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THE ROLE OF SOCIAL SCIENCE RESEARCH IN REDUCING THE BURDEN OF TUBERCULOSIS IN HIGH HIV PREVALENCE SETTINGS

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Abstract: Tuberculosis (TB) is a global public health problem. The HIV/AIDS epidemic negatively affects tuberculosis control in many countries. The United Nations has set the Millennium Development Goals (MDGs) aiming to halve TB prevalence and mortality by the year 2015. In this paper, the authors summarize the global situation of TB associated with HIV/AIDS (TB/HIV), WHO's interim policy on TB/HIV, as well as the status and needs of social science research. The authors reviewed two major social interventions which are critical for TB control in HIV high prevalence settings, namely those to reduce stigma and those to promote adherence to TB/HIV medication. The review suggests that more social science research should be implemented in resource limited countries.

Key words: tuberculosis, HIV/AIDS, TB/HIV, social science research, social interventions

"The battle against AIDS will not be won unless the international community does more to fight TB as well".
Nelson Mandela, former president of South Africa and former tuberculosis patient
The 15th International AIDS Conference, Bangkok, Thailand, 15 July 2004

Why does fighting AIDS need to involve fighting TB?

HIV/AIDS and tuberculosis (TB) are the world's first and second leading causes of death from infectious diseases. If there had been no HIV epidemic, TB would have been controlled. In 2005, it is estimated that about 40.3 million adults and children were living with HIV and about 3.1 million have died from AIDS [1]. About8.8million new cases of TB occurred in 2003 with an estimated 1.7 million dying from the disease. In the same year, about 674,000 new cases of TB were associated with HIV (TB/HIV), and 229,000 people with HIV/AIDS (PHA) died from TB [2]. Tuberculosis is the leading cause of morbidity and mortality among PHA. At least one in three will develop TB [3]. The alarming global crisis of HIV/AIDS and TB has prompted the United Nations and the international community to set a global target to reduce these priority diseases. The Millennium Development Goals (MDGs) aim to reverse the incidence and halve the mortality of these two diseases by the year 2015 [4].

Biologically, it is well known that TB enhances HIV replication and accelerates HIV progression, thereby shortening the life expectancy of PHA [5]. The close interaction between TB and HIV/AIDS indicates the need to reduce the burden of both HIV/AIDS and TB. Most Sub-Saharan African countries with high HIV prevalence have suffered the negative impact of the interaction between HIV/AIDS and TB for more than a decade. WHO recently published the first interim policy on collaborative TB/HIV activities to tackle the dual epidemics [6]. Table 1 presents the WHO's recommended interventions to reduce the burden of TB and HIV/AIDS. The biomedical interventions to reduce TB and HIV burden include antiretroviral therapy (ARV) and cotrimoxazole preventive therapy (CPT) for HIV-positive TB patients and isoniazid (INH) to prevent TB among PHA. The results of clinical research show that CPT can prolong lives and ARV can reduce death among HIV-positive TB patients [7-10]. INH can reduce the risk of developing TB among PHA [11]. Despite the efficacy of these medical interventions, the task of reducing the TB/HIV burden in resource-limited settings is a great challenge and necessitates interventions suggested by social science research.

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Why social science research?

To control the epidemic of TB and HIV/AIDS, we must not only deal with the HIV virus and the TB bacteria but also manage patients who carry these germs and educate the health worker who deliver health services to the population. Human behavior and social environments are complicated, and biomedical interventions alone are not sufficient for disease control. It has consistently been proposed that TB and AIDS are social diseases whose patterns of transmission must be understood, not only through the clinical or laboratory studies of bacteria and virus, but also through the study of attitudes, behavior and social organization [12-19]. In particular, HIV/AIDS provides a tragic example of a complex interactions between the disease agent and human behavior, which further complicates the effort to control tuberculosis. How can social science research contribute to TB prevention and care in high HIV prevalence settings?

Based on the social science research in the northernmost province of Thailand where HIV epidemic fuels the TB epidemic (HIV prevalence among pregnant women was 3.7% and TB incidence was 140/100,000), we summarized the psycho-social interactions between HIV/AIDS and TB and the negative impact on TB and HIV/AIDS prevention and care (table 2) [20]. It is noteworthy that this social science research was carried out before ARV was available to the poor people of Thailand. Increased access to antiretroviral therapy among people with AIDS in Thailand and other resource-limited countries might reduce AIDS related fatalism and stigma [21, 22]. But even though several high HIV prevalence countries in sub-Saharan Africa successfully mobilized free ARV for poor patients, stigma and discrimination, especially among women resulted in a low level of participation in HIV testing and access to ARV. Gender inequality is such that a poor woman is placed in an even worse social situation if her husband or in-laws become aware of her HIV status [1]. These complicated circum-

Table 1: Interventions to reduce the burden of TB and HIV/AIDS recommended by the World Health Organization [6]

A. Interventions for collaboration between TB and AIDS programs
A.1. Set up a coordinating body for TB/HIV activities effective at all levels
A.2. Conduct surveillance of HIV prevalence among tuberculosis patients
A.3. Carry out joint TB/HIV planning
A.4. Conduct monitoring and evaluation
B. Interventions to decrease the burden of tuberculosis in people living with HIV/AIDS
B.1. Establish intensified tuberculosis case-finding
B.2. Introduce isoniazid preventive therapy
B.3. Ensure tuberculosis infection control in health care and congregate settings
C. Interventions to decrease the burden of HIV in tuberculosis patients
C.1. Provide HIV counseling and testing
C.2. Introduce HIV prevention methods
C.3. Introduce co-trimoxazole preventive therapy
C.4. Ensure HIV/AIDS care and support
C.5. Introduce antiretroviral therapy

Table 2: The psycho-social interactions between HIV/AIDS and TB and the negative impact on TB and HIV/AIDS prevention and care in Thailand (before a lunching the government's policy on access to ARV in 2003) [20, 22, 33-35]

Psycho-social interactions between HIV/AIDS and TB
• The social stigma attached to HIV/AIDS is enormous and results in denial of HIV testing and delay in access to TB and HIV care
• Serious AIDS stigma and an inadequate knowledge about TB symptoms results in delays in seeking TB care because TB symptoms are comparable to AIDS and people with TB symptoms were afraid of HIV/AIDS
• Most HIV negative TB patients were stigmatized as having AIDS.
• The high mortality among HIV-positive TB patients during TB treatment discredited the TB treatment efficacy. Health staff had a low motivation to care for the patients because the treatment results were so discouraging.
• Fatalism attached to HIV/AIDS hindered the patients access to health care patients felt hopeless and lacked the motivation to adhere to their TB treatment.

Table 3: List of some interventions to reduce TB and HIV/AIDS stigma and desired outcomes [36-40]

Interventions to reduce stigma	Desired outcomes
Mass communication interventions Television, radio, newspaper or poster presenting the followings: - photos and news of countries' leaders or popular persons telling about their experiences of having TB or HIV/AIDS; showing close embrace with patients; showing acceptance for HIV blood testing; showing TB is curable or showing that they are surviving from HIV/AIDS - photos and news of Miss HIV/AIDS stigma contest	- increase public's acceptance and reduce discrimination - promote early HIV testing - reduce delay in seeking care
Training/workshop -Intensive training course to reduce stigma among health workers -Community awareness-raising through participatory training -Involving HIV-positive long-term survivors in education and training	-improve health workers' attitude towards TB and AIDS patients and willingness to care for them. -increase HIV testing and access to care
Counseling -Individual or family counseling by health workers or by HIV-positive counselor	-reducing self-stigma; reduce anxiety and stress; disclosure HIV to spouse and family members
Social mobilization and community participation -Establishing network (self-help group) of PHA and motivate PHA to join the network -Financial assistance to PHA and family -Involving PHA, religion leaders and community leaders in policy making, and in the development and implementation of programs.	-reducing self-stigma -empowering PHA -increasing HIV testing and disclosure of HIV status
Improving health service system and promoting access to treatment -Free and effective treatment -Integrating HIV/AIDS care with other chronic diseases clinics -PHA-Friendly hospital	-increase access to HIV care -increase HIV testing -reduce discrimination feeling
Law and regulation intervention - Law or regulation against stigma in general - Law or regulation for the work places - Code of professional ethics/code of practice - Standard or universal guidelines	- protection of patients' right - patients are eligible for petition the court and receive support for complaint due to stigma.

Table 4 Scope of adherence to medication in TB/HIV care [41]

Scope of adherence to medication in TB/HIV care	Targeted HIV-positive person	Expected health outcome of good adherence	Potential negative impact of non-adherence
Adherence to TB preventive Therapy	HIV-infected persons with latent TB infection (no clinical TB symptoms)	- Reducing risk to become sick with TB	- drug resistance
Adherence to TB treatment	HIV- positive TB patients (having TB symptoms)	-cure from TB and do not transmit TB to others	-death -treatment failure -drug resistant -continue transmitting TB to others
Adherence to antiretroviral therapy (ARV)	AIDS patients (usually CD4 < 200cells/mm^3)	-prolong life, better quality of life -avoid opportunistic infection -reduce risk of HIV transmission	-drug resistant -treatment failure -death

Table 5 Interventions for improving adherence to medication [13, 20, 42-44].

Interventions for enhancing adherence to medication
Improving health service systems -Eliminating or lowering user fees -Providing directly observed therapy (DOT) -Organizing service hours convenient for patients and minimizing waiting time -Active follow-up system for non-adherent patients -Offering health education and counseling service by using linguistically and culturally appropriate messages. -Hospitalization may prevent non-adherence among patients exhibiting the profile of defaulter (e.g. homeless, alcoholic patients) -Involving people with HIV/AIDS network and community leaders in delivery services (e.g. providing medication education, follow up non-adherent cases, home visit)
Improving attitude and performance of health care providers -Good relationship between health providers and patients significantly improves patient adherence. -The providers should render service with courtesy and respect for patients. -The providers should understand patients' needs and constraints, understand patients' cultural differences in attitudes to disease. -The providers should spend more time listening to patients. -Giving rewards to health provider who achieve high adherence rate
Facilitating patient medication -Providing special packages of medicine such as a daypack for easier medication. -Prescribing medication once a day and fixing a time such as before breakfast or before bed. -Providing several medicine reminding system (alarm clock, calendar, reminding through pager or cell phone, linking medication time to daily life activity)
Providing incentives to patient and community -Providing transportation support to attend clinics, shelter support for homeless people, offering meals and assistance with job skills for poor patients -Giving rewards to patients who adhere well to the treatment. -Paying a deposit at the start of their treatment, which entitles the patient to cheaper drugs and is refundable on good adherence to prescribed course.

Table 6 Literature on social science research in TB in comparison with AIDS cited by the National Library of Medicine (NLB) website [26].

Searching keywords ..... AND TB ..... AND AIDS	TB (no. of papers)	AIDS (no. of papers)
Social sciences AND	1837	25,028
Behavioral research AND	4	515
Qualitative research AND	13	141
KAP AND (Knowledge, Attitude, Practice)	4	132
Poverty AND	137	1007
Stigma AND	31	433

Table 7 Study topics and geographic location of tuberculosis behavioral and social science research (n=175) [45]

Research settings	
-USA-based	47%
-International-based	36%
-Non-location specific (e.g. concept, position papers)	17%
Study topics	
-Patient adherence	47%
-Social, cultural factors (including Knowledge-Attitude-Belief)	45%
-Structural influences	33%
-Health seeking behavior	19%
-Provider adherence	14%
-Others	12%

stances require social science research to identify socially and culturally sensitive behavioral interventions.

UNAIDS and WHO identify stigma and adherence to medication as the major challenges in controlling the HIV/AIDS epidemic [1, 23]. In this article, we discuss two major social and behavioral interventions, namely interventions to reduce stigma (table 3) and interventions to promote adherence to TB/HIV medications (table 4, 5). These two social interventions are important prerequisites for implementing the medical interventions recommended by WHO (table 1). For example, interventions for reducing AIDS stigma can facilitate HIV testing for TB patients, and interventions for enhancing adherence are important to ensure treatment efficacy and to prevent drug resistance when patients receive ARV, CPT and INH.

Status of social science research in TB and TB/HIV

In 1975, the Special Program for Research and Training in Tropical Diseases (TDR), a globally coordinated effort of the United Nations Children's Fund (UNICEF), United Nations Development Program (UNDP), World Bank and World Health Organization (WHO), was established to combat neglected tropical diseases and diseases of the poor and disadvantaged [24]. In view of the importance of research in social sciences to control communicable diseases, TDR started supporting social science research in 1979. However, social science research for tuberculosis is still quite new to TDR, as shown by the fact that TDR added tuberculosis to the tropical disease portfolio only in 1999 [25]. Table 6 clearly shows that TB has received much less attention from social science researchers in comparison to HIV/AIDS. According to the United States National Library of Medicine (the world's largest literature database for medical and public health research), the number of TB social science research articles in scientific journals is 7 to 128 times less than HIV/AIDS articles [26]. Obviously, social science research in HIV/AIDS has received ample recognition because results of this research help to identify interventions to reduce the HIV/AIDS burden [27]. However, the role of social science research in improving TB care, especially in developing countries, is oddly limited for such an old disease as TB. A recent review of the 175 social science articles on TB (table 7) shows that half of the studies were conducted in the United State (Rawls and Booker, 2005), although 95 percent of global TB cases and99percent of TB deaths occur in the developing world [2]. Clearly, therefore, it is imperative that more social science research be conducted in high burden and resource-limited countries.

The difficulty of achieving the global target for TB control is mainly due to the lack of human resources and qualified staff [28]. At the global level, the published information on human resources and TB control are limited and almost none relate to HIV-TB control [29]. Training is essential to the development of a health workforce geared to TB control, but regular international TB training courses are organized by a limited number of organizations [30, 31]. Most international training courses focus on clinical or laboratory training and the management of tuberculosis programs. To our knowledge, none of these courses include social science subjects in the training curriculum, except the international courses organized continuously by the Research Institute of Tuberculosis (RIT) Japan Anti-TB Association (JATA) since 1963. The RIT has incorporated social science subjects into the training curriculum for TB program managers from the start of training on the basis of the view that clinicians and TB program managers should apply a broader and more holistic perspective to TB services and TB programs. Currently, social science topics include concepts and practical examples about community beliefs and perceptions, social stigma, health systems and health-seeking behavior, gender, adherence to treatment, community participation and interpersonal communication skills. These topics are relevant to WHO's newly recommended Stop TB Strategy for achieving the MDGs [2].

CONCLUSION:

HIV/AIDS biologically and socially interacts with TB. Reducing TB associated HIV burden requires both biomedical and social interventions. The current involvement of social scientists in research and training for TB control in high HIV prevalence settings is limited. To facilitate the Millennium Development Goal for TB, social science research and training should be implemented in countries which are affected by these dual epidemics. Social science research can promote understanding regarding the complex psychosocial interplay of TB and HIV/AIDS. In addition to addressing these problems, social science research can help to identify the interventions which are effective in urging vulnerable patients, including women and the poor to take advantage of TB/HIV prevention and care services. Social science research should show how to implement these interventions on a large scale and how to influence national policy and care strategy [2,27,32]. The training courses designed for TB and HIV/AIDS program managers and service providers should include social science subjects to ensure that the program and services are responsive to the complicated biological and the psycho-social interactions of TB and HIV/AIDS.

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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 genotype at nucleotide position								No. (%) of subjects	
CYP2B6 status	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)

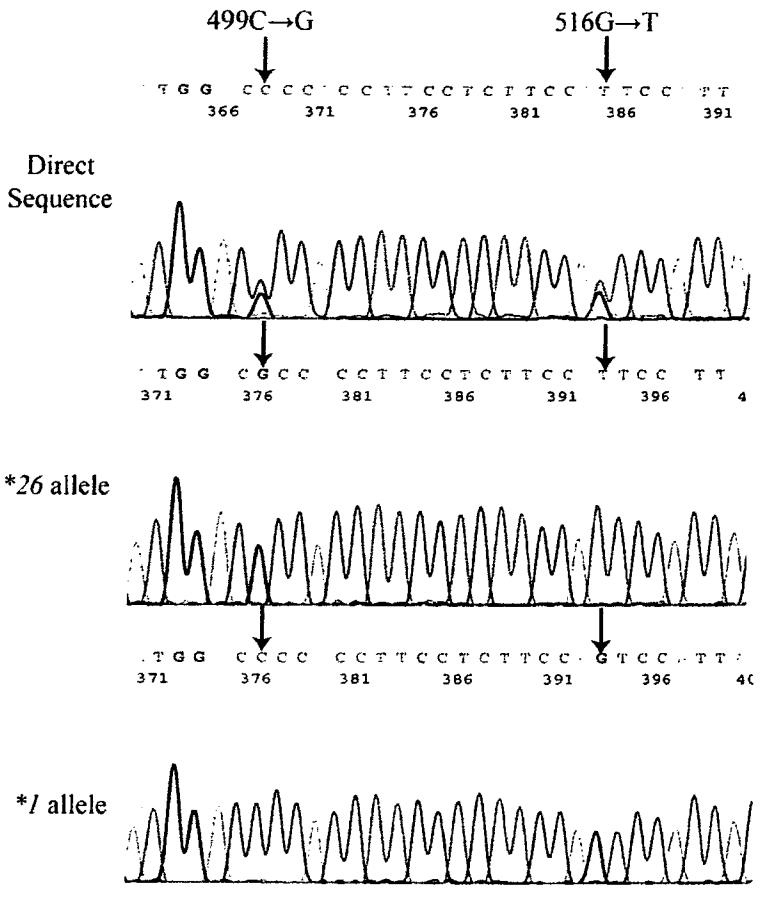

Figure 1. Direct *(top panel)* and subclonal *(middle and bottom panels)* sequences of *CYP2B6* in 499C→G carriers. The genotypes 499G, 516T, and 785G (not shown) exist in the same allele, newly designated as *"CYP2B6 *26."* The same results were obtained in all 9 patients with the 499CG, 516GT, and 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,

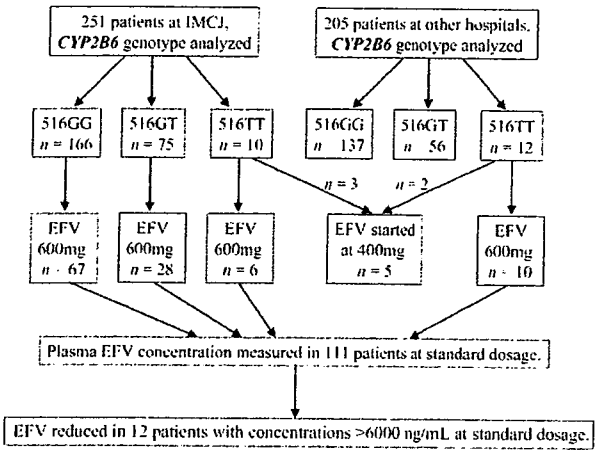

Figure 2. Flow diagram of study participants. The *CYP2B6* genotype was analyzed in 251 patients at the International Medical Center of Japan (IMCJ) and in 205 patients at other hospitals. Standard dosage of EFV was administered in 101 patients at IMCJ, including sixty-seven *CYP2B6* 516GG, twenty-eight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

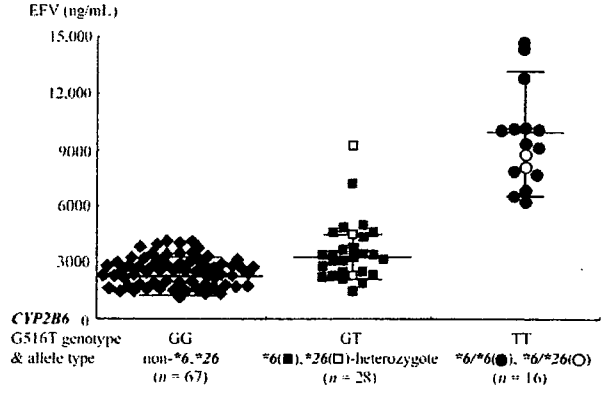

Figure 3. Plasma efavirenz (EFV) concentrations measured during EFV treatment with standard dose (600 mg). A total of 111 HIV-1–infected patients treated with EFV-containing regimens were divided into 3 groups on the basis of nucleotide genotype at *CYP2B6* position 516 (GG, GT, or TT), and their plasma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

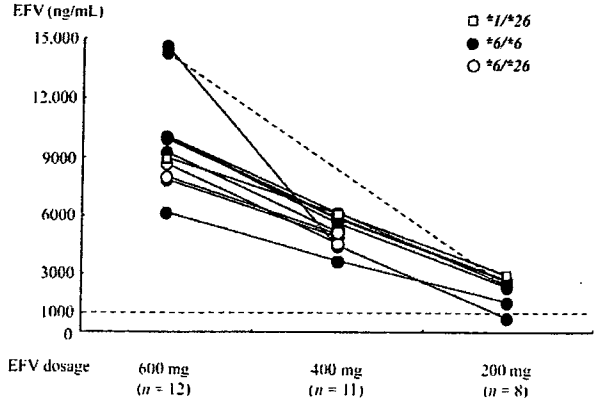

Figure 4. Dose reduction of efavirenz (EFV) in 12 patients whose concentrations were extremely high while receiving treatment with standard dose (600 mg). EFV dose was reduced from 600 to 400 mg in 11 patients and was further reduced, to 200 mg, in 7 patients. In one patient who had severe CNS symptoms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

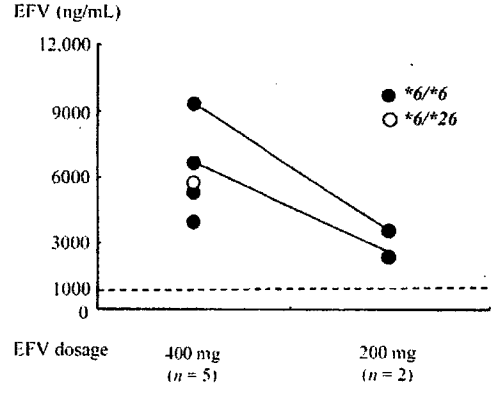

Figure 5. Introduction of low-dose efavirenz (EFV)-containing antiretroviral regimen to *CYP2B6 *6/*6* and **6/*26* carriers. Treatment was started in 4 EFV-naive carriers *CYP2B6 *6/*6* and one **6/*26* carrier, with 400-mg EFV-containing regimens. EFV dose was further reduced, to 200 mg, in 2 patients 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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compared with four in the experienced group. Four of these five patients had HCV co-infection. Two events arose after one month of treatment and the other three after a year, confirming the multifaceted mechanisms causing this toxicity. In all these cases the treatment had to be stopped, and the patients regressed.

To the best of our knowledge, this study comprises the biggest series to date of patients treated with lopinavir/ritonavir and followed prospectively outside clinical trials. In addition, this HIV-positive population had a high prevalence of co-infection with hepatitis viruses.

The frequency of hepatotoxicity was actually low, unlike in other studies. This might partly be the result of methodological differences, reflecting how the data were collected. Retrospective studies can suffer major selection bias. Gonzalez-Requena *et al.* [11] also reported a low incidence of adverse events, but their case series was small and was followed up for not more than one year.

In conclusion, the present study found that lopinavir/ritonavir caused only limited hepatic toxicity in this population of HIV-positive patients with a high prevalence of co-infection with hepatitis B virus or HCV.

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Premature sister chromatid separation in HIV-1-infected peripheral blood lymphocytes

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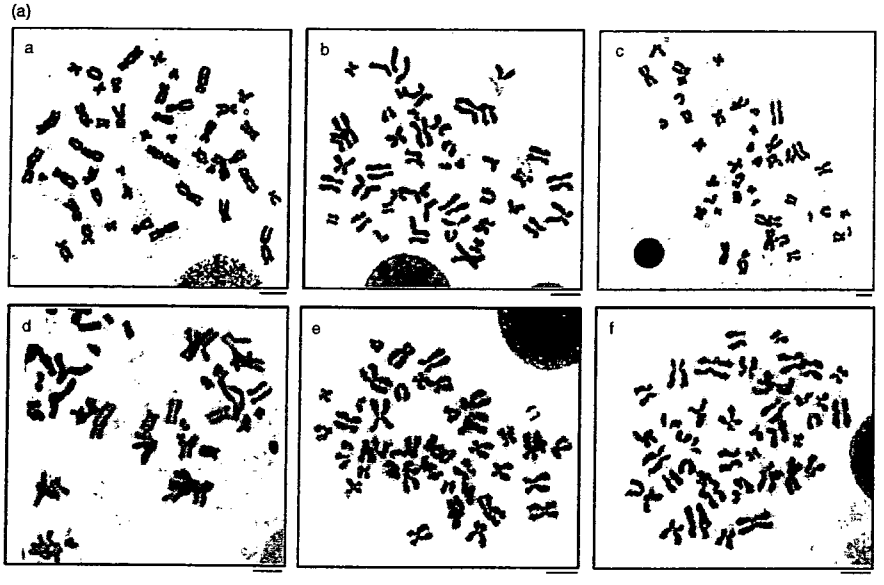
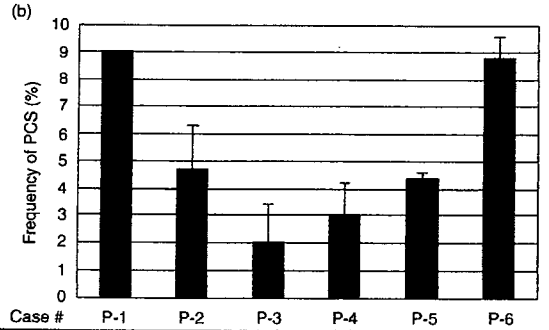
To investigate the mechanism of aneuploidy that is frequently observed in AIDS, we examined premature sister chromatid separation (PCS), a sign of genomic instability, in peripheral blood cells of HIV-1-infected individuals. PCS was found in all

six HIV-1 individuals at a high incidence. When peripheral blood cells from healthy volunteers were infected with HIV-1 *in vitro*, the incidence of PCS increased. This suggests that HIV-1 infection causes PCS and has the potential to induce aneuploidy.

Malignancy in HIV infection influences the prognosis of AIDS patients. These neoplasms are the result of various diseases that accompany immunodeficiency, such as co-infections with Epstein–Barr virus or human herpes virus 8 [1–4]. Besides these AIDS-defining cancers, several non-AIDS-defining cancers also occur at a higher incidence in HIV-infected individuals [5–9]. Moreover, it has been reported that HIV-1 itself is tumorigenic in immortalized B cells in nude mice [10,11]. These reports lead to the hypothesis that HIV-1 has the potential to induce neoplasms before AIDS develops.

Aneuploidy is a phenomenon of chromosome instability that is frequently reported in HIV-1-infected individuals





Case #	P-1	P-2	P-3	P-4	P-5	P-6
Total WBC	3800	5500	8400	5700	6100	2700
HIV-1 mRNA (copies/ml)	27,000	< 50	76	< 50	< 50	35,000

Fig. 1. Metaphase spreads of blood cells in HIV-1 infection. (a) Representative metaphase spreads of peripheral blood cells from HIV-1-infected individuals (b, c, d, e, and f are from cases nos. P-1, 2, 4, 5, and 6, respectively, see Fig. 1b). (a). (b) Frequency of premature sister chromatid separation (PCS). The frequency of PCS (black bar), and number of HIV-1 messenger RNA copies and total white blood cells (WBC) are shown. (c) Metaphase spreads of peripheral blood mononuclear cells (PBMC) from healthy volunteers. Representative metaphase spreads of PBMC from healthy volunteers with (a/+, b/+, and c/+) or without (a, b, and c) vesicular stomatitis virus G protein (VSV-G)-pseudotyped HIV-1 infection are shown. (d) Aneuploidy in HIV-1-infected cells. Metaphase spreads from P-1, P-6 and from PBMC with VSV-G-pseudotyped HIV-1 infection were positive for aneuploidy with numbers of chromosomes of 85, 75 and 65, respectively. The scale bar represents 5 μm.