and non-Japanese HIV/AIDS patients.

	Nationality (*n*)			Odds ratio
	Japanese	Non-Japanese	Unknown	
Gender				
Male	2224	151	22	11.45*
Female	95	74	1	
Unknown[b]			6	
Transmission category				
Male-to-male sexual contact	1691	73	9	5.60[a,*]
High-risk heterosexual contact	399	114	7	
Sexual contact	72	4	0	
Other	29	10	2	
Unidentified[b]	128	24	11	
Subtype				
B	2051	118	25	8.85*
Non-B	198	101	3	
Unidentified[b]	70	6	1	
BED assay (*n* = 640)				
Recent	220	13	0	2.31*
Not recent	351	48	8	
Drug-resistant HIV-1				
Detected	173	16	5	1.05
Not detected	2146	209	24	

[a] Odds ratios for the transmission category were calculated between male-to-male sexual contact and other categories which include high-risk heterosexual contact, sexual contact, and other.
[b] Unknown and Unidentified cases were omitted in calculation of odds ratio.
* $p < 0.01$.

tion conferred by PIs, NRTIs, or NNRTIs. The annual prevalence of drug-resistant mutations shown in Fig. 1 had an overall tendency to increase from 5.9% (16/273) in 2003 to 8.3% (50/605) in 2008. The most prevalent mutation in each year was NRTI-associated resistance, with 11 (4.0%), 12 (4.0%), 21 (5.0%), 23 (5.2%), 28 (5.9%), and 23 (3.7%) cases, followed by PI- and NNRTI-associated mutations. PI-resistant major mutations were detected in 63 cases (2.5%), and NNRTI-associated mutations were detected only in 20 cases (0.8%). These data reflect the type of antiretrovirals being prescribed in treated population. In other words, NRTIs have a long history of being prescribed including the period of mono and dual therapy; thus, NRTIs have been more frequently used. As a consequence, NRTI-resistant HIV-1 has emerged and been transmitted more frequently to treatment-naïve patients. Regarding the drug-resistant mutations shown in Table 3, T215 revertants (T215X) (3.2%), M184I/V (0.5%), K103N (0.6%), and M46I/L (1.7%) accounted for the majority of detected mutations in contrast to other muta-

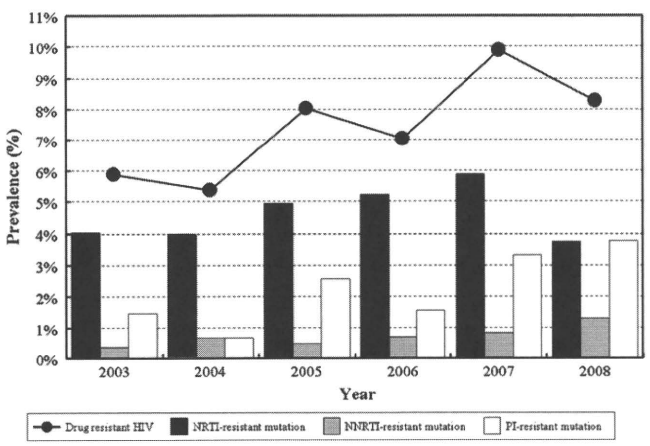

Fig. 1. Annual overall prevalence of drug-resistant HIV-1 (solid circles) in Japan increased in treatment-naïve patients in Japan from 2003 to 2008. The most prevalent mutation in each year was associated with resistance to nucleoside reverse transcriptase inhibitor (NRTI) treatment. Annual prevalence of drug-resistance mutations was categorized by antiretroviral drug class (NRTIs, solid black bars; non-nucleoside reverse transcriptase inhibitors [NNRTIs], horizontally striped bars; protease inhibitors [PIs], solid white bars). Drug-resistant HIV-1 was counted once even when the strain contained multiple drug-resistant mutations. Each drug-resistant mutation was counted even when multiple mutations were detected in one patient.

Table 3
Drug-resistant mutations in newly diagnosed HIV/AIDS patients, by class of antiretroviral drugs.

	6-Year total (2573)	
	n	(%)
NRTI[a]		
M41L	11	(0.4)
K65R	1	(0.0)
D67N/G/E	7	(0.3)
T69D	8	(0.3)
69INS	1	(0.0)
K70R/E	2	(0.1)
L74V/I	3	(0.1)
V75A/M	2	(0.1)
Y115F	3	(0.1)
M184V/I	12	(0.5)
L210W	5	(0.2)
T215X	81	(3.2)
K219Q/E/N/R	4	(0.2)
NNRTI[a]		
L100I	1	(0.0)
K101E	2	(0.1)
K103N	14	(0.6)
V106A/M	1	(0.0)
Y181C/I/V	3	(0.1)
P225H	1	(0.0)
P236L	1	(0.0)
PI[a]		
L24I	1	(0.0)
D30N	5	(0.2)
V32I	3	(0.1)
M46I/L	44	(1.7)
I47V/A	2	(0.1)
V82A/L	2	(0.1)
I85V	5	(0.2)
N88D/S	7	(0.3)
L90M	4	(0.2)

[a] Numbers of cases and the proportions in parentheses are listed.

Table 4
Predictive factors for transmission of drug-resistant HIV-1.

	Drug-resistant HIV-1 (*n*)		Odds ratio
	(+)	(−)	
Gender			
Male	183	2214	1.92
Female	7	163	
Nationality			
Japanese	173	2146	1.05
Non-Japanese	16	209	
Transmission category			
Male-to-male sexual contact	130	1643	0.91
High-risk heterosexual contact	37	484	
Sexual contact	15	60	
Other	1	40	
Unidentified[a]	11	152	
Subtype			
B	180	2014	2.36**
Non-B	11	291	
Unidentified	3	77	

[a] For calculation of odds ratio, unidentified cases were omitted.
** $p < 0.01$.

tions that were detected only sporadically throughout the study period (supplementary Table 2).

Analysis of possible predictive factors for transmission of drug-resistant HIV-1 showed that individuals infected with subtype B HIV-1 had a significantly higher tendency to harbor drug-resistant HIV-1 than non-B subtypes (OR = 2.36; 95% CI = 1.27–4.88; $p < 0.01$) (Table 4). Other possible predictive factors, including male gender (OR = 1.92; 95% CI = 0.89–4.93; $p = 0.1$), Japanese nationality (OR = 1.05; 95% CI = 0.62–1.92; $p = 1$), and MSM behavior (OR = 0.91; 95% CI 0.66–1.26; $p = 0.57$), were not significant predictive factors in our study population. These results indicate that the chance of getting infected with drug-resistant HIV-1 was the same for anyone regardless of gender, nationality, or risk behavior.

3.4. MSM are diagnosed earlier than heterosexually infected individuals

To examine awareness of HIV infection, especially of risk behavior, and to characterize HIV-testing patterns among the HIV-infected population, we estimated the time of seroconversion by quantifying the amount of anti-HIV antibody in plasma samples. Of 640 randomly selected samples in 2007 and 2008, 233 (36.4%) were classified by BED assay with a cut-off value of 0.8 as recently infected (<155-day seroconversion), while the remaining 407 (63.4%) were classified as not recently infected (Table 5). For the recently and not recently infected groups, the average $CD4^+$ T cell count and HIV-1 viral load were 285 and 215 cells/μL and 5.1×10^5 and 1.4×10^5 copies/mL, respectively. Recently infected individuals were shown by ANCOVA with $CD4^+$ T cell counts as the covariate, to have significantly higher HIV-1 viral loads than not recently infected cases (Fig. 2). These data support that the BED assay had precisely determined early infected cases.

With respect to risk behavior, the highest rate of recent infection was in MSM (39.2%), followed by either homo- or heterosexual contacts (38.9%), and heterosexual contacts (25.0%). No patients infected through a risk behavior other than sexual contacts were categorized as recently infected. Whereas 37.8% of male patients were determined to be recently infected, only 13.8% of female patients were categorized as recently infected. These findings were reinforced by statistical analysis. Recent HIV-1 infection was significantly predicted by male gender (OR = 3.79; 95% CI 1.29–15.17; $p < 0.01$), MSM behavior (OR = 1.67; 95% CI = 1.11–2.54; $p = 0.01$), Japanese nationality (OR = 2.31; 95% CI 1.20–4.76; $p < 0.01$), and infection with subtype B HIV-1 (OR = 5.64; 95% CI = 2.37–16.33; $p < 0.01$) (Table 5). In other words, Japanese males, especially those who were MSM, were more aware of being at high risk of HIV-1 infection and got tested more often than non-Japanese. In contrast, females, individuals of non-Japanese origin, heterosexuals, and non-subtype-B-infected persons, had low awareness of the risks of HIV-1 infection.

Table 5
Predictive factors for recent or not-recent seroconversion determined by BED assay, $n = 640$.

	Seroconversion (*n*)		Odds ratio
	Recent (*n* = 233)	Not recent (*n* = 407)	
Gender			
Male	229	377	3.79**
Female	4	25	
Unknown[b]	0	5	
Nationality			
Japanese	220	351	2.31**
Non-Japanese	13	48	
Unknown[b]	0	8	
Transmission category			
Male-to-male sexual contact	189	293	1.67[a,*]
High-risk heterosexual contact	24	70	
Sexual contact	7	11	
Other	0	4	
Unidentified[b]	13	29	
Subtype			
B	224	350	5.64**
Non-B	6	53	
Unidentified[b]	3	4	
Drug-resistant HIV			
Detected	14	37	0.64
Not detected	219	370	

[a] Odds ratio for the transmission category was calculated between male-to-male sexual contact and other categories which include high-risk heterosexual contact, sexual contact, and other.
[b] Unknown or unidentified cases were omitted in calculation of odds ratio.
* $p < 0.05$.
** $p < 0.01$.

Regarding associations between the time of diagnosis and drug-resistant HIV transmission event, time of diagnosis did not differ significantly between those harboring and those not harboring drug-resistant HIV-1 (OR = 0.64; 95% CI = 0.31–1.24; $p = 0.18$) (Table 5), suggesting that transmission of drug-resistant HIV-1 is not a recent trend, but has been ongoing since the first antiretroviral, AZT, was introduced in 1986.

4. Discussion

Our study results show that the proportion of drug-resistant HIV-1 among newly diagnosed cases in Japan increased slightly (by 2.4%) from 2003 to 2008, with fluctuations from year to year. Drug-resistant HIV-1 in HAART-naïve patients are transmitted from HAART-experienced patients with inadequate adherence or from other treatment-naïve individuals with drug-resistant strains, but not yet diagnosed or tested for drug-resistant HIV-1 (de Mendoza et al., 2005). Hence, drug-resistant mutations detected in the naïve population should be tightly related to trends in antiretroviral use in the treated population. Antiretrovirals available in the early days of the HAART era, especially, had short half-lives and low genetic barriers for drug resistance acquisition, making the viruses easily resistance prone. On the other hand, new antiretroviral drugs, such as lopinavir, atazanavir, amprenavir and darunavir, have been developed so that they have improved pharmacokinetics and higher genetic barriers, thus the viruses have less chance of developing drug resistance (Dunn et al., 2008; Lima et al., 2008; Zajdenverg et al., 2009). In the present study, we found that drug-resistant mutations detected among treatment-naïve patients were

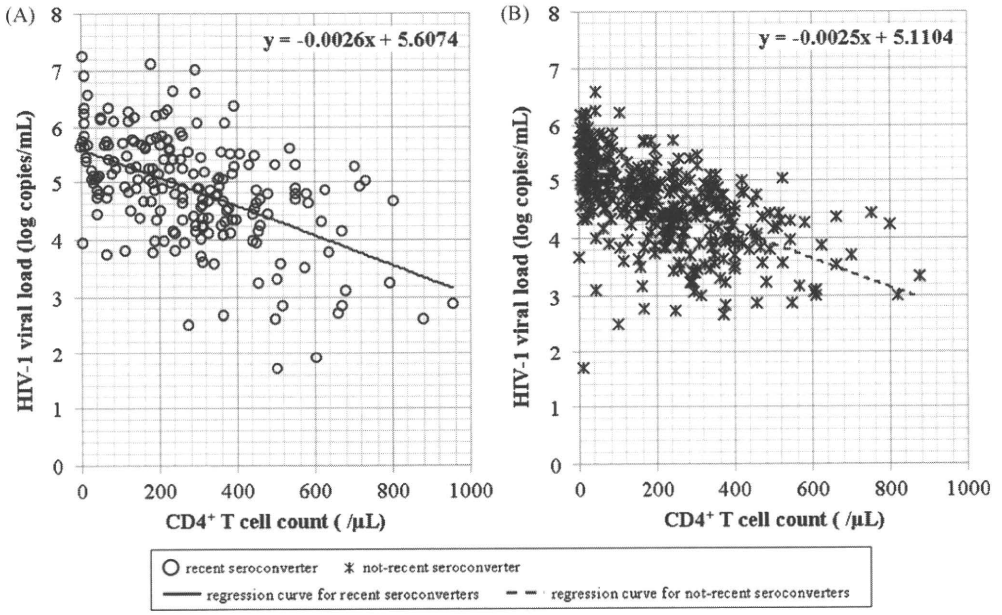

Fig. 2. Scatter plots of viral load and CD4+ T cell counts for (A) recently seroconverted patients (○), and (B) not recently seroconverted patients (*) determined by BED assay. Regression curves and their equations are shown for each group.

associated especially with antiretrovirals used prior to and early in the HAART era. It should be noted that contrary to the reports from the United States and many of European countries (Audelin et al., 2009; Vercauteren et al., 2009; Wheeler et al., 2010), the prevalence of NNRTI-resistant variants have been determined to be low in Japan, less than 1% in the study period 2003–2007 and 1.3% in 2008 being the highest. This difference is due to the situation in Japan that delavirdine had never been used and even nevirapine is only rarely prescribed. Nonetheless, strains with T215X, M46I/L, K103N, and M184V/I mutations were detected every year, suggesting that these strains are stably maintained in individuals and in high-risk populations even under antiretroviral drug-free environments. This finding is supported by the insignificant difference in prevalence of drug-resistant HIV-1 between recently and not recently infected groups. These results raise the concern that such drug-resistant strains may have become some epidemic strains actively transmitted among newly diagnosed HIV/AIDS patients. Furthermore, considering the presence of low frequent variants, the prevalence of drug-resistant mutations in this report may be higher if more sensitive techniques, such as allele-specific PCR and ultra-deep sequencing, are applied to test the samples (Halvas et al., 2010; Varghese et al., 2009). Further studies employing such techniques are needed to understand the detailed epidemic in Japan.

In investigating predictive factors for transmission of drug-resistant strains, we found that the only predictive factor was subtype B HIV-1 (OR = 2.36, $p < 0.01$). The lower transmission risk of drug-resistant strains in non-B HIV-1 can be explained by patients' countries of origin. We observed a significant relationship between non-B subtype HIV-1 and non-Japanese patients, most of whom were from developing countries with limited access to antiretrovirals. Thus, our finding agrees with reports of low prevalence drug-resistant HIV-1 transmission in developing countries (Bártolo et al., 2009; Ishizaki et al., 2009; Mintsa-Ndong et al., 2009; Ndembi et al., 2008; Nouhin et al., 2009; Pillay et al., 2008).

Interestingly, a high proportion of Japanese MSM was diagnosed as recently infected compared to patients of non-Japanese origin, and females determined by BED assay. This result may be due to successful prevention programs targeting the MSM community, so that they have become more aware of their risks of HIV-1 infection. On the other hand, many of non-Japanese patients are seen at hospitals long after HIV infection is established. In addition, women tend to be ignorant of the risks of HIV infection, thus they are often diagnosed upon a prenatal HIV screening test.

Although MSM was not a predictive factor for transmission, this group included 130 cases with drug-resistant HIV-1, the highest prevalence among all the transmission categories. Therefore, those who are involved in prevention programs should take one step further to remind the MSM community about drug-resistant HIV-1 and the limited choice of effective antiretrovirals. HIV-1 transmission has been reported to be prevented in models that assessed the effect of HIV-1 testing for wider populations and immediate initiation of antiretroviral therapy (Granich et al., 2009). Although this model seems very appealing, our results suggest the importance of not forgetting the emergence and transmission of drug-resistant HIV-1 and the limited selection of antiretroviral drugs. It is important to continue surveying newly diagnosed HIV/AIDS patients to keep track of trends in drug-resistant HIV-1 transmission, to reveal high-risk populations with low awareness of HIV infection, to propose effective programs to prevent transmission of drug-resistant HIV-1, and to develop antiretroviral drugs with improved pharmacokinetics/pharmacodynamics. All these efforts may bring us one step closer to eradicating HIV-1.

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