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Role of Genetic Diversity amongst HIV-1 Non-B Subtypes in Drug Resistance: A Systematic Review of Virologic and Biochemical Evidence

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Abstract

The genetic diversity of HIV-1 has required its classification into types and subtypes. There is controversy as to how and to what extent genetic diversity may affect the emergence of antiretroviral drug resistance in HIV-1 subtypes other than B. To better understand the impact of genetic diversity (represented by different HIV-1 subtypes) on resistance to reverse transcriptase and protease inhibitor drugs, a systematic review was conducted on virologic and biochemical evidence obtained from work with non-B HIV-1 subtypes. We searched 11 databases and retrieved 3,486 citations on all aspects of non-B subtype-related resistance research. Twenty-seven studies with virologic and/or biochemical data met the eligibility criteria for our systematic review. Nineteen studies were found that reported phenotypes in non-B subtypes (304 from naive isolates and 242 from drug-exposed isolates) and 11 studies that used molecular biology techniques to study non-B resistance to antiretroviral drugs. Compared to the NL4-3 laboratory strain, lower baseline susceptibilities of recombinant A/G subtype virus to protease inhibitors were observed and a substantial proportion of subtype C isolates displayed higher IC_{50} at baseline for atazanavir. Some A/G isolates were found to have reduced susceptibility to abacavir. Mutations not typical of B subtypes include the reverse transcriptase mutation V106M and the protease mutations M89I/V and N83T. Virologic and biochemical data suggest that K65R is more likely to emerge in subtype C HIV-1. There is evidence to suggest differential effects of other mutations according to subtype, e.g. the protease inhibitor mutations I93L and M89I/V. Importantly, the most widely used commercial phenotyping systems do not take into account gag variations among natural isolates, which could limit the accuracy of measured susceptibility. Enzymatic and virologic data support the concept that naturally occurring polymorphisms in different non-B subtypes can affect the susceptibility of HIV-1 to different antiretroviral drugs, the magnitude of resistance conferred by major mutations, and the propensity to acquire some resistance mutations. Tools may need to be optimized to accurately measure drug susceptibility of non-B subtypes, especially for protease inhibitors. **(AIDS Rev. 2008;10:212-23)**

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Key words

Genetic diversity. HIV-1. Non-B subtypes. Drug resistance.

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Background

Almost all HIV genetic sequences can be classified into subtypes or recombinant forms, even though problems in classification can occasionally arise. Due to genetic relatedness, it is reasonable to think that viruses belonging to a certain subtype will respond in a similar way to antiretroviral drug pressure. However, responsiveness of HIV-1 to antiretroviral drugs might vary among subtypes, particularly if major dissimilarities are present. It might also be expected that responsiveness might be predictable for each individual subtype.

Genotyping of HIV obtained from plasma samples has been used for two important purposes. First, genotyping has permitted the detection of mutations responsible for drug resistance in patients failing therapy. Second, it allows surveillance of epidemiological trends in various geographic areas and populations. Therefore, HIV-1 subtyping may become important for the prediction of resistance patterns. Since the best opportunity for long-lasting antiretroviral treatment efficacy is the first therapeutic regimen, a clear knowledge of the potential for resistance and cross-resistance in different subtypes is important.

Data used to define drug resistance mutations have been generated almost entirely from subtype B HIV-1[1,2]. However, non-B subtypes are dominant throughout the world and are also increasingly prevalent in countries that had traditionally been affected primarily by subtype B[3-7]. This has resulted in a need to understand the following: (i) how the frequency of resistance mutations among certain subtypes may be different than those described in subtype B; (ii) whether the timing of the emergence of resistance may differ among subtypes; (iii) how the efficacy of second-line drugs might be affected to differential extent by resistance mutations within each subtype; and (iv) the influence of genetic characteristics among people infected by different subtypes on clinical outcome (virologic failure and/or CD4 responses).

The interpretation of degrees of resistance by popular algorithms is widely accepted and used in clinical practice in all countries in which B subtypes predominate. However, such algorithms may be less accurate in assessing resistance in non-B subtypes, in part because of differences in the genetic backgrounds among subtypes. In fact, several studies comparing different HIV resistance algorithms revealed high degrees of discordance in the interpretation of protease inhibitor resistance when applied to non-B subtypes[8-11].

Our primary goal was to assemble the relevant virologic and biochemical evidence on the role of polymorphisms of non-B subtypes on resistance to reverse transcriptase inhibitors (RTI) and protease inhibitors (PI). Therefore, we conducted a systematic review of all literature published in English language on non-B subtypes. Our secondary objectives were to (i) summarize and evaluate available knowledge in regard to antiretroviral resistance to non-B subtypes, (ii) identify gaps in knowledge, and (iii) delineate research that might be needed to fill such gaps. Herein we review the literature on virologic and biochemical aspects of HIV drug resistance in non-B HIV-1 subtypes.

Review strategy

This review strategy covers the period January 1996 to December 2007. We limited our search to publications in English. Eleven electronic databases for full text articles and conference abstracts were searched: PUBMED (1996-2007), Web of Science (1996-2007), EMBASE (1996-2005), BIOSIS (1996-2007), AIDSLINE (1996-2007), OVID (1996-2007), Psychinfor (1996-2007), Cochrane controlled trials register (1996-2007), DARE (1996-2007), COCHRANE (1996-2007), ILLUMINA (1996-2007). Bibliographies and references from primary studies and review articles were also searched. If full-text articles were not available, abstracts or letters were used, provided they contained relevant information and were of sufficient completeness. Conference websites were also searched and abstracts with pertinent data were reviewed. Abstracts which were later found as full-text publications were excluded and the full-text publication was examined instead. We gave primary importance to sensitivity of the search. Our search string included key words and search terms as follows:

Search #1: "HIV"[MeSH] OR "HIV-1"[MeSH] OR "HIV-1"[TI]

Search #2: "non-b"[TIAB] OR "subtype*"[TI] OR "clade"[TI] OR "strain*"[TI] OR "variant*"[TI] OR "non-B subtype*"[TIAB]

The same key words were used for searches in databases in which MeSH terms are not applicable including conference search engines. We also extracted additional information from reference lists of reviews on non-B subtype HIV-1.

Study selection

The study selection methodology is shown in figure 1. After pooling, a total of 5,288 citations were identified. After excluding duplicates, 3,486 citations were selected for further review. From these, 405 were related to resistance to antiretrovirals in non-B subtype HIV-1. All citations had full reports and were reviewed. After applying our inclusion and exclusion criteria, consistent with the goals of our review, 30 articles were included and carefully examined.

Inclusion criteria

Based on our objectives, we included two types of studies: (i) those that identified *in vitro* drug susceptibility of isolates (viral phenotype), and (ii) studies using molecular biological techniques such as site-directed mutagenesis,

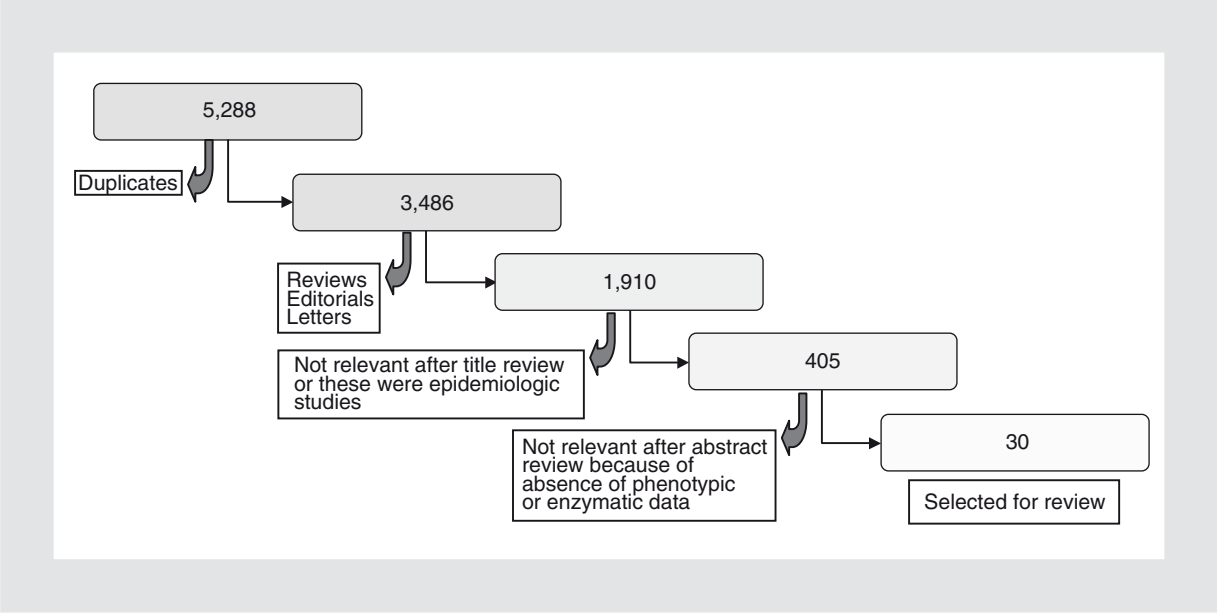

Figure 1. *Selection of studies in the systematic review.*

enzymatic assays, and competition assays in order to understand the effect of resistance mutations on viral and/or enzymatic function.

Exclusion criteria

We excluded news reports, duplicates, animal experiments, modeling studies, methods papers, comorbidity studies, reviews, editorials, perspectives, and opinion pieces. We also excluded (i) studies that dealt only with genetic and epidemiologic diversity and that did not evaluate impact on antiretroviral resistance, (ii) vaccine studies related to non-B subtypes, and (iii) studies that combined all non-B subtypes into a single analysis.

Results

The process of data selection is shown in figure 1. A total of 30 studies were found to meet our inclusion criteria. The studies of drug susceptibility testing of non-B subtypes are summarized in table 1 and the studies that used molecular biological methods and drug resistance selection are summarized in table 2.

Studies of drug susceptibility testing of non-B subtype HIV-1

Nineteen studies that measured HIV-1 susceptibility of non-B subtype strains to antiretrovirals were included. The total number of samples whose phenotypes have been published and included in this review is 546, of which 242 were from patients who had been exposed to antiretrovirals. Overall, the majority of non-B HIV-1 subtypes possessed wild-type susceptibilities similar to those of subtype B wild-type isolates (Table 1).

In untreated patients, decreased susceptibility to PI in some non-B subtype isolates was found, though infrequently. Compared to subtype B, diminished susceptibilities among wild-type isolates have been found for A/G recombinant viruses in two different studies. In the first, lower susceptibilities to nelfinavir and lopinavir were reported, while in the second, four of 12 isolates had lower susceptibility to atazanavir[12,13]. The first study did not test atazanavir. Neither study was designed to test statistical significance related to drug susceptibility levels based on the presence of polymorphisms. The study by Kinomoto, et al.[12] also performed molecular modeling and suggested that distortions in the K26 pocket of A/G proteases appear to be responsible for a lower binding energy of nelfinavir and hence lower susceptibility of A/G viruses to this drug. Vergne, et al.[14] reported isolates with susceptibilities slightly above the biological test cutoffs for ritonavir as follows: one of two CRF06 isolates (FC = 3), one of two CRF13 isolates (FC = 4.7), and one of two F isolates (FC = 2.4) (ritonavir biological cutoff = 2.4). For nelfinavir and saquinavir, respectively, this was true for one of two B isolates (FC = 2.6) (nelfinavir biological cutoff = 2.3) and a single URF A/G/J isolate (FC = 2.1) (saquinavir biological cutoff = 1.7).

With regard to RTI, the study by Vergne, et al.[14] reported that one of three G isolates had low susceptibility to efavirenz but not nevirapine, one of 12 CRF02 had low susceptibility to efavirenz and nevirapine, one URFA/D/A isolate

Table 1. Phenotype-genotype studies

Study	Type of phenotyping test used	Type of isolates	Drugs tested	Samples (n)	Subtypes tested (n)	Findings
Abecasis, 2005[16]	Antivirogram®	PI-treated patients	RTV, NFV, APV, IDV, LPV, ATV	96	G (55), F (9), AG (18), C (9), URF (5)	M89I/V only associated with higher susceptibility to all PIs tested (especially ATV), except to LPV. The combination M89I/V+L90M was associated with higher IC_{50} FC for APV, ATV, IDV, but to lower IC_{50} FC for NFV, RTV and IDV.
Abecasis, 2006[17]	Antivirogram®	Naive individuals	APV, IDV, LPV, NFV, SQV, ATV, RTV, TPV	42	G (19), AG (10) F(6), C(7) For TPV, G (14), AG (10) F(4), C(4)	AG samples were found more susceptible to NFV and RTV. Hypersusceptibility to NFV and RTV was associated with the 70R polymorphism. 37D/S/T was associated to lower susceptibility to IDV and 89M to reduced susceptibility to LPV. Subtype F had the lowest susceptibility to TPV.
Agwale, 2006[43]	PhenoSense™	Naive individuals	ABC, ADV, ddI, 3TC, d4T, ddC, ZDV, DLV, EFV, NVP, APV, IDV, NFV, RTV, SQV	18	AG (14) and G (4)	2 subtype G isolates had high IC_{50}. One for RTV, NFV and APV and the other for RTV and NFV.
Apetrei, 1998[44]	In-house phenotype susceptibility assay	Naive individuals	ZDV, 3TC, DLV, NVP, TIBO, SQV, RTV	5	Type O. Subtypes B and F	Although the IC_{50} were higher for the subtype F strains than for the subtype B strains, all the subtype F isolates were susceptible to PI. All were also sensitive to the NNRTI NVP and DLV.
Caride, 2000[45]	Recombinant virus technology VIRCO	Treated patients failing HAART	AZT, 3TC, ddI, ddC, d4T, ABC, ADV, NVP, DLV, EFV	14	A (1), F (4), B (9)	Same mutation pattern for NRTI and NNRTI found in non-B subtype isolates. Although patients received similar PI drugs, non-B subtypes did not present the L90M and I84V mutations and used mainly G48V and V82A/F to achieve drug resistance.
Caride, 2001[46]	Recombinant virus technology VIRCO	Treated patients failing HAART	IDV, RTV, NFV, SQV	14	A (1), F (4), B (9)	Primary mutations in B similar to A and F. The L90M mutation was rare in A and F. The IC_{50} values for A and F isolates were high similar to those found in B isolates
Eshleman, 2006[41]	PhenoSense™ HIV assay	Patients who received NVP for mother-to-child transmission prevention	DLV, EFV, NVP	10 isolates, 51 infectious clones were generated; and 29 were selected for analysis.	A (29)	26 of 29 isolates had resistance mutations consistent with those typically observed in B subtype. One clone had 5-8 fold increase in IC_{50} and had wild-type genotype; one clone had wild-type susceptibility and had K103N; and one clone had the G190A mutation and had hypersusceptibility to DLV.

(continue)

Table 1. Phenotype-genotype studies *(continued)*

Study	Type of phenotyping test used	Type of isolates	Drugs tested	Samples (n)	Subtypes tested (n)	Findings
Fleury, 2006[13]	Phenoscript™	Naive individuals	AZT, 3TC, d4T, ddI, ABC, TFV, NVP, EFV, IDV, NFV, APV, SQV, LPV, ATV, ENF	37	AE (12), AG(12), C or AC (13)	1 CRF01_AE, 2 CRF02_AG, and 4 subtype C strains exhibited FC above the cut-off for ATV.
Grossman, 2004[47]	PhenoSense™	7 naive and 16 IDV and NFV treated patients failing regimen	APV, IDV, LPV, NFV, RTV, SQV	23	C (23)	D30N mutation caused large reductions in susceptibility to NFV but not to other PI were seen. When the L90M mutation was present in combination with additional mutations (e.g. M46I, I54V, A71V, V82A, and/or I84V), isolates demonstrated reduced susceptibility to several PI.
Holguin, 2004[48]	Phenosense™ HIV. Virologic™	Naive individuals	RTV, IDV, SQV, NFV, LPV, APV, LPV	58	G (29), C(22), A92), J(2), D(2), F(1)	All isolates were susceptible to tested PI.
Holguin, 2006[49]	PhenoTect™ (InPheno, Switzerland) and PhenoSense™(HIVTM, Monogram, USA)	Naive individuals	APV, IDV, LPV, NFV, RTV, SQV	16	G (9), AG (1), C (1), H (1), A (4)	Only 1 isolate showed substantially decreased susceptibility to a PI drug. One G subtype had a 2.9 IC_{50} FC. 10 of 16 samples (with Phenotect™) and 5 of 16 samples (with PhenoSense™) showed hypersusceptibility to APV.
Kinomoto, 2005[12]	In-house phenotype susceptibility assay	Naive individuals	NFV, IDV, RTV, LPV, SQV, APV	39	AG (39)	Ghanaian HIV-1 CRF02_AG are differentially less susceptible to the PI originally designed and tested for subtype B, in the following order: NFV=LPV>IDV>SQV>RTV>APV.
Palmer, 2001[50]	In-house phenotype susceptibility assay	Naive individuals	AZT, TFV, ADV	25	A (4), AC, B (3), C (3), D (3), AE (3), F (3), G (3), O (3)	Naive isolates were similarly susceptible to tested drugs.
Palmer, 1998[51]	In-house phenotype susceptibility assay	Naive and treated patients; AZT only (5), PFA (1), AZT+ddI (1), AZT+3TC (1)	AZT, 3TC, ddI, RTV, NVP, PFA	31	A, B, C, D, E (AE) n of each subtype not available	Isolates from naive patients were susceptible to all tested drugs. Reduced susceptibility was found only in 3 of 5 isolates from patients who had received therapy.
Richard, 2004[52]	Virologic™	Treated patients	Only drugs to which viruses had decreased susceptibility were reported	6	D (6)	Phenotype was consistent with prediction by genotypic analysis used*.

(continue)

Table 1. Phenotype-genotype studies *(continued)*

Study	Type of phenotyping test used	Type of isolates	Drugs tested	Samples (n)	Subtypes tested (n)	Findings
Shafer, 1999[53]	In-house phenotype susceptibility assay	Naive individuals	SQV, RTV, IDV, NFV, AZT, ddI, 3TC, NVP	5	C (Zimbabwe)	All isolates were fully susceptible to the tested drugs.
Tanuri, 1999[54]	In-house phenotype susceptibility assay	Naive individuals	SQV, IDV	15	B(9), F(6)	Similar susceptibility ranges.
Vergne, 2006[14]	Antivirogram® (Virco, Belgium)	Naive	APV, ATV, IDV, LPV, NFV, RTV, SQV, 3TC, ABC, ZDV, d4T, ddI, FTC, TFV, DLV, EFV, NVP	35	A (3), B (2), D (2), F (2), G (3), CRF02_AG (12), CRF06 (2), CRF09 (1) , CRF11 (4),13 (2), URFA/D/A (1), URFA/G/J (1)	Minor mutations did not influence wild-type PI susceptibilities in vitro, despite their number. 4 strains had in vitro susceptibilities with an IC_{50} FC above the biological cutoffs.
Weidle, 2003[15]	Antivirogram® (VIRCO, Belgium)	Treated patients 2 NRTI + ABC, 1 NNRTI, and/or 1 PI. Therapy was categorized as two NRTI if the regimen included 2 NRTI with or without hydroxyurea	NVP, DLV, EFV, IDV, RTV, SQV, APV, LPV	94	D/D (42), A/A (41), A/D (4), C/C (3), D/A (2), D/C (1).	24 specimens had HIV with phenotypic resistance to at least one drug. Resistance to > one class was found in 18%.

*Phenotypic assay by Virologic™ and at the genotypic interpretation by the Joint Clinical Research Centre in Kampala, Uganda.
AZT: zidovudine; 3TC: lamivudine; FTC: emtricitabine; d4T: stavudine; ddI: didanosine; ddC: zalcitabine; ABC: abacavir; TFV: tenofovir; ADV: adefovir; NVP: nevirapine; EFV: efavirenz; DLV: delavirdine; IDV: indinavir; NFV: nelfinavir; APV: amprenavir; SQV: saquinavir; LPV: lopinavir; ATV: atazanavir; TPV: tipranavir; ENF: enfuvirtide; TIBO: a tetrahydroimidazo[4,5,1-*jk*][1,4]-benzodiazepin-2(1*H*)-one and thione derivate; PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor.

Table 2. Studies using molecular biology techniques to characterize impact of mutations

Study	Technique	Drugs tested	Mutations studied	Findings
Brenner, 2003[25]	B vs. C subtype HIV-1 drug resistance selections, phenotyping.	NVP, EFV	Drug-selected mutations during tissue culture of HIV-1.	In tissue culture, and in addition to K103N, the mutation V106M emerges in HIV-1 subtype C upon EFV drug pressure. Such mutation confers resistance to also NVP and DLV. V106M is not selected by NVP nor DLV. V106M did not emerge in B subtype HIV.
Brenner, 2006[23]	Drug resistance selections.	TFV	Drug-selected mutations during tissue culture of HIV-1.	K65R arose under TNF drug pressure by week 12 in four subtype C selections. In contrast, no TNF resistance arose in four subtype B (> 34-74 weeks), one each of CRF2_AG and G (> 30-33 weeks), and three HIV-2 (> 27-28 weeks) selections. K65R appeared after 55 and 73 weeks in two CRF1_AE selections with TNF.
Clemente, 2006[22]	Crystallographic and kinetic studies.	RTV, IDV, NFV, SQV, APV, LPV, ATV, TPV	Resistance mutation 82F in B and AE subtype proteases. Reversion of 82F→V in a post-therapy AE protease.	Inhibition analysis with PI revealed that the natural polymorphisms found in A/E can influence inhibitor resistance.
Coman, 2007[21]	Crystallographic analysis, kinetic studies of subtype B and C HIV-1 proteases.	RTV, IDV, NFV, SQV, APV, LPV, ATV, TPV	Resistance mutations D30N, L90M of N88D or double and triple mutants with such mutations within the natural B and C protease background polymorphisms.	Preexistent polymorphisms in subtype C protease, by themselves, do not provide for a greater level of resistance. Conversely, the natural polymorphisms found in subtype C protease, in combination with drug resistance mutations can influence enzymatic catalytic efficiency and inhibitor resistance.
Coutsinos, 2007[24]	Reverse transcription with B and C RT on B and C RNA and cDNA templates.	TFV	Subtype B vs. C	Either subtype C or B RT is more prone to introduce the mutation K65R when synthesizing DNA from a subtype C RNA template.
González, 2003[20]	B(3) and C(8) clinical isolates phenotyping and site-directed mutagenesis. One C subtype clone (C6) carrying the C consensus polymorphisms (I15V, M36I, R41K, H69K, L89M, and I93L) and one C clone with all C consensus polymorphisms except I93L.	LPV, SQV, NFV, APV, RTV, IDV	Clinical isolates of C and subtype carrying and not carrying I93L mutation.	The conclusion was that hypersusceptibility to LPV in C subtype HIV-1 is strongly associated to presence of I93L mutation.

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Table 2. Studies using molecular biology techniques to characterize impact of mutations *(continued)*

Study	Technique	Drugs tested	Mutations studied	Findings
González, 2004[55]	B,C replication kinetics.	NFV	Effect of mutations D30N, L90M, D30N/N88D in B and C, and mutations D30N/ N83T, D30N/M89L in B and C proteases.	D30N and L90M have a stronger negative impact in replicative capacity of C subtype than in B subtype. N88D restores replicative capacity of C and B carrying D30N. Also, only in C, N83T and M89L partially restore replicative capacity.
Holguin, 2006[49]	Site-directed mutagenesis and viral competition assays.	SQV, RTV, NFV, APV, IDV, LPV	NL4-3 with and without K20I, M36I or K20I/M36I.	K20I and M36I were associated with a diminished *in vitro* susceptibility to SQV and IDV, and thereby provided a significant replication benefit (up to 3.5-fold) in comparison to the wild-type clone.
Quan, 2003[56]	Site-directed mutagenesis and drug susceptibilities in cell-free assays.	3TC, NVP, DLV, EFV	Subtypes B, AE and C.	Subtype C RT-16 containing K103N/V106M was as resistant as subtype B, that is, RT-2 containing L100I/K103N, and showed high-level NNRTI cross-resistance. Of note, subtype C RT-16 and RT-17, harboring V106M together with other mutations, showed high-level NNRTI cross-resistance with > 16-fold, 3-fold, and > 200-fold increases for NVP, DLV, and EFV, respectively.
Velazquez-Campoy, 2001[18]	Catalytic activity of A, B, C subtype proteases.	IDV, RTV, SQV, NFV	Polymorphisms present in proteases of each subtype.	PI have higher Kis for the A and C subtype proteases. Biochemical fitness of these proteases is higher than that of the B subtype protease.
Velazquez-Campoy, 2002[19]	Calorimetric and kinetic parameters of A, B, C proteases carrying mutation V82F/I84V.	IDV, NFV, SQV	V82F/I84V within the background of natural polymorphisms of different HIV-1 protease subtypes.	The changes in binding affinity induced by the V82F/I84V mutation are similar and independent of the subtype background. However, the background polymorphisms in A and C proteases amplify the effect of mutations V82F/I84V when compared to B proteases.

AZT: zidovudine; 3TC: lamivudine; FTC: emtricitabine; d4T: stavudine; ddI: didanosine; ddC: zalcitabine; ABC: abacavir; TFV: tenofovir; NVP: nevirapine; EFV: efavirenz; RTV: ritonavir; IDV: indinavir; NFV: nelfinavir; APV: amprenavir; SQV: saquinavir; LPV: lopinavir; ATV: atazanavir; TPV: tipranavir; ENF: enfuvirtide; PI: protease inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor.

had reduced susceptibility to efavirenz and nevirapine, and one CRF02 isolate had reduced susceptibility to zidovudine. Fleury, et al.[13] described that several viruses had above average cutoff values in the absence of typical resistance mutations. One of 12 A/E isolates, which had decreased susceptibility to nevirapine and efavirenz, possessed an I135T substitution. Of 12 A/G subtype isolates, two had an IC_{50} FC above the cutoff for abacavir. The D123N + I135V mutations were associated with a decrease in susceptibility to this drug. The I135T mutation was also associated with lower susceptibility to nonnucleoside reverse transcriptase inhibitors (NNRTI) in subtype C.

Regarding isolates obtained from ART-treated patients, a study by Weidle, et al.[15] reported that 96% (n = 94) of isolates resistant to stavudine or zidovudine had at least one commonly recognized mutation for zidovudine resistance. Eleven of 17 had typical NNRTI resistance mutations. Seventeen specimens had phenotypic resistance to at least one PI. Two patients, who had never received PI, had low-level PI resistance and had no major mutations. All patients who received nelfinavir (n = 9) had resistance to nelfinavir. Six of these possessed subtype D viruses, all with the D30N mutation. Three were subtype A and did not have mutations at position 30 (two had M46I and one had N88S). Abecasis, et al.[16] studied the effect of the M89I mutation on viral susceptibility to PI. Such a mutation, which has been found to emerge in F, G, and C subtypes after nelfinavir failure, was also associated with increased susceptibility to other PI, except for lopinavir. Also, the combination of mutations at position 89 together with the L90M mutation had a differential effect on susceptibility to other PI (Table 1). In addition, the same authors reported that other polymorphisms were associated with either reduced or increased susceptibility to PI in a differential fashion in A/G, C, and F subtypes (Table 1)[17].

Studies using molecular biological and drug resistance selection techniques

Thermodynamic studies performed on target-inhibitor interactions in proteases have specifically described a lower affinity of some non-B subtype proteases for PI and for selective amplification of primary resistance mutations on the basis of background polymorphisms (Table 2).

Velasquez-Campoy, et al.[18,19] reported two important findings: (i) the enzymatic efficiency or vitality of subtype C proteases was higher than those of subtype B, and (ii) natural polymorphisms in the subtype C and A protease genes amplified the effect of resistance mutations on PI binding. This suggests that subtype C proteases processed their substrates faster that B proteases; for example, the effect of the V82F and I84V resistance mutations V82F and I84V in subtype A and C viruses was to reduce binding to substrate by twofold to sevenfold more than occurred in subtype B. Background polymorphisms differentially affected the effect of the PI saquinavir, nelfinavir, indinavir, and ritonavir, and the double mutation V82F/I84V improved the relative vitality of the protease by ~ 100 in the presence of tested PI. This effect was more pronounced in the protease of subtypes A and C, in which relative vitality sometimes reached 1000-fold[19]. Taken together, these results suggest that natural polymorphisms contribute to the HIV resistance phenotype by both lowering drug discrimination and by restoring enzyme function.

González, et al.[20] found that the IC_{50} of a number of subtype C isolates carrying the I93L mutation were 16.2 to 2.6 times lower than that of a reference strain HXB2/NL4-3-PR ($p < 0.0001$; two-tailed *t* test). However, when subtype B or C isolates lacking this specific substitution were analyzed, the IC_{50} were only 1.2 to 0.7 times less than that of HXB2/NL4-3 ($p = 0.77$; two-tailed *t* test). Nor was this phenotypic effect observed when I93L was naturally present in subtype B (isolate Br-RS2172). The authors suggested that the I93L substitution might cause hypersusceptibility to lopinavir in subtype C HIV-1 rather than resistance[20].

Coman, et al.[21] performed important comparative enzymatic and structural analyses of proteases derived from subtypes B and C that carried one or several nelfinavir-selected resistance mutations (D30N, N88S, L90M). They showed a differential effect of these resistance mutations on the kinetics of B and C proteases as follows: (i) the effect varied according to whether only a single mutation or combinations of mutations were present, and (ii) the effect differed based on each PI tested. Structural analyses of subtype C protease bound to nelfinavir and indinavir showed that these inhibitors form similar interactions with residues in the active site of subtype B protease, and that naturally occurring polymorphisms could alter the position of the outer loops of the subtype C protease, especially the “60” loop. Clemente, et al.[22] reported that high levels of PI resistance in A/E and B proteases were due to combinations of active site and non-active site mutations that involve background polymorphisms.

Some mutations may also have important roles only in certain subtypes. For example, N83T and M89L restored replicative capacity only in subtype C. The M89I/V substitutions that occur in subtypes G, F, and C had a differential effect, depending on which PI was tested and whether or not the L90M substitution was present[16] (Table 1).

In regard to reverse transcriptase, one drug resistance selection study showed that subtype C viruses selected the K65R mutation faster under tenofovir pressure compared to subtype B[23]. A template-based mechanism for this phenomenon has been proposed[24]. Certain NNRTI mutations also appear to emerge preferentially in certain subtypes, e.g. V106M in subtype C instead of V106A in subtype B[25]. Furthermore, V106A in subtype B is mainly associated with resistance to nevirapine but not efavirenz, while V106M confers broad NNRTI cross-resistance.

Discussion

Biochemical and virologic data provide compelling evidence as to the differential effect of viral genetic background on both the type and degree of antiretroviral drug resistance in HIV-1. In regard to protease, genetic background can affect the degree of protein binding caused by primary mutations and also help to restore enzymatic activity based on the presence of background polymorphisms and subtype. This effect was not discernible in the absence of typical major resistance mutations, but rather only when particular backgrounds of combinations of major resistance mutations and background polymorphisms were present. In this regard, it seems that some background polymorphisms can act as secondary resistance mutations.

In addition, HIV phenotypic assays have failed to reveal major differences in the susceptibilities of B versus non-B subtypes, consistent with molecular data (Table 2). However, there are only few data on relative susceptibility levels among subtypes carrying specific major resistance mutations. More information is needed since many polymorphisms in non-B viruses are considered to be secondary resistance mutations, based on the fact that they emerge in B subtype viruses after drug exposure. However, the effect of such polymorphisms within different genetic backgrounds cannot always be extrapolated to B subtypes. They might sometimes contribute to higher levels of resistance in certain genetic backgrounds, but could also have either a neutral effect or hypersensitize HIV to certain antiretroviral drugs (e.g. I93L is a secondary resistance mutation in subtype B but causes hypersusceptibility to PI in subtype C)[20].

Novel NNRTI resistance mutations have been found in subtype C that had not been recognized in subtype B. In tissue culture, subtype C can acquire a different mutation (i.e. V106M) under NNRTI drug pressure compared to what it is seen in subtype B, and this mutation confers broad NNRTI cross-resistance. Resistance surveillance of South African patients has confirmed the importance of the V106M mutation in subtype C HIV-1[26]. Similarly, rates of acquisition of resistance could have important implications in regard to durability of response to therapy. *In vitro,* the emergence of the K65R mutation is faster in subtype C than in B. Biochemical mechanisms have been proposed to explain this observation based on subtype C template usage and processing by reverse transcriptase[24]. In the clinic, K65R has been seen in approximately 70% of patients failing didanosine- and stavudine-containing nucleoside backbones in Botswana[27], and this has now been confirmed in a larger study performed in Malawi in patients with subtype C viruses who received stavudine/lamivudine and nevirapine as first-line therapy. Of these individuals, 19% developed either the K70E or K65R mutations. However, K65R did not appear to emerge frequently in subtype C patients who participated in large clinical trials in which they received either tenofovir or tenofovir/emtricitabine as part of a triple therapy regimen[29]; this substantiates the view that suppression of viral replication will forestall the appearance of drug resistance regardless of subtype. However, larger numbers of patients and longer follow-up will be required to assess the situation in patients receiving therapy in subtype C endemic areas.

To date, most drug-susceptibility phenotypes in non-B isolates have been determined by commercial and/or in-house assays that were developed primarily to measure B subtype drug susceptibilities and that are based on the laboratory adapted strains HXB2 or NL4-3. These studies employed modified molecular clones of laboratory strains that lack both the terminal portion of Gag and most of Pol, in order to generate a chimeric clone[12,30-33]. Only one study used an assay that included a substantially more complete Gag gene of a clinical isolate[13] and, interestingly, is the only one to have reported higher frequencies of resistance to atazanavir on the part of subtype C isolates. The lack of a complete Gag gene in the constructs to measure non-B drug susceptibility is a potential limitation of studies in regard to the accuracy of PI susceptibility determinations in non-B isolates (Fig. 2).

Multiple clinical and *in vitro* studies have confirmed that protease and gag are a functional unit and co-evolve when HIV is subject to drug pressure. Both genes mutate under PI pressure and Gag mutations can act as compensatory substitutions that may increase levels of resistance and viral replication capacity[34-38]. None of the recombinant phenotyping systems used to date for clinical samples included the complete original Gag gene in the chimeric virus used for determining phenotype. One subtype B study demonstrated that inclusion only of the p7/p1 and p1/p6 regions (as is the case with current commercial assays) instead of the complete Gag gene may underestimate the reduction of drug susceptibility by at least twofold in some viral isolates[39]. Although this difference appears small, non-B subtypes might develop compensatory Gag mutations different from those seen in subtype B, establishing a need to take the entire Gag region into account in phenotyping assays used to assess drug susceptibilities with non-B viruses.

A wide variety of mutations can impact on drug sensitivity to differential extent and only few data on the potential for cross-resistance to PI among non-B subtypes have been published. In regard to nelfinavir, there may be a tendency to select for the L90M pathway in some subtype C isolates, instead of D30N, which could reduce the potential for salvage ability of PI therapy[47]. In addition, the D30N mutation was not found in A/G recombinants from Ivory Coast that instead possessed N88S after exposure to nelfinavir[40]. N88S unlike D30N can cause cross-resistance with other PI, including atazanavir. Therefore, accurate cross-resistance information is not yet available for most non-B subtypes due to a scarcity of paired genotypic and phenotypic data, yet this subject should now be assumed to be of clinical significance. In regard to NNRTI,

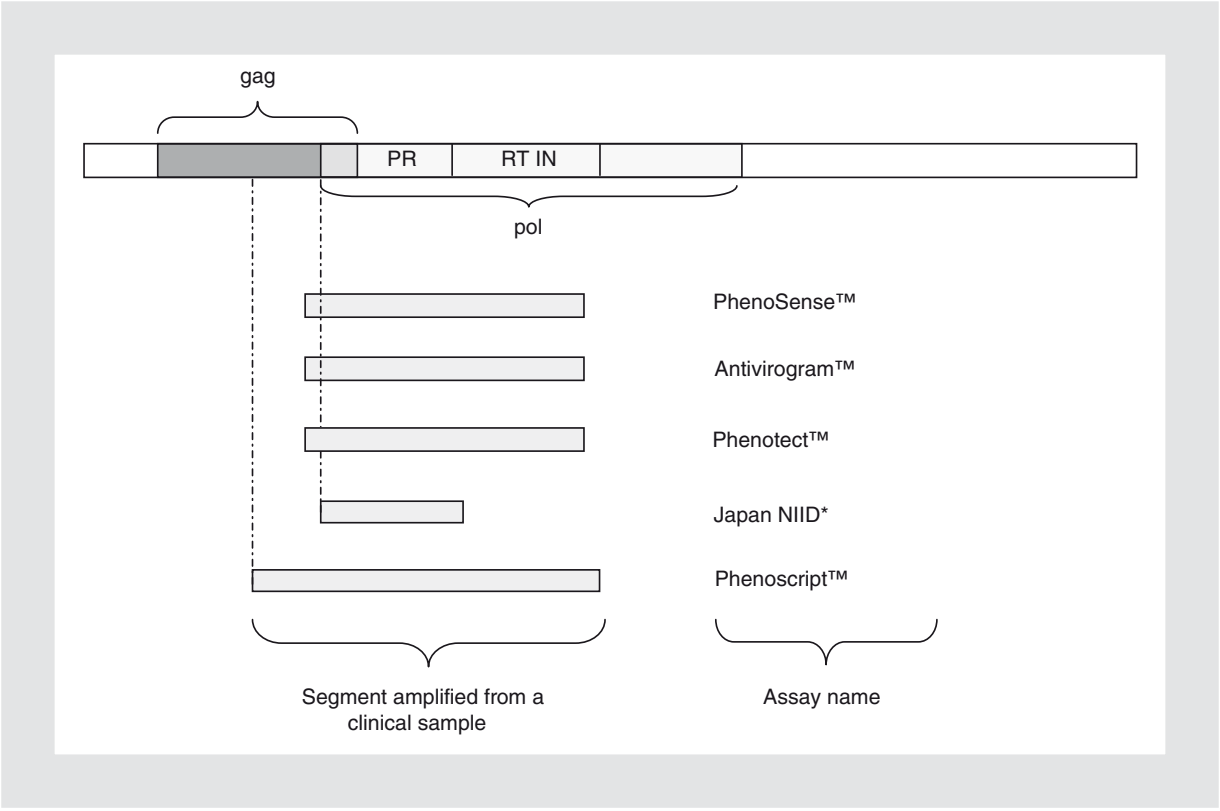

Figure 2. *Phenotype-based susceptibility systems used to analyze the non-B viruses included in this review. Phenotype susceptibility assays vary in the length of the gag gene that is incorporated into the recombinant construct to produce chimeric viruses. The longest gag segment introduced is that used in the Phenoscript assay. NIID: National Institute for Infectious Diseases.*

only one study analyzed the genotypes and phenotypes of non-B subtypes obtained from a clinical trial of use of single-dose nevirapine for prevention of mother-to-child transmission[41].

In conclusion, the genetic diversity of HIV-1 can affect the type of resistance mutations, degree of resistance, and timing of emergence of antiretroviral resistance. However, the accumulated published evidence is insufficient to adequately assess the contribution of the innate genetic diversity of HIV-1 on resistance and cross-resistance, particularly for PI in non-B HIV-1. The potential for extensive cross-resistance acquires further importance in settings with limited access to antiretroviral drugs in which PI may be the only realistic option for salvage therapy[42]. No *in vitro* selection study has ever been published for PI in non-B subtypes, yet such data may be crucial to understanding cross-resistance for specific drugs. In addition, large numbers of paired samples need to be systematically collected from naive and treated patients infected with subtypes C, A/E, A/G, A, and G, in order to generate genotypic and phenotypic data sets that will have applicability for both established drug classes as well as for newer classes such as integrase inhibitors.

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