免疫再構築症候群とその対応

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強力な抗HIV療法（HAART）によってHIV感染症/AIDSの予後は一段と改善されたが，一方で免疫不全が改善する過程で，免疫再構築症候群（IRS）と呼ばれる病態が起こることが知られている。今回，名古屋医療センターで治療したAIDS症例においてIRSを併発した症例について検討し，IRSの対処法，予防法などにつき報告する。

【対象および方法】

2004年1月から2006年10月までに受診された新規患者267名中，初診時AIDSと診断された82症例を対象とした。IRSの頻度，AIDS指標疾患，HAART開始時のCD4陽性リンパ球数・HIVウイルス量，IRSへの対応などを検討した（帯状疱疹は除外とした）。

【結果と症例】

AIDS82症例は，ニューモシスティス肺炎（PCP）35例，食道カンジダ15例，非定型抗酸菌症（MAC）ならびに結核8例，サイトメガロウイルス感染症・網膜症8例，HIV脳症5例，トキソプラズマ脳症4例など（重複感染を含む）であった。IRSの評価可能症例はAIDS82例中64例，うち10例（16%）にIRSを併発した。10例のAIDS指標疾患は粟粒結核を含む結核関連・MAC7例（8例中7例：88% IRS発症），PCP4例（1例はMACと重複合併）（評価可能31例中4例：13% IRS発症）であった。

PCP4例中IRSにより実際PCPが悪化したのは2例であり，PCP治療終了からART導入までの日数がそれぞれ11日，12日と短く，また導入前のβDグルカンが不明，1,860 pg/mL，導入前のCD4が60/μL，5/μLと低値，HIVウイルス量が1.5×10^5，1.3×10^6とかなり高値であった。症状は2例ともART 7日目に高熱を来たし，NSAIDまたはART続行+PSL 1 mg/kgの投与にて軽快した。一方，IRSを発症しなかった27例は，PCP治療終了からART導入までの日数が中央値37日（10-83日）であり，導入前のβDグルカンも中央値151 pg/mL（6-2250）と低かった（導入前のCD4：中央値17/μL（12-149），HIVウイルス量中央値2.9×10^5 copies/mL（2.4×10^4-3.9×10^6））であった。

症例1（図1）：62歳，男性。主訴：呼吸困難。現病歴：咳が続き呼吸困難にて総合病院に入院。PC肺炎，CMV肺

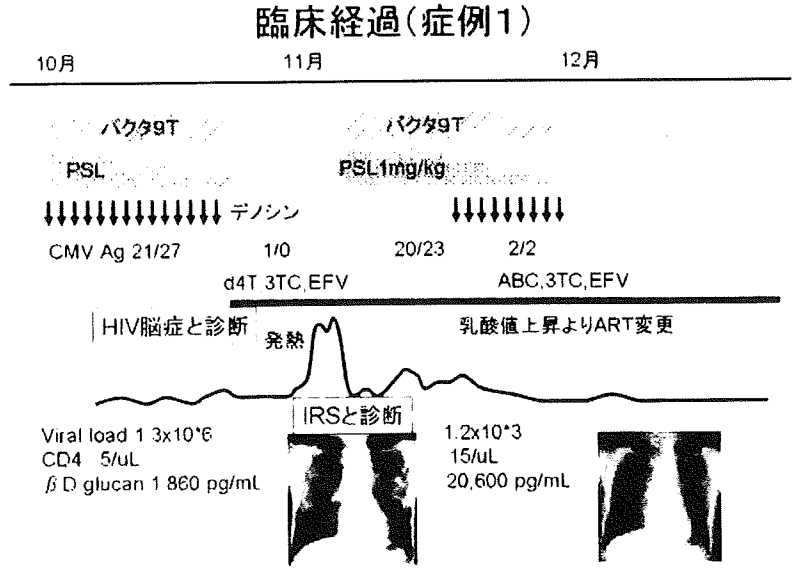

図1　症例1（PCP，HIV脳症）の臨床経過
IRSにはバクタ9T，プレドニゾロン（PSL）1 mg/kg投与にて対処し，抗HIV療法後，顕著に意識レベル改善，会話も可能となった。PCPも軽快した。

表 1 結核・非定型抗酸菌症で発症した免疫再構築症候群

症例	CD4 値（/μL）	HIV-RNA	臨床経過
36 歳，男性	19	9.2×10^5	粟粒結核にて近医入院。TB 軽快後当院へ転院。ART 導入してより血球貪食症候群，クリプトコッカス髄膜炎など IRS 発症し，ART 中断，mPSL パルスも奏効せず死亡。
34 歳，男性	60	7.6×10^5	MAC 敗血症として治療，ART 開始していたが，6 カ月後皮下に結核性膿瘍（ガフキー 2 号）を発症し，TB 治療に変更。
27 歳，男性	154	50＞	症例提示（症例 2）
57 歳，男性	15	5.8×10^5	結核性リンパ節炎。血液培養も陽性。HIV 脳症を疑い，ART。ART 開始後 8 日目より高熱，リンパ節腫大のため IRS と診断し，NSAID 投与も改善せず，PSL 投与（1 mg/kg）し軽快，以後漸減。
51 歳，男性	4	2.1×10^5	HIV 脳症と MAC 敗血症。ART を当初より開始。12 日目より高熱呈し，NSAID にて軽快。HIV 脳症も改善。
21 歳，男性	7	2.0×10^6	PCP に MAC 敗血症・CMV 脳脊髄炎合併。ART 開始後 12 日目より高熱出現。IRS と診断し，NSAID 投与するも改善せず，PSL（1 mg/kg）にて軽快。以後漸減。
63 歳，男性	90	2.1×10^5	右結核性胸膜炎。胸水細胞診で PEL（primary effusion lymphoma）の疑いがあり ART を早期から開始。胸水増量・高熱を来たすが，抗結核療法で改善。

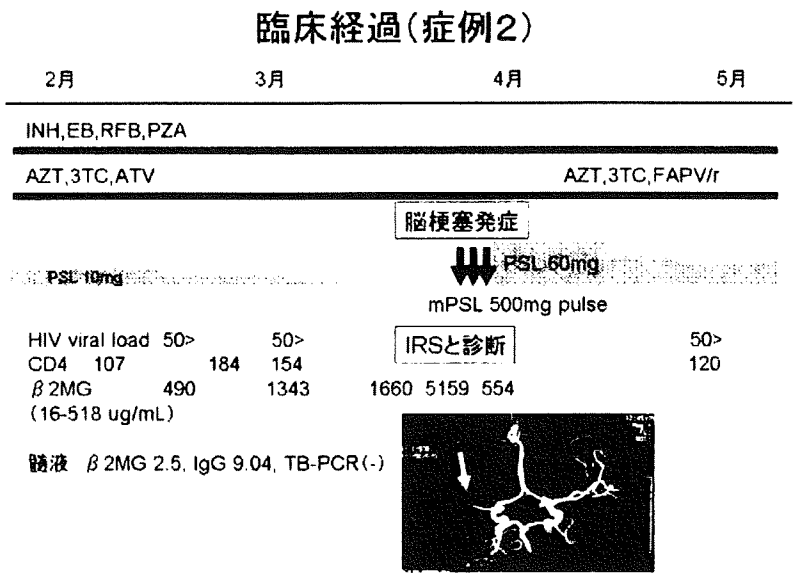

図 2 症例 2（結核）の臨床経過と頭部 MRI
右中大脳動脈（MCA）水平部で血管造影が途絶し，また末梢の描出があり，他の血管病変を認めず，動脈硬化の危険因子もなく，プレドニゾロン（PSL）減量・中止後に発症し，発熱などの症状が先行していたこと，髄液および血清の β2 ミクログロブリンが上昇，結核腫が右シルビウス裂付近に多発していたことから，結核性髄膜炎が PSL 減量・中止によって悪化し，右中大脳動脈付近に血管炎を起こし，梗塞を発症と判断した。メチルプレドニゾロン 500 mg のパルス療法つづいて PSL1 mg/kg 投与により，左片麻痺は改善を認め，MR 血管造影検査でわずかに右中大脳動脈の血流の再開が認められた。

炎と診断された。HIV 抗体検査陽性が判明し，名古屋医療センターに転院。昏明なるいそうを認め，日時，場所など正答できなかった。四肢運動機能，発語は顕著な障害を認めなかった。βD グルカン 797μg/mL. CD4 5/μL, HIV ウイルス量 1.3×10^6 copies/mL, CMV 抗原（C10, 11）21, 27/150,000 cells であった。CMV 脈絡網膜炎の所見は認めなかった。HIV 脳症と診断し，その治療を優先，IRS を覚悟で ART（d4T＋3TC＋EFV）を開始した。

結核・播種性非定型抗酸菌（MAC）感染症の場合は，高率（88%）に IRS を発症した（表 1）。原因として結核菌または MAC の培養結果に時間がかかるため，そして他の合併症を有することが多く，余儀なく抗 HIV 療法（ART）を開始せねばならない状況であることが挙げられる。

症例 2（図 2）：27 歳，男性。主訴：左半身の脱力。現病歴：PCP にて総合病院入院したが，血液培養で結核菌が検出され，結核専門病院に移り治療を受けた。軽快後当院転院（頭部 MRI で結核腫あり）。初診医で HAART 導入され，IRS 生じたため ART 中断となっていた。当院でプレドニゾロン（PSL）併用 ART 再導入。PSL 減量すると発熱，頭痛があった。10 ケ月かけて漸く PSL 中止したが，発熱が間歇的にあり，2006 年 4 月突然左上下肢の脱力が出現し，救急入院となる。意識は清明。軽度の構音障害と顔面を含む左半身麻痺を認めた。プレドニゾロン減量・中止したことによる IRS，すなわち血管炎による脳梗塞と診断し，メチルプレドニゾロン 500 mg のパルス療法ついて PSL 1 mg/kg を投与した。6 月になり左片麻痺は改善（上肢伸展挙上と手指は協同屈曲運動可能，下肢は伸展挙上）を認め，排泄もトイレで自立した。MR 血管造影検査でわずかに右中大脳動脈の血流の再開が認められた。

【考　察】

名古屋医療センターでは ART 後の免疫再構築症候群発症を AIDS 64 例中 10 例（16%）に認めた。PCP 症例で IRS 発症を認めたのは，PCP 治療終了後 1 日目と 12 日目の極めて早期に ART を導入したケースであり，それぞれ 7 日目に高熱症状が出現した。PCP の治療終了後 ART 導入しても IRS 発症が認められなかったのは，導入までの間隔が中央値 37 日であった。十分な間隔または βD グルカンの低値が確認されれば ART 導入後の IRS は予防できると考えられた。一方で結核，播種性 MAC 感染症では極めて高率（88%）に IRS を発症した（ほとんどの症例で ART 開始 8-12 日目に症状出現）。結核，MAC 症例では TB, MAC の培養結果が判明する前に他の合併症のため，ART を導入せざるを得ない症例が多かった　IRS には NSAID, 高熱が持続すれば PSL（0.5-1 mg/kg）投与にて対処した。ART 中断は 1 例のみだった。IRS が長期化する症例も認められ，PSL の長期投与が必要となる場合があった。

服薬アドヒアランス形成支援—失敗例，成功例を通して考える—

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はじめに

抗 HIV 療法は，治療ガイドラインをもとに開始が予定されるが，最終的な開始の決め手となるのは，患者が規則正しく服薬を継続できるための準備ができているかということである。患者が積極的に治療の決定に参加し，問題を解決し，自分の意志で服薬を開始できるための支援が医療者側には必要である。それらをふまえ服薬アドヒアランス形成支援について検討した

方　法

1997 年から 2006 年 3 月までに当院を受診して，現在まで定期的に通院している患者 122 名中，服薬開始後 3 ケ月以上経過している患者 84 名について，患者の治療成績や服薬状況などを診療録から調査した。

結果/考察

当院の服薬中の患者 84 名について，アドヒアランスの状況別に開始後より良好のまま継続できている群を良好群（71 例）とし，服薬開始後から不良になったが改善したものを改善群（9 例），開始後より不良のまま改善できないものを不良群（3 例）の 3 つに分け，服薬アドヒアランスに影響すると考えられる影響因子との状況を比較と共に，個々の症例を振り返った。「一般病院などの他施設での治療開始」「他者に病名告知していない」「身近なサポーターがいない」「仕事多忙による受診調整困難や服薬時間確保困難」「受診中断歴がある」「日常生活に支障をきたすような副作用著明」の数値が 55% 以上と高くでた。改善群では，服薬アドヒアランスを形成，維持する要因として上がってきたものは，副作用などが最小限に押さえられているか，生活にいかに服薬スケジュールを組みこめるか，問題が生じた

時の対処法が理解できているか，患者をサポートする体制ができているか，経済的問題はクリアできているか，医療とうまくつながれているか（正確なセルフレポートが言える，定期受診等），患者の病気の理解と共に受入れができているかなどのように，現在私達HIV医療に携わるものが，必須としている抗HIV療法を開始するにあたって押さえなければならい内容であった。特に重要で支援としては難しい病気を受け入れるにはどうすればよいかという点で見たときには，病気とつきあっていける前向きな意識を持つために生活を保障するために必須との認識をもつ，生活の一部として服薬を組み込め，自己管理意識をもつサポートを受けると共に，家族等の大切な人のためにという前向きな意識をもつ，日和見感染症などの入院経験，死への恐怖をもつなどの生命の危機意識をもつなどを患者自身が認識できることも重要である。逆に不良群3症例の継続できない理由としてあげられる副作用，服薬と生活リズムの調整，受診などにより仕事や日常生活が弊害される事を嫌う，顕著に効果が現れるわけではなく，辛さ・わずらわしさなどが表に出やすく，長期的見通しのなさによるモチベーション維持困難，薬により，病気であることを認識させられる拒否感など受容できていない，自己の行為で感染したという罪悪感，喪失感から生への希望が見出せていない，病気を知られたくないため他者の視線が気になるなどの服薬の回避などの心理面が大きく，病気と治療と自分の生活，今後がつなげられない，受け入れられない状況も考えられた。

ま と め

1. 患者背景も複雑になり，生活基盤の不安定，社会生活不適応者が増えている。社会的サポートが必要なケースについては病院，家族，地域，行政と連携しての支援が必要である。良好群の中でも精神科の介入を早期にする事で安定がはかられているケースもあるように，専門職につなぐことも必要。
2. 症例のアドヒアランス不良群のように，介入しても改善がみられず，多剤耐性ウイルスを獲得していく患者への支援，とくに，CD4が200を下回った状況で体制が再調整するまで服薬中止するという判断の難しさや他者のサポートを拒み続ける患者へどう介入するか。
3. 治療良好な患者についても，長期化する治療に対して先の見通しのなさや服薬疲れなど問題も出現し，モチベーションの維持困難が出てくる可能性も認識して関わっていく必要がある。

患者の中には，様々な状況により病気や治療に対して，受け入れができずに前に進むことができない人もいる。服薬アドヒアンスを良好に維持していくための医療者の支援は，患者個々が抱える問題を早期にとらえ，指示者，アドバイザー，サポーター，評価者などという多面的関わりをしながら患者がセルフマネージメントができ，問題解決に自ら向かえるように支援していくことである。服薬開始時期は，個々により様々だが，医療者は初診時より先を見据えて，服薬開始に向けて意図的に関わっていく必要がある。

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MAJOR ARTICLE

Successful Efavirenz Dose Reduction in HIV Type 1–Infected Individuals with Cytochrome P450 2B6 *6 and *26

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Background. Efavirenz (EFV) is metabolized primarily by cytochrome P450 2B6 (CYP2B6), and high plasma concentrations of the drug are associated with a G→T polymorphism at position 516 (516G→T) of *CYP2B6* and frequent central nervous system (CNS)–related side effects. Here, we tested the feasibility of genotype-based dose reduction of EFV.

Methods. *CYP2B6* genotypes were determined in 456 human immunodeficiency virus type 1 (HIV-1)–infected patients who were receiving EFV treatment or were scheduled to receive EFV-containing treatment. EFV dose was reduced in *CYP2B6* 516G→T carriers who had high plasma EFV concentrations while receiving the standard dosage (600 mg). EFV-naive homozygous *CYP2B6* 516G→T carriers were treated with low-dose EFV. In both groups, the dose was further reduced when plasma EFV concentration remained high.

Results. *CYP2B6* 516G→T was identified in the **6* allele (found in 17.9% of our subjects) and a novel allele, **26* (found in 1.3% of our patients). All EFV-treated *CYP2B6* **6/*6* and **6/*26* carriers had extremely high plasma EFV concentrations (>6000 ng/mL) while receiving the standard dosage. EFV dose was reduced to 400 mg for 11 patients and to 200 mg for 7 patients with persistently suppressed HIV-1 loads. EFV-containing treatment was initiated at 400 mg in 4 *CYP2B6* **6/*6* carriers and one **6/*26* carrier. Two of them still had a high plasma EFV concentration while receiving that dose, and the dose was further reduced to 200 mg, with successful HIV-1 suppression. CNS-related symptoms improved with dose reduction in 10 of the 14 patients, although some had not been aware of the symptoms at initial dosage.

Conclusions. Genotype-based EFV dose reduction is feasible in *CYP2B6* **6/*6* and **6/*26* carriers, which can reduce EFV-associated CNS symptoms.

Efavirenz (EFV) is an important anti–HIV-1 agent in current combination treatment and is usually prescribed at a fixed dosage of 600 mg once daily [1, 2]. The plasma concentration of EFV varies widely in individuals, and the prevalence of CNS symptoms is higher in those with high concentrations [3]. EFV is metabolized mainly by cytochrome P450 2B6 (CYP2B6), and its concentration was reported to be associated with the *CYP2B6* 516G→T genetic polymorphism [4–8]. Previously, we reported that all Japanese patients with the 516TT genotype had extremely high EFV concentrations (>6000 ng/mL), without exception [4]. However, other studies reported some exceptional cases of subjects with the 516TT genotype with normal concentrations, although most of the

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516TT carriers had high concentrations [5–8]. The difference between our data and those of others may reflect polymorphisms other than 516G→T in *CYP2B6*. If this is the case, analysis of other polymorphisms and determination of the *CYP2B6* haplotype may be helpful in predicting EFV plasma levels. In the present study, we determined the *CYP2B6* haplotype of 456 HIV-1–infected patients and analyzed its relationship with EFV concentration in 111 of them. Furthermore, we reduced the EFV dose in 12 patients whose EFV concentrations had been high while receiving the standard dosage. We also used reduced doses of EFV in 5 EFV-naive patients in whom EFV concentration was predicted to become extremely high while receiving the standard dosage, on the basis of *CYP2B6* haplotype determination.

SUBJECTS, MATERIALS, AND METHODS

Patients. This analysis included 60 previously reported HIV-1–infected individuals at the International Medical Center of Japan (IMCJ) [4] and another group of 396 HIV-1–infected patients who were receiving treatment of the standard dosage (600 mg once daily) of EFV or were scheduled to begin receiving EFV-containing treatment at the following 11 hospitals in Japan: Hokkaido University (Sapporo), Sendai Medical Center (Sendai), Niigata University (Niigata), Higashi Saitama Hospital (Hasuda), IMCJ (Tokyo), Ishikawa Prefecture Central Hospital (Kanazawa), Nagoya Medical Center (Nagoya), Osaka National Hospital (Osaka), Hiroshima University (Hiroshima), Kyushu Medical Center (Fukuoka), and Kumamoto University (Kumamoto). The ethics committee of each hospital approved this study, and each participant gave written informed consent.

CYP2B6 *genotype.* DNA samples were extracted from peripheral blood specimens obtained from participants, and genotyping of *CYP2B6* 64C→T (*rs8192709*), 415A→G (*rs12721655*), 499C→G (*rs3826711*), 516G→T (*rs3745274*), 777C→A (*rs* number not available), 785A→G (*rs2279343*), 1375A→G (*rs* number not available), and 1459C→T (*rs3211371*) was performed by allele-specific fluorogenic 5′ nuclease chain reaction assay with predesigned primers and TaqMan MGB probes (TaqMan SNP Genotyping Assay; Applied Biosystems) or previously published primers and MGB probes [4]. In subjects confirmed to carry 499C→G, all 9 exons of the *CYP2B6* gene were amplified with previously published primers [9], and their DNA sequences were directly determined. For haplotype analysis of the *CYP2B6* allele, PCR amplification of the genomic region (3130 bp) containing exons 4 and 5 was performed using sense primer 5′-AACTGTACTCACTCCCAGAGT-3′ and antisense primer 5′-CTCCCTCTGTCTTTCATTCTGT-3′. The amplified PCR product was subjected to subcloning, and the DNA sequence of each clone was determined. For genotyping of *CYP2B6* 983T→C (*rs28399499*), new primers and probes were designed as follows: forward primer, 5′-GCCTGAAATGCCTCTTTAAAATGAGATTC-3′; reverse primer, 5′-GCGATGTGGGCCAATCAC-3′; VIC probe for 983T, 5′-CTGTTCAATCTCCC-3′; and FAM probe for 983C, 5′-CTGTTCAGTCTCCC-3′. The obtained genotyping results of *CYP2B6* 983T→C for >10 patients were confirmed by direct sequencing of exons 7 and 8 with use of primers published elsewhere [9].

Plasma EFV concentration. Samples of peripheral blood were collected during a daytime office visit (9–16 h after the patient took EFV) from the patients who had received EFV treatment at 600-mg dose at bedtime for >4 weeks. EFV concentration was measured by the reverse-phase high-performance liquid chromatography (HPLC) method [10]. For cases of EFV-dose reduction, plasma concentration was measured >2 weeks after the change in EFV dose. Differences in EFV concentrations between groups were examined for statistical significance with Student's t test. A P value <.05 denoted the presence of a statistically significant difference.

RESULTS

Novel* CYP2B6 *allele. The *CYP2B6* genotype was analyzed in 456 HIV-1–infected patients, including 442 Japanese, 8 other Asians, and 6 others. During the analysis, we noticed that some patients had the *CYP2B6* 499C→G polymorphism, substituting Ala for Pro at the 167th amino acid, which is already registered in the SNP Database, although the *CYP2B6* allele containing 499G had not been determined yet. TaqMan Genotyping Assay indicated that *CYP2B6* 449G was heterozygous with 499C in 12 individuals (2.6%), who were all Japanese (table 1). Direct sequencing of all the exons confirmed the results of TaqMan Genotyping Assay and showed that 8 subjects had 516GT, 785AG, and 1375AA genotypes; 3 had 516TT, 785GG, and 1375AA genotypes; and 1 had 516GT, 785AG, and 1375AG genotypes without any other mutation. Subcloning analysis of the PCR products confirmed that 499G always coexisted in the same allele with 516T and 785G (figure 1). Therefore, it was concluded that the novel haplotype containing 499C→G had 2 other single-nucleotide polymorphisms (SNPs): 516G→T and 785A→G. We formally registered this novel allele with the Human Cytochrome P450 Allele Nomenclature Committee, and it was designated "*CYP2B6* *26" (http://www.cypalleles.ki.se/). With use of this nomenclature, the *CYP2B6* haplotype of the twelve 499C→G carriers were identified as eight *1/*26 heterozygotes, three *6/*26 heterozygotes, and one *23/*26 heterozygote (table 1). The allelic frequency of *26 was 1.3% in our study participants.

CYP2B6 *haplotype determination.* In 456 HIV-1–infected individuals, we determined the genotypes of 9 SNP positions (64C→T, 415A→G, 499C→G, 516G→T, 777C→A, 785A→G, 983T→C, 1375A→G, and 1459C→T) in *CYP2B6* (table 1). No *CYP2B6* genetic polymorphism was detected in 211 patients, and their haplotype was determined to be *1/*1. The haplotypes

Table 1. *CYP2B6* haplotype and allele frequencies in study participants.

CYP2B6 status	*CYP2B6* genotype at nucleotide position								No. (%) of subjects	
	415	499	516	777	785	983	1375	1459	All[a]	Japanese
Haplotype:										
**1/*1*	AA	CC	GG	CC	AA	TT	AA	CC	211 (46.3)	205 (46.4)
**1/*2*	AA	CC	GG	CC	AA	TT	AA	CC	30 (6.6)	30 (6.8)
**1/*4*	AA	CC	GG	CC	AG	TT	AA	CC	43 (9.4)	42 (9.5)
**1/*5*	AA	CC	GG	CC	AA	TT	AA	CT	4 (0.9)	3 (0.7)
**1/*6*	AA	CC	GT	CC	AG	TT	AA	CC	104 (22.8)	101 (22.9)
**1/*23*	AA	CC	GG	CC	AA	TT	AG	CC	2 (0.4)	2 (0.5)
**1/*26*	AA	CG	GT	CC	AG	TT	AA	CC	8 (1.8)	8 (1.8)
**2/*4*	AA	CC	GG	CC	AG	TT	AA	CC	6 (1.3)	5 (1.1)
**2/*5*	AA	CC	GG	CC	AA	TT	AA	CT	1 (0.2)	1 (0.2)
**2/*6*	AA	CC	GT	CC	AG	TT	AA	CC	5 (1.1)	5 (1.1)
**4/*4*	AA	CC	GG	CC	GG	TT	AA	CC	5 (1.1)	5 (1.1)
**4/*6*	AA	CC	GT	CC	GG	TT	AA	CC	12 (2.6)	12 (2.7)
**5/*5*	AA	CC	GG	CC	AA	TT	AA	TT	1 (0.2)	1 (0.2)
**5/*6*	AA	CC	GT	CC	AG	TT	AA	CT	1 (0.2)	1 (0.2)
**6/*6*	AA	CC	TT	CC	GG	TT	AA	CC	19 (4.2)	17 (3.8)
**6/*26*	AA	CG	TT	CC	GG	TT	AA	CC	3 (0.7)	3 (0.7)
**23/*26*	AA	CG	GT	CC	AG	TT	AG	CC	1 (0.2)	1 (0.2)
Total									456	442
Allele:										
**1*	A	C	G	C	A	T	A	C	613 (67.2)	596 (67.4)
**2*	A	C	G	C	A	T	A	C	42 (4.6)	41 (4.6)
**4*	A	C	G	C	G	T	A	C	71 (7.8)	69 (7.8)
**5*	A	C	G	C	A	T	A	T	8 (0.9)	7 (0.8)
**6*	A	C	T	C	G	T	A	C	163 (17.9)	156 (17.6)
**23*	A	C	G	C	A	T	G	C	3 (0.3)	3 (0.3)
**26*	A	G	T	C	G	T	A	C	12 (1.3)	12 (1.4)
Total									912	884

[a] Including 442 Japanese, 8 other Asians (5 Thai, 2 Koreans, and 1 Filipino), 4 Hispanics, and 2 non-Hispanic whites.

of single-SNP carriers with 64CT, 785AG, 1375AG, and 1459CT were determined to be **1/*2*, **1/*4*, **1/*23*, and **1/*5*, respectively. Those of homozygous polymorphism carriers with 785GG only, 1459TT only, and both 516TT and 785GG were determined to be **4/*4*, **5/*5*, and **6/*6*, respectively. When the fact that **2* is the only allele harboring 64C→T is considered, patients with 64CT and 785AG; 64CT and 1459CT; and 64CT, 516GT, and 785AG were identified as **2/*4*, **2/*5*, and **2/*6* heterozygotes, respectively. Patients with both 516GT and 785GG genotypes but without other polymorphisms were determined to have **4/*6* heterozygotes. There were 104 patients (22.8%), including 101 Japanese, who held both 516GT and 785AG genotypes without other polymorphisms. There were 2 possible haplotypes, **1/*6* and **4/*9*, in this genotypic pattern. When the fact that **9* had not been reported in Japanese subjects was considered [11], we found that all 101 Japanese were **1/*6* heterozygotes. Haplotype analysis by subcloning of PCR products described above was performed in the 3 others, and their haplotype was determined as **1/*6*. One Japanese patient had 516GT, 785AG, and 1459CT genotypes without other polymorphisms, and there were 2 possible haplotypes, **1/*7* and **5/*6*, in this genotypic pattern. Because **7* had not been reported in Japanese subjects [11], the haplotype in this patient was determined to be **5/*6*. Overall, the allelic frequency of **6* was 17.9% in our study participants. The 415A→G, 777C→A, and 983T→C polymorphisms, which are the determinants of **8*, **3*, and **18*, respectively, were not observed in our subjects.

CYP2B6 *and EFV concentration.* We determined the *CYP2B6* haplotype in 251 patients at IMCJ and in 205 patients at the other 10 hospitals. Of the 251 genotype-analyzed patients at IMCJ, 101 were being treated or were beginning treatment with a standard dose of EFV during this study period (figure 2). Plasma EFV concentrations were measured in all 101 patients, including sixty-seven 516GG holders, twenty-eight 516GT holders, and six 516TT holders. To clarify the effect of the 516TT genotype, EFV concentration was also measured in ten 516TT holders undergoing treatment with the standard dose of EFV at other hospitals. The mean concentration (± SD)

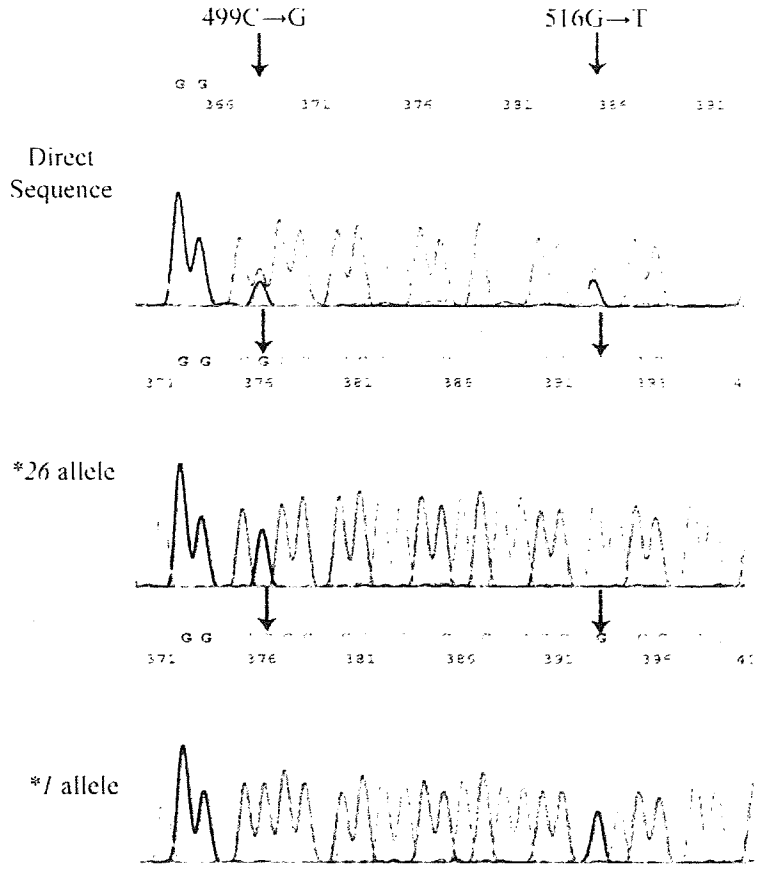

Figure 1. Direct *(top panel)* and subclonal *(middle and bottom panels)* sequences of *CYP2B6* in 499C→G carriers. The genotypes 499G, 516T, and 785G (not shown) exist in the same allele, newly designated as "*CYP2B6 *26*." The same results were obtained in all 9 patients with the 499CG, 516GT, and 785AG genotypes, and the patients were identified as eight **1/*26* carriers and one **23/*26* carrier. Although shown are the sense-strand sequences only, both strands were sequenced. Arrows indicate the variant nucleotide positions 499 and 516.

of EFV in all patients was 3740 ± 2800 ng/mL. When divided by the genotype of position 516, striking discreteness was observed (figure 3). All (95% CI 91.1%–100%) of the 16 carriers of 516TT genotype, including fourteen **6/*6* carriers and two **6/*26* carriers, had extremely high EFV concentrations (>6000 ng/mL). Their mean concentrations (9500 ± 2580 ng/mL) were many orders of magnitude higher than those of the other genotype carriers ($P < 10^{-4}$). There was no significant difference in EFV concentration between **6/*6* carriers and **6/*26* carriers. On the other hand, there were only 2 patients who had such high EFV concentrations among the other genotype carriers. One was a **1/*6* carrier (7140 ng/mL), and the other was a **1/*26* carrier (9710 ng/mL). Direct sequencing of all *CYP2B6* exons showed no polymorphism other than 499C→G, 516G→T, and 785A→G in these individuals. The mean concentrations of EFV of the twenty-eight 516GT carriers, including twenty-five **6*-heterozygotes (3320 ± 1240 ng/mL; $P < 10^{-4}$) and three **26*-heterozygotes (5470 ± 3840 ng/mL; $P < 10^{-4}$), were significantly higher than those of the sixty-seven 516GG genotype carriers (2450 ± 770 ng/mL). None (95% CI 0%–0.1%) of the 516GG carriers had a high EFV concentration (>6000 ng/mL). Considered together, it was concluded that high plasma EFV concentrations were associated with *CYP2B6 *6* and **26* and that *CYP2B6 *6/*6* and **6/*26* carriers had extremely high plasma EFV concentrations at standard dosage, without exception.

EFV dose reduction from 600 mg. To determine whether the EFV dose can be reduced in patients who have a high concentration while receiving the standard dose, a dose-reduction protocol was applied in 12 patients with high plasma concentrations (>6000 ng/mL [range, 6170–14,690 ng/mL]), including one **1/*26* heterozygote, nine **6/*6* homozygotes, and two **6/*26* heterozygotes. Before the dose reduction, plasma HIV-1 load was undetectable (<50 copies/mL) in all patients for >1 month with treatment of a standard antiretroviral regimen containing 600 mg of EFV. In these 12 patients,

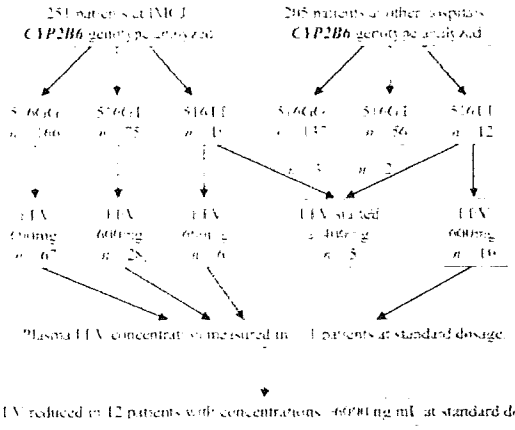

Figure 2. Flow diagram of study participants. The *CYP2B6* genotype was analyzed in 251 patients at the International Medical Center of Japan (IMCJ) and in 205 patients at other hospitals. Standard dosage of EFV was administered in 101 patients at IMCJ, including sixty-seven *CYP2B6* 516GG, twenty-eight 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

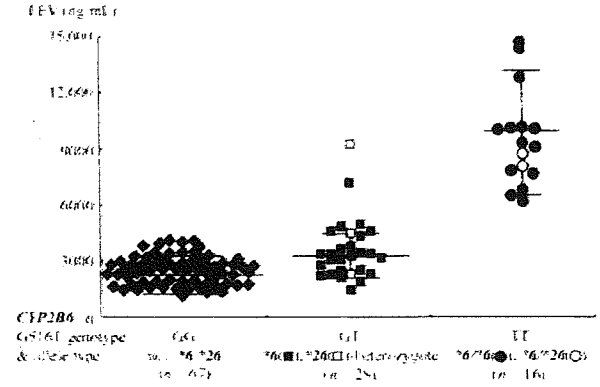

Figure 3. Plasma efavirenz (EFV) concentrations measured during EFV treatment with standard dose (600 mg). A total of 111 HIV-1–infected patients treated with EFV-containing regimens were divided into 3 groups on the basis of nucleotide genotype at *CYP2B6* position 516 (GG, GT, or TT), and their plasma 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.

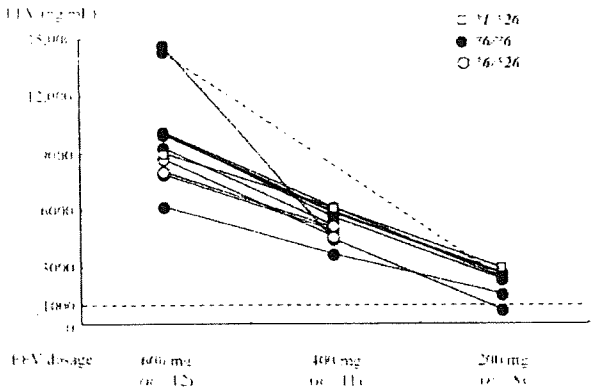

Figure 4. Dose reduction of efavirenz (EFV) in 12 patients whose concentrations were extremely high while receiving treatment with standard dose (600 mg). EFV dose was reduced from 600 to 400 mg in 11 patients and was further reduced, to 200 mg, in 7 patients. In one patient who had severe CNS symptoms 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

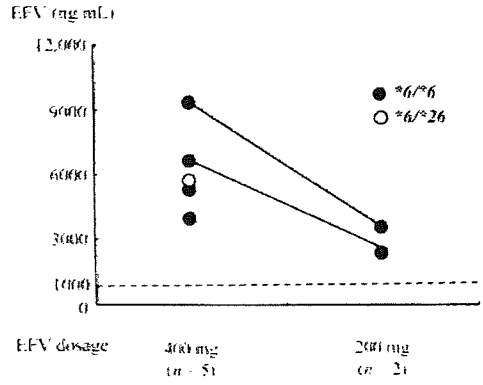

Figure 5. Introduction of low-dose efavirenz (EFV)-containing antiretroviral regimen to *CYP2B6 *6/*6* and **6/*26* carriers. Treatment was started in 4 EFV-naive carriers *CYP2B6 *6/*6* and one **6/*26* carrier, with 400-mg EFV-containing regimens. EFV dose was further reduced, to 200 mg, in 2 patients 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.

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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 M46I 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[e]	1.6
T215E	1[e]	0.2
T215S	2	0.3
NNRTI		
Any	4	0.7
L100I	1	0.2
K103N	2[e,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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