Comparison of Drug Resistance Mutations and Their Interpretation in Patients Infected With Non-B HIV-1 Variants and Matched Patients Infected With HIV-1 Subtype B

Brigitte Montes, PhD, Laurence Vergne, PhD,† Martine Peeters, PhD,† Jacques Reynes, MD, PhD,* Eric Delaporte, MD, PhD,*† and Michel Segondy, PhD**

Objective: To compare the prevalence of mutations associated with resistance to antiretroviral drugs and their interpretation in patients infected with non-B HIV-1 variants versus HIV-1 subtype B–infected patients with similar treatment regimens.

Methods: The reverse transcriptase (RT) and protease genes of HIV-1 were sequenced, and subtypes were determined by phylogenetic analysis. Each sequence belonging to a non-B variant was matched with a sequence belonging to subtype B. Patterns of resistance mutations were interpreted in terms of drug resistance using the HIV db algorithm.

Results: RT mutations M41L, L210W, and, to a lesser extent, T215Y were less prevalent in patients infected with non-B variants. This lower prevalence was associated with subtypes A (A1/A2), C, F (F1/F2), and CRF06_cpx. A lower prevalence of high-level resistance to zidovudine was also observed in patients infected with these HIV-1 variants. In the protease gene, differences between patients infected with B or non-B strains were mainly observed for mutations playing a minor role in drug resistance and known to occur mainly as a natural polymorphism in non-B strains: K20R/M/I, M36I, L63P, A71V/T, and V77I. Interpretation of genotypes using the HIV db algorithm indicated that resistance to saquinavir, ritonavir, indinavir, and amprenavir was more frequently a high-level resistance for subtype B and an intermediate-level resistance for non-B variants, but this difference was only significant for amprenavir.

Conclusion: Our results suggest that the genetic diversity of HIV-1 does not play a major role in the development of resistance to antiretroviral drugs.

Key Words: HIV-1, non-B variants, mutations, reverse transcriptase, protease, resistance

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From the *Departments of Virology and Infectious Diseases, University Hospital of Montpellier, Montpellier; and †Retrovirus Laboratory, UR036, Institut pour la Recherche et le Développement (IRD), Montpellier, France.

Reprints: Michel Segondy, Laboratoire de Virologie, Hôpital Saint-Eloi, 80 avenue Augustin Fliche, 34295 Montpellier Cedex 05, France (e-mail: m-segondy@chu-montpellier.fr).

Subtype B is the predominant variant of HIV-1 in western countries. This subtype plays a minor role in the HIV-1 pandemic, however, because most HIV-1 infections worldwide are caused by other subtypes or circulating recombinant forms (CRFs) such as subtype A (central and western Africa), subtype C (eastern and southern Africa, India, and China), CRF02_AG (western Africa), or CRF01_AE (Thailand and Southeast Asia).[1]

Genotypic tests for HIV-1 resistance are now routinely incorporated in the follow-up of HIV-1–infected patients to identify the role of resistance mutations in treatment failures and to guide the choice of a rescue regimen.[2,3] Genotypic studies have been mainly conducted in populations predominantly infected with HIV-1 subtype B, and drug resistance interpretation algorithms have not been validated on a large scale in populations infected with non-B variants.

It has been reported by several studies that minor drug resistance mutations in the reverse transcriptase (RT) and protease genes of HIV-1 are frequent in treatment-naive patients infected with non-B HIV-1 strains,[4–8] but these differences seem to have only a minor impact on drug susceptibility in vitro[9,10] or on the response to antiretroviral therapy in vivo.[11–14] The differences that could exist between subtype B and non-B subtypes in the development of drug resistance mutations in patients with treatment failure remain poorly documented, however. Taking into consideration the increasing access to antiretroviral therapy and the introduction of sequence-based genotypic assays in countries where non-B HIV-1 variants predominate, it is important to evaluate the possible impact of HIV-1 genetic diversity on genotypic testing of drug resistance. Therefore, the aim of the present study was to compare the RT and protease drug resistance mutations that developed in patients infected with B or non-B HIV-1 variants.

METHODS

Study Design

This study was conducted on HIV-1–infected patients attending the University Hospital of Montpellier between October 1999 and October 2002 and for whom demographic data, treatment history, CD4 cell counts, plasma viral load, and sequences of the RT and protease genes were available.

To compare populations of patients infected with B and non-B HIV-1 variants, each sequence obtained from a patient infected with a non-B strain was matched with a sequence obtained from a patient infected with a subtype B strain; non-B sequences belonging to the same patient but obtained after different treatment regimens were matched independently. Criteria for matching were the number and nature of the drugs administered, the number of previous treatment regimens, the drug combination received at the time of testing, and the duration of the failing regimen. Disease stage, CD4 cell count, viral load, and age and sex of patients were also taken into account as much as possible.

Gene Sequencing and Phylogenetic Analysis

HIV-1 subtyping was based on phylogenetic analysis of the *pol* gene.[15,16] RT (codons 1–240) and protease genes were amplified from plasma samples and sequenced as previously described.[15,17] The sequences were aligned with known HIV-1 RT sequences representing the different subtypes and CRFs by use of CLUSTAL W.[18] Phylogenetic trees were constructed by the neighbor-joining method, and reliability of the branching orders was assessed by the bootstrap approach with CLUSTAL W. For samples giving an ambiguous result by phylogenetic analysis of the *pol* gene, subtype characterization was obtained by amplification, sequencing, and phylogenetic analysis of the envelope V3 to V5 region as previously reported.[19] Sequences were submitted to the GenBank under the accession numbers AJ577726 to AJ577747, AJ577857 to AJ577996, and AJ578156 to AJ578179.

Data Analysis

In the present study, we considered mutations previously reported to be associated with drug resistance. In the protease gene, the mutations considered were L10I/F/V/R, K20R/M/I, L24I, D30N, V32I, L33F, M36I, M46I/L, I47V, G48V, I50V, F53L, I54V/T/L/M, L63P, A71V/T, G73S, V77I, V82A/T/F/S, I84V, N88S/D, L90M, and I93L/M/N. In the RT gene, the mutations considered were M41L, E44D/A, A62V, K65R, D67N, T69D/N/S/A, insertion 69, K70R, L74V, V75T/I/A/M/S, F77L, Y115F, F116Y, V118I, Q151M, M184V/I, L210W, T215Y/F, and K219Q/E for resistance to nucleoside analogue RT inhibitors (NRTIs) and A98G, L100I, K101E, K103N, V106A/M, V108I, Y181C/I, Y188C/L/H, G190A/S/E, P225H, and P236L for resistance to nonnucleoside RT inhibitors (NNRTIs). On the basis of the high frequency of their selection by antiretroviral treatments and/or their involvement in HIV-1 resistance to 1 or several antiretroviral drugs, mutations D30N, G48V, I50V, V82A/T/F/S, I84V, and L90M in the protease gene and M41L, K65R, D67N, insertion 69, K70R, L74V, K103N, V106A/M, Q151M, Y181C/I, M184V/I, Y188C/L/H, G190A/S/E, L210W, T215Y/F, and K219Q/E in the RT gene were considered as major resistance mutations; the other mutations were considered as minor resistance mutations.

Each protease and RT sequence was interpreted in terms of drug resistance by using the on-line HIV db algorithm (*http://hivdb.stanford.edu*), which, similar to the other currently available algorithms, is essentially based on data obtained from patients infected with HIV-1 subtype B. The high, intermediate, and low levels of resistance were considered, whereas viruses for which the algorithm indicated a potential low-level resistance to a drug were not considered as resistant to the drug.

The χ^2 test was used for categoric variables to compare proportions between patients infected with non-B HIV-1 variants and the matched subtype B–infected patients; the Yate continuity correction factor was applied to 2×2 contingency tables. The 2-tailed Fisher exact test was used when the sizes were too small. For continuous variables, comparisons were based on the nonparametric Mann-Whitney U test. All statistical tests were done by Statgraphics Plus software, version 5.1 (Manugistics, Rockville, MD). $P < 0.05$ was considered to be significant.

RESULTS

Patients and HIV-1 Genetic Variants

A total of 1586 sequences from 1109 patients were analyzed. Ninety-three sequences from 74 patients that did not cluster with HIV-1 subtype B and for which the corresponding demographic, therapeutic, clinical, and biologic data were available were retained for the present study. Among the patients infected with non-B HIV-1 variants, the subtype/CRF distribution in decreasing order of importance was as follows: 18 (24.3%) CRF02_AG; 9 (12.2%) CRF01_AE; 8 (10.8%) subtype D; 7 (9.5%) subtype G; 6 (8.1%) subtype A1; 6 (8.1%) subtype C; 5 (6.8%) CRF06_cpx; 5 (6.8%) CRF11_cpx; 4 (5.4%) subtype F1; 2 (2.7%) subtype A2; 1 (1.3%) of each subtype F2, K, and CRF12_BF; and 1 unique recombinant involving multiple subtypes.

Each non-B variant sequence was matched with a sequence obtained from a patient infected with HIV-1 subtype B. Overall, 93 RT and 83 protease sequences obtained from 74 patients infected with non-B HIV-1 variants were matched with 93 RT and 83 protease sequences from 93 subtype B–infected patients, respectively. Within each group, the protease gene was not sequenced for 10 protease inhibitor (PI)–naive patients. As shown in Table 1, the 2 groups of patients were similar in terms of therapeutic, demographic, clinical, virologic, and immunologic characteristics; they only differed in sex ratio and in the median duration of exposure to lamivudine, nevirapine, efavirenz, and nelfinavir.

Prevalences of Mutations Associated With Drug Resistance

Only mutations previously reported to be associated with drug resistance were considered in the present study.

TABLE 1. Characteristics Associated With the 93 Sequences Obtained From Patients Infected With Non-B HIV-1 Variants and the Matched Sequences Obtained From Patients Infected With HIV-1 Subtype B

Characteristic	HIV-1 Subtype		P*
	Non-B	B	
Age, median years (25th–75th percentiles)	40.3 (31.4–52.0)	40.7 (35.3–49.1)	0.51
Sex, % men	59.1	80.6	0.001
CDC class, no. (%)			0.45
A	19 (20.4)	26 (28.0)	
B	27 (29.0)	26 (28.0)	
C	47 (50.6)	41 (44.0)	
Viral load, median 10^3 copies/mL (25th–75th percentiles)	29.9 (7.9–94.1)	36.8 (5.1–111.0)	0.69
CD4 cell count, median cells/mm^3(25th–75th percentiles)	286 (140–395)	285 (160–442)	0.47
Number of antiretroviral regimens, median	2	2	0.83
Number of antiretroviral drugs, median	6	6	0.81
No. (%) patients with prior treatment with†			1.0
Zidovudine	80 (86.0)	82 (88.2)	
Didanosine	72 (77.4)	70 (75.3)	
Zalcitabine	19 (20.4)	19 (20.4)	
Stavudine	71 (76.3)	68 (73.1)	
Lamivudine	79 (84.9)	79 (84.9)	
Abacavir	39 (41.9)	38 (40.9)	
Nevirapine	35 (37.6)	35 (37.6)	
Efavirenz	19 (20.4)	22 (23.7)	
Saquinavir	42 (45.2)	37 (39.8)	
Ritonavir	39 (41.9)	38 (40.9)	
Indinavir	31 (33.3)	30 (32.3)	
Nelfinavir	41 (44.1)	40 (43.0)	
Amprenavir	4 (4.3)	7 (7.5)	
Lopinavir	11 (11.8)	9 (9.7)	
Cumulative exposure, median days (25th–75th percentiles) to			
Zidovudine	836 (458–1295)	780 (570–1200)	0.88
Didanosine	705 (338–1170)	600 (360–920)	0.36
Zalcitabine	360 (242–422)	360 (150–570)	0.75
Stavudine	660 (300–900)	690 (450–1080)	0.35
Lamivudine	661 (293–1118)	840 (585–1215)	0.03
Abacavir	296 (150–646)	345 (150–578)	0.97
Nevirapine	256 (97–330)	420 (210–570)	0.03
Efavirenz	184 (120–300)	375 (345–488)	0.01
Saquinavir	624 (225–735)	600 (368–1110)	0.55
Ritonavir	240 (90–780)	390 (188–796)	0.32
Indinavir	390 (236–840)	480 (203–735)	0.97
Nelfinavir	210 (106–390)	390 (228–668)	0.004
Amprenavir	137 (110–148)	90 (60–120)	0.18
Lopinavir	267 (158–344)	255 (173–366)	0.86
Duration of failing regimen, median days (25th–75th percentiles)	202 (75–390)	225 (90–405)	0.84

*χ^2 test or Mann-Whitney *U* test.
†None of the patients had received tenofovir at the time of testing.
CDC, Centers for Disease Control and Prevention.

Prevalences of the drug resistance mutations are presented in Table 2. In the RT gene, mutations M41L and L210W were significantly more prevalent in subtype B–infected patients ($P < 0.02$ and $P < 0.01$, respectively). Mutation T215Y/F was also more prevalent in patients infected with HIV-1 subtype B, but this difference was not significant ($P = 0.08$). The difference concerned only the T215Y mutation, which was identified in 27 and 40 patients infected with non-B and B HIV-1 strains, respectively ($P = 0.07$), whereas mutation T215F was identified in 10 patients from each group ($P = 1.0$).

Analysis of the prevalence of the RT mutations at codons 41, 210, and 215 among the different non-B subtypes or CRFs showed that the lower prevalence of the RT mutations M41L and L210W in non-B strains was associated with subtypes A1/A2, C, F1/F2, and CRF06_cpx ($P = 0.005$ and $P = 0.001$ for M41L and L210W, respectively) and that the lower prevalence of T215Y was associated with subtypes A1/A2 and C ($P = 0.002$).

At codon 106 of the RT gene, the V106M mutation was observed in 2 patients infected with non-B HIV-1 variants: 1 patient was infected with subtype C and the other was infected with CRF02_AG; both patients were treated with an efavirenz-containing regimen.

In the protease gene, mutations L63P, A71V/T, and V77I were significantly more prevalent in subtype B–infected patients, whereas the K20R/M/I and M36I mutations were significantly more prevalent in patients infected with non-B HIV-1 variants. At codon 20, the K20I substitution was significantly more prevalent in non-B–infected patients as compared with subtype B–infected patients (51.8% vs. 6.0%; $P < 0.0001$).

The lower prevalence of the L63P protease mutation in patients infected with non-B HIV-1 variants was associated with all the non-B subtypes and CRFs except subtype D and CRF06_cpx ($P < 0.001$). The lower prevalence of the A71V/T protease mutation was associated with all the non-B subtypes and CRFs except CRF06_cpx ($P = 0.007$), and the lower prevalence of the protease V77I mutation was associated with all the non-B subtypes and CRFs except D and F1 ($P < 0.001$). The higher prevalence of the K20R/M/I protease mutation in non-B viruses, mainly represented by the K20I substitution, was associated with subtype G, CRF02_AG, CRF06_cpx, and CRF11_cpx ($P < 0.0001$), and the higher prevalence of the protease M36I mutation was associated with all the non-B subtypes and CRFs ($P < 0.001$).

Interpretation of Mutations Associated With Resistance

The prevalences of the different levels of resistance to the antiretroviral drugs as determined by sequence interpretation using the HIV db algorithm are presented in Figure 1. Although not significant, non-B HIV-1 strains were less frequently resistant to zidovudine, stavudine, and tenofovir but more frequently resistant to nevirapine and efavirenz than HIV-1 subtype B strains. A lower prevalence of high-level resistance to zidovudine (6.6% vs. 40% for the matched patients; $P = 0.002$) was observed in the group of patients infected with subtypes A1/A2, C, F1/F2, and CRF06; no significant difference was observed for the other NRTIs between this group of patients and the matched HIV-1 subtype B–infected patients. It was also observed that resistance to saquinavir, ritonavir, indinavir, and amprenavir was more frequently a high-level resistance for subtype B viruses and an intermediate-level resistance for non-B viruses. This difference was only significant for amprenavir ($P = 0.03$), however.

DISCUSSION

Drug resistance mutations in non-B subtypes of HIV-1 have been analyzed in previous studies.[4–8,11–14,20–23] Few studies compared resistance mutations developed in patients infected with subtype B versus non-B HIV-1 variants. however. In this study, patients infected with non-B and B HIV-1 variants were matched for similar treatments. Between the 2 groups, only a difference in the median duration of exposure to lamivudine, nevirapine, efavirenz, and nelfinavir was observed, but this difference had no impact on the prevalence of mutations conferring specific resistance to lamivudine (M184V/I), NNRTIs, or nelfinavir (D30N and N88D). This apparently paradoxic observation can be explained by the rapid selection of the mutations conferring resistance to these drugs.[24–26] Patients in both groups were also similar for age; disease stage; and clinical, immunologic, and virologic status; they differed only in sex ratio. The higher proportion of women in the group of patients infected with non-B HIV-1 subtypes can be explained by the fact that the subtype B–infected patients acquired HIV-1 infection predominantly by homosexual contact in France, whereas patients infected with non-B variants were predominantly heterosexuals. In this latter group, many individuals were from African origin or had an epidemiologic link with this continent, where non-B strains predominate. We assume that this difference in sex ratio has no influence on the development of the different drug resistance mutations. Differences in adherence to treatment as well as differences in pharmacologic determinants that could exist between the 2 groups of patients might have an impact on the development of resistance mutations. These data were not available, however, and could not be taken into account for the sequence-matching process.

The major difference observed between B and non-B strains for drug resistance mutations in the RT gene was a lower prevalence of the M41L and L210W mutations and, to a lesser extent, the T215Y mutation in patients infected with non-B HIV-1 strains, particularly in those infected with subtypes A (A1, A2), C, F (F1, F2), and CRF06_cpx. A close association between mutations M41L, L210W, and T215Y and a mutual exclusion between mutations K70R and L210W

TABLE 2. Frequency of the Drug Resistance Mutations in Sequences Obtained From Patients Infected With non-B HIV-1 Variants and the Matched Sequences Obtained From Patients Infected With HIV-1 Subtype B

Mutation	HIV-1 Subtype		*P*
	Non-B	B	
RT*			
NRTIs			
M41L	29 (31.2)	45 (48.4)	0.02
E44D/A	10 (10.8)	12 (12.9)	0.82
A62V	2 (2.2)	2 (2.2)	1.0
K65R	3 (3.2)	0 (0.0)	0.24
D67N	34 (36.6)	38 (40.9)	0.65
T69D/NS/A	6 (6.5)	10 (10.8)	0.43
Insertion 69	1 (1.1)	4 (4.3)	0.36
K70R	23 (24.7)	19 (20.4)	0.60
L74V	10 (10.8)	9 (9.7)	1.0
V75T/I/AM/S	4 (4.3)	2 (2.2)	0.68
F77L	1 (1.1)	0 (0.0)	1.0
Y115F	0 (0.0)	4 (4.3)	0.13
F116Y	1 (1.1)	0 (0.0)	1.0
V118I	13 (14.0)	23 (27.7)	0.09
Q151M	1 (1.1)	0 (0.0)	1.0
M184V/I	49 (52.7)	48 (51.6)	1.0
L210W	16 (17.2)	32 (34.4)	0.01
T215Y/F	37 (39.8)	50 (53.8)	0.08
K219Q/E	25 (26.9)	16 (17.2)	0.16
NNRTIs			
A98G	8 (8.6)	8 (8.6)	1.0
L100I	2 (2.2)	2 (2.2)	1.0
K101E	8 (8.6)	4 (4.3)	0.37
K103N	20 (21.5)	18 (19.4)	0.85
V106A/M	4 (4.3)	1 (1.1)	0.36
V108I	1 (1.1)	4 (4.3)	0.36
Y181C/I	16 (17.2)	13 (14.0)	0.69
Y188C/L/H	1 (1.1)	2 (2.2)	1.0
G190A/S/E	17 (18.3)	15 (16.1)	0.85
P225H	2 (2.2)	1 (1.1)	1.0
P236L	0 (0.0)	1 (1.1)	1.0
Protease†			
L10I/F/V/R	34 (41.0)	31 (37.3)	0.75
K20R/M/I	53 (63.9)	14 (16.9)	0.04
L24I	0 (0.0)	0 (0.0)	—
D30N	4 (4.8)	6 (7.2)	0.74
V32I	0 (0.0)	1 (1.2)	1.0
L33F	2 (2.4)	3 (3.6)	1.0
M36I	71 (85.5)	22 (26.5)	<0.0001
M46I/L	20 (24.1)	19 (22.9)	1.0
I47V	1 (1.2)	1 (1.2)	1.0
G48V	2 (2.4)	6 (7.2)	0.28
I50V	0 (0.0)	1 (1.2)	1.0
F53L	5 (6.0)	1 (1.2)	0.21
I54V/T/L/M	19 (22.9)	16 (19.3)	0.70
L63P	33 (39.8)	57 (68.7)	0.0003
A71V/T	14 (16.9)	29 (34.9)	0.013
G73S	4 (4.8)	5 (6.0)	1.0
V77I	5 (6.0)	18 (21.7)	0.007
V82A/T/F/S	16 (19.3)	18 (21.7)	0.85
I84V	9 (10.8)	13 (15.7)	0.49
N88D‡	3 (3.6)	5 (6.0)	0.72
L90M	18 (21.7)	23 (27.7)	0.23
I93L/M/N	14 (16.9)	18 (21.7)	0.55

*N = 93.
†N = 83.
‡The N88S mutation was not observed.
Data are no. (%) sequences.
Major resistance mutations are indicated in bold.

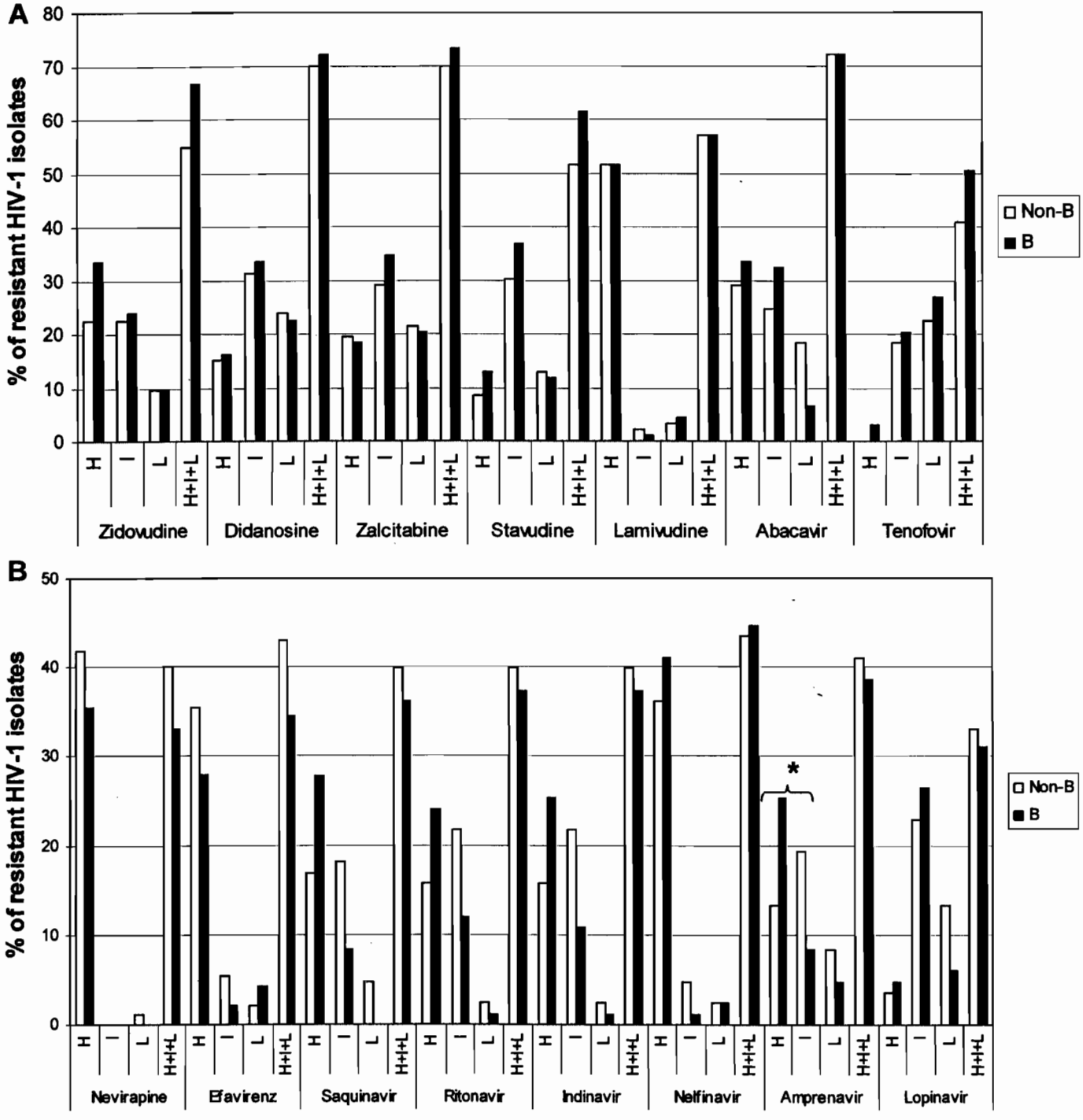

FIGURE 1. Prevalence of resistance levels of HIV-1 isolates to nucleoside analogue reverse transcriptase inhibitors (A) and non-nucleoside reverse transcriptase inhibitors and protease inhibitors (B), as interpreted by using the HIV db algorithm. H, high level of resistance; I, intermediate level of resistance; L, low level of resistance. *$P < 0.05$.

have been previously reported.[27] On the other hand, an extensive polymorphism at codons not involved in drug resistance has been identified in the RT gene of non-B HIV-1 variants.[8,23] Therefore, it can be hypothesized that the differences in the RT amino acid sequences between HIV-1 subtypes resulting from natural polymorphism could have a negative impact on the development of the M41L, L210W, and T215Y mutational patterns. Alternatively, the underrepresentation of M41L and

L210W in patients infected with non-B subtypes could be explained by a preference for developing T215F rather than T215Y in this group of patients. Our results do not validate this hypothesis, however, because no difference was observed in the prevalence of the T215F mutation between the 2 groups of patients. Mutations M41L, L210W, and T215Y/F are important for resistance to NRTIs (nucleoside-associated mutations [NAMs]), particularly for resistance to zidovudine.[28] The lower prevalence of these mutations in patients infected with non-B subtypes of HIV-1 seems to have a low impact on interpreted resistance to NRTIs, however. Indeed, differences in resistance to NRTIs observed between the groups of patients infected with B or non-B HIV-1 subtypes were not significant. A significant lower frequency of high-level resistance to zidovudine was only observed in the group of patients infected with subtypes A1/A2, C, F1/F2, and CRF06, characterized by a decreased prevalence of mutations M41L, L210W, and T215Y. The lack of difference in the prevalence of the other NAMs such as D67N, K70R, and K219Q/E can explain the lack of significant difference in the frequency of resistance to zidovudine (all levels of resistance combined) between patients infected with B and non-B HIV-1 strains.

Recently, it has been reported that patients infected with subtype C develop the V106M mutation, which confers cross-resistance to NNRTIs after treatment with efavirenz[29] or nevirapine.[30] In the present study, the V106M mutation was identified in 2 patients infected with non-B HIV-1 strains treated with an efavirenz-containing regimen. One of these patients was infected with a CRF02_AG strain, suggesting that the V106M mutation can also be selected by subtypes/CRFs other than C.

The higher prevalence of the protease mutations K20R/M/I and M36I and the lower prevalence of the protease mutations L63P, A71V, and V77I in treatment-naive patients infected with non-B strains have been previously reported.[7,8,23,31–33] Therefore, the differences in the prevalence of the mutations in the protease gene observed in the present study between patients infected with B and non-B HIV-1 strains probably result from the natural polymorphism of the protease gene and not from the selection of these mutations by antiretroviral therapy. The different prevalences of these minor resistance mutations to PIs, mainly as a result of natural polymorphism in non-B HIV-1 strains, seems to have a low impact on genotype-based resistance interpretation. Indeed, no difference was observed in the frequency of resistance (all levels of resistance combined) to PIs between patients infected with B or non-B HIV-1 viruses, whereas differences in the levels of resistance to PIs were only significant for amprenavir. On the other hand, patients infected with B or non-B HIV-1 viruses showed no difference in the prevalence of the major resistance mutations to PIs. This finding suggests that the development of the major resistance mutations to PIs during the course of therapy is similar between patients infected with B or non-B HIV-1 variants.

At codon 88 of the protease gene, N88S and N88D mutations have different phenotypic effects.[34] In a study comparing the resistance mutation patterns in patients infected with HIV-1 subtype B or CRF01_AE, it has been reported that the N88D mutation was found exclusively in patients infected with subtype B, whereas mutation N88S was more frequently identified in patients infected with CRF01_AE.[35] In the present study, the N88D mutation was rarely observed in patients infected with subtype B (5 patients) and non-B subtypes (1 subtype D, 2 subtype G), and the mutation N88S was not identified.

Our results show that the genetic diversity of HIV-1 has a minor influence on drug resistance levels determined by sequence interpretation. Resistance interpretation depends on the algorithm used, however. In the present study, resistance interpretation was based on a widely used algorithm, but it cannot be excluded that the differences between patients infected with subtype B and non-B variants would have been more significant if an alternative algorithm had been used.[36] On the other hand, the currently available algorithms are essentially based on data obtained with HIV-1 subtype B, and their relevance for interpreting sequences obtained from non-B subtypes remains to be confirmed.

Differences in naturally occurring or drug-selected resistance mutations can exist between the different non-B subtypes/CRFs. Sample sizes of the different non-B variants identified in the present study were too small to be individually analyzed, however; additional studies conducted in countries where these non-B subtypes/CRFs are highly prevalent could find additional differences and identify novel mutations associated with resistance.

Our results suggest that the genetic diversity within HIV-1 group M probably does not play a major role in the development of resistance to antiviral drugs. Resistance to antiretroviral drugs was not higher in patients infected with non-B viruses; to the contrary, a tendency to a lower level of resistance to zidovudine and PIs was observed in patients infected with non-B HIV-1 variants. Consequently, the efficacy of antiretroviral therapy should not be impaired in countries where non-B HIV-1 strains are highly prevalent.

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