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Polymorphism in HIV-1 Non-subtype B Protease and Reverse Transcriptase and its Potential Impact on Drug Susceptibility and Drug Resistance Evolution

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Abstract

HIV-1 non-subtype B viruses are predominant worldwide. At least 9 different HIV-1 group M subtypes and 14 circulating recombinant forms differ from one another by 10-15% in their *pol* gene, which includes the coding regions for the viral protease and reverse transcriptase (RT), the current targets of antiretroviral drugs. Inter-subtype genotypic diversity includes polymorphism at amino acid residues known to be related to drug resistance in HIV-1 subtype B. Whether polymorphism alters protease and RT function, drug susceptibility, or clinical response to treatment, is unclear. Worldwide dissemination of non-subtype B viruses and increasing availability of antiretroviral drugs in the developing world will expand drug use and the likelihood of drug resistance in non-subtype B viruses. In this review we define and characterize inter-subtype RT and protease polymorphism, and examine the evidence for genotypic and phenotypic differences between HIV-1 subtypes as well as the potential for different clinical responses and evolution of drug resistance among non-B infected individuals.

Key words

HIV-1 subtypes. Polymorphism. Drug resistance.

Introduction

HIV-1 is characterized by broad genetic diversity, both within infected individuals as well as between viral subtypes[1-5]. Phylogenetic analyses of global isolates demonstrate three distinct groups of HIV-1: M (main), O (outlier) and N (non-M, non-O). The M group, which accounts for most infections worldwide[6], is further divided into at least 9 distinct subtypes (A, B, C, D, F, G, H, J, K) and 14 circulating recombinants forms (CRFs), the most common of which are CRF01_AE and CRF02_AG[7]. These subtypes and intersubtype recombinants differ from one another by 25-35% in the *env* gene, a recognized challenge to vaccine development[2,8-10]. In the *pol* gene, functional constraints of the protease and reverse transcriptase (RT) enzymes limit the variation in these genes to 10-15%[11,12]. However, although the *pol* gene is the most conserved region of HIV-1[13], there is sufficient diversity in protease and RT of global isolates to allow phylogenetic subtype identification[14-19] (Fig. 1).

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Variation between subtypes, extending to functional enzymes, is likely the result of a "starburst" radiation of numerous viral lineages following a single transmission event of a group M ancestor virus from a chimpanzee to a human[20]. Continued divergence of HIV-1 subtypes is sustained by selective immunologic pressure on diverse quasispecies[21,22], as well as the cumulative acquisition of single base pair mutations due to the lack of RT proofreading capability and high viral replication rates. In addition, insertions, deletions and recombination events contribute significantly to HIV-1 diversity[18].

Widespread use of antiretroviral drugs is an additional selective pressure on protease and RT, which may exert different effects on distinct HIV-1 subtypes. Intersubtype polymorphism may modulate antiretroviral drug susceptibility, the probability that drug resistance will arise during therapy, and the fitness of HIV-1 variants that acquire drug resistance mutations. While there is little evidence, thus far, that specific drugs are more, or less active in persons infected with different HIV-1 subtypes, it is likely that increasing antiretroviral drug use will be an additional selective pressure in the evolution of HIV-1 worldwide.

The use of drug resistance testing in resource rich countries has led to a great deal of information about drug resistance among subtype B isolates, although these represent only a small fraction of global HIV-1 infection[6]. Non-B subtypes and CRFs, which already predominate in Africa, Asia and much of Eastern Europe, are identified with increasing frequency in the Western world[23-27]. With the increasing drug availability in developing countries and the growing numbers of non-B infected individuals in developed countries, sequence differences between subtypes have already been shown to alter the sensitivity and accuracy of virus load measurements[28,29] and of genotypic drug resistance detection[30] as diagnostic tools in patient management.

In this review, we define and characterize the inter-subtype polymorphism in HIV-1 protease and RT, and review the available data on the influence of subtype and genetic variation on susceptibility to antiretroviral drugs, and on the evolution of drug resistance following antiretroviral treatment.

Consensus sequences and polymorphism in B and non-B HIV-1 subtypes

The Protease and RT consensus sequences within an HIV-1 subtype are derived from comparison and alignment of sequences from untreated persons, to predict the specific amino acid residue occurring most frequently at each position.

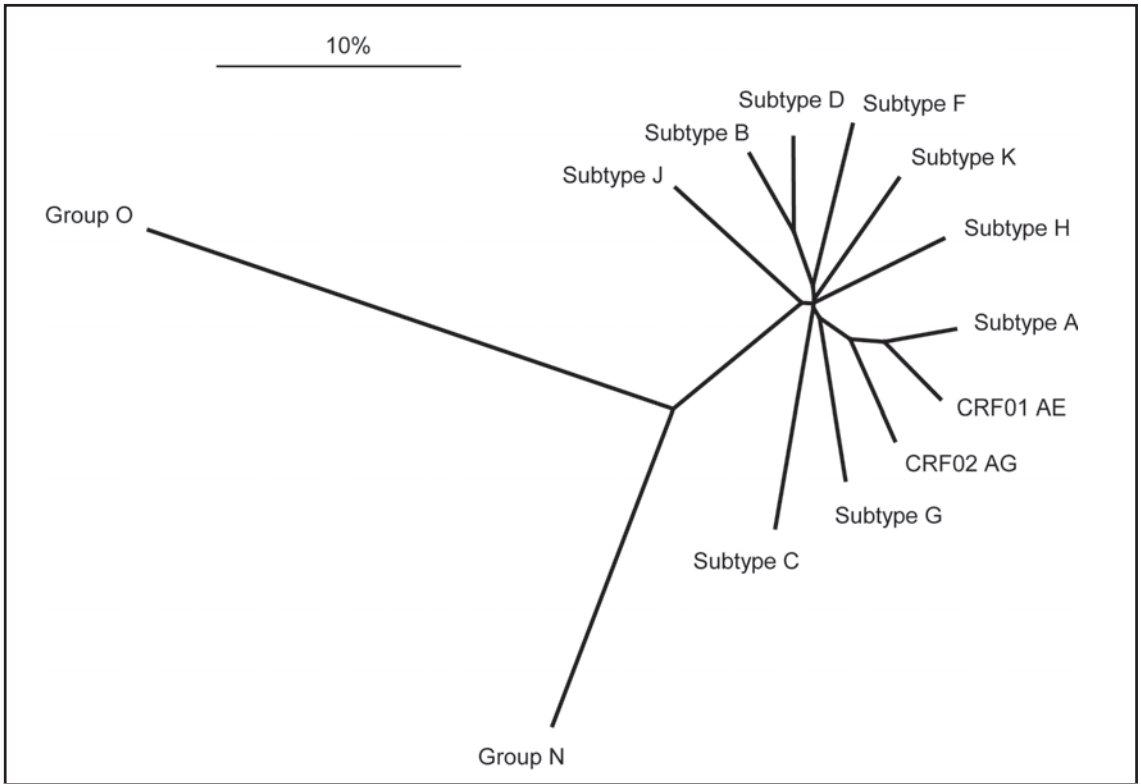

Figure 1. *Neighbor-joining tree constructed from pol sequences of 13 reference isolates (subtype A isolate U455, CRF01_AE isolate U54771, CRF02_AG isolate L39106, subtype B isolate HXB2, subtype C isolate C2220, subtype D isolate NDK, subtype F isolate 93BR020, subtype G isolate SE6165, subtype H isolate 90CR056, subtype J isolate SE9173c, subtype K isolate 97EQTB11C, group N isolate YBF30, group O isolate AZT70C). The scale bar represents a 10% nucleotide difference.*

Polymorphism is the variation from the consensus sequence within the subtype being evaluated, arbitrarily defined as position specific differences occurring in > 1% of sequences.

The degree of change in a protein's primary structure, as reflected by the nucleic acid sequence is proportional to the biological importance of each residue. Highly conserved residues in enzymes are most directly involved in biological function while variable amino acid residues are distant from the active site and are not directly involved in catalytic processes or in functional interactions with other molecules[31]. Genetic polymorphism is expected to appear at sites that are less directly critical for enzyme activity and where variation within the protein may be driven by immune selection and functional adaptation.

A number of studies have assessed the genetic baseline diversity of protease and RT from non-subtype B HIV-1 infected persons not exposed to antiretroviral therapy (Table 1). Most of these published sequences have been compiled in the Stanford Protease and RT Database (http://hivdb.stanford.edu)[32]. To illustrate the variation within and between subtypes, figure 2 shows conserved and polymorphic sites in the protease (Fig. 2A) and RT (Fig. 2B), from 1400 subtype B and 830 non-B infected persons (including subtypes A, C, D, F, G, CRF01_AE and CRF02_AG), who were not exposed to antiretroviral therapy. Within each subtype, frequencies of observed changes in amino acids are distinguished from the expected residues at each position of the current subtype-specific consensus sequence (http://hiv-web.lanl.gov).

Table 1. *Published studies with protease and RT sequences from HIV-1 non-B infected antiretroviral-naïve persons*

Study	Years	Geography	No. Persons	Main Subtypes
Apetrei[65]	<'98	Romania	14	F
Auswinporn[83]	'99-'00	Thailand	25	AE
Balotta[24]	<'01	Italy	19	F, AG
Becker-Pergola[17]	'98-'94	Uganda	27	A, D
Brindeiro[84]	'96	Brazil	9	AE, F, C
Cane[68]	<'01	UK	12	C
Carr[85]	<'01	South America	5	F
Caumont[86]	'99	Vietnam	23	AE
Cornelissen[18]	'92	Global	30	AE, D, A
Cuevas[87]	<'02	Cuba	8	D, G
Dowling[88]	'99-'00	Kenya	41	A, C, D
Ellenberger[89]	'95	Cote d'Ivoire	20	AG
Fonjungo[90]	'98	Cameroon	110	AG, J, G, F
Frater[79]	<'01	Africa	79	A, C, D
Gao[91]	'84-'94	Africa, Brazil	10	All
Grossman[55]	'99-'00	Israel	56	C
Hoelscher[92]	'97	Tanzania	9	A, C
Holguin[93]	'98	Spain	15	G, C, H
Holguin[26]	'86-'00	Spain	71	G, C, A, D
Koch[94]	<'01	Burundi	18	C
Lole[95]	'92-'95	India	6	C
Masciotra[96]	'97	Argentina	24	F
McCutchan[97]	'94-'95	Nigeria	5	AG
Nkengasong[98]	'97	Cote d'Ivoire	12	AG
Pandrea[99]	'97-'98	Moldova	6	A
Perez-Alvarez[100]	'99	Spain	12	G
Pieniazek[101]	'96	Lebanon	14	A
Pieniazek[15]	'86-'98	Global	187	A, D, F, AE, C, AG
Pillay[102]	'00	South Africa	37	C
Rodenburg[103]	'94-'98	Global	13	C
Shafer[12]	'95	Zimbabwe	12	C
Shafer[11]	'95	Zimbabwe	12	C
Si-Mohamed[104]	'92-'98	France	12	AG, J
Sirivichayakul[105]	'90-'00	Thailand	63	AE
Tanuri[59]	'93-'97	Brazil	23	F
Tebit[106]	'99	Cameroon	19	A, AG
Thomson[107]	'99	Spain	13	G
Toni[60]	'97-'00	Cote d'Ivoire	99	AG, A
Triques[108]	'95-'97	Africa	5	F, K
Vergne[16]	'95-'99	Africa, France	129	AG, F, A, D, J, G, C
Vergne[109]	'00	Gabon	13	AG
Vicente[110]	'96	Nigeria	10	A

No.– Number; AE denotes CRF01_AE; AG denotes CF02_AG.

Protease polymorphism

The HIV-1 protease is a dimeric and symmetric molecule made up of two 99-residue polypeptide chains responsible for the post-translational processing of the viral *gag* and *gag-pol*-encoded polyproteins, to yield the structural proteins and enzymes of the virus[33]. The substrate cleft of the protease is formed by residues at positions 8, 23, 25-27 (the active site), 28-30, 32, 47-52 (the flexible "flap"), 53, 80, 82 and 84[34]. Except for the V82I mutation, discussed below, these predicted amino acid residues appear to be highly conserved across subtypes, in the absence of protease inhibitor (PI) use.

Of the 99 protease amino acids, 30 positions in subtype A (30%), 31 in B (31%), 26 in C (26%), 34 in D (34%), 33 in F (33%), 28 in G (28%), 14 in CRF01_AE (14%) and 35 in CRF021_AG (35%) are polymorphic in sequences from drug naïve persons (Fig. 2A). Compared to 1129 subtype B protease sequences, significantly less variation is seen in 53 CRF01_AE protease sequences ($p = 0.004$, Chi Square test), which may be a result of the temporal and geographic clustering of CRF01_AE isolates, most of which are from the explosive expansion of infection in Thailand in the last decade[35].

There are a number of amino acid substitutions that occur at high rates in certain non-subtype B viruses at positions associated with subtype B drug resistance (Table 2, Fig. 3A). Based on observations of differences between sequences from untreated and treated persons, mutations at protease positions 10, 20, 36, 63, 71, 77 and 93 are characterized as "secondary protease mutations" in subtype B[36,37]. While mutations at these positions do not cause high-level drug resistance themselves, they contribute to drug resistance when present together with certain primary protease mutations, or have been shown to compensate for the decrease in catalytic efficiency caused by PI selected primary protease mutations[38-42].

Differences between HIV-1 subtypes have been observed in the protease at residues with a more prominent role in drug resistance as well. Although the V82I mutation occurs in about 1% of untreated individuals infected with subtype B, it has been identified at 6% in subtype C, 9% in subtype F, and is the consensus (>50%) in subtype G isolates from untreated persons. Preliminary data suggest that this mutation alone confers minimal resistance to the available PIs in subtype B[33]. However amino acid changes at V82 resulting in V82A, F, S or T are associated with high level resistance to most PIs[37]. In subtypes F and G, the M46L or I substitution, which leads to resistance to most of the PIs in subtype B[36], has been identified in 4 and 7% of sequences from untreated individuals, respectively.

In addition to this inter-subtype diversity, comparison of non-B protease consensus sequences to the subtype B protease consensus (http://hiv-web.lanl.gov) shows differences in overall 16 residues, of which 5 are in positions related to subtype B drug resistance (Fig. 3A). The consequences of polymorphism in non-B subtypes with respect to the activity of current PIs are not known. However, as discussed below, there is increasing evidence for differences between subtypes, which may be driven by underlying polymorphism.

Reverse transcriptase polymorphism

The RT enzyme is responsible for RNA-dependent DNA polymerization and DNA-dependent DNA polymerization[33,43]. RT is a heterodimer consisting of p66 and p51 subunits. The p51 subunit is composed of the first 440 amino acids of the RT gene. The p66 subunit is composed of all 560 amino acids of the RT gene. The p66 subunit contains the DNA-binding groove and the active site, and the p51 subunit functions as a scaffold for the enzymatically active p66 subunit. Both subunits are required for optimal polymerase activity.

The active site of the RT is a highly conserved region encoded by amino acids from codons 183-186 (YMDD motif). Polymerase activity is associated with orderly binding of substrates, followed by the deoxynucleotide triphosphate binding related with amino acids 65, 72, 110, 113, 115, 116, 151, 160, 183, 184, 185, 186, 219 and 43. It is noteworthy that none of these codons are polymorphic (in the absence of antiretroviral drugs) (Fig. 2B), emphasizing their role in the enzyme function.

Of the first 240 RT amino acids, 41 positions in subtype A (17%), 45 in B (19%), 56 in C (23%), 43 in D (18%), 35 in F (15%), 32 in G (13%), 46 in CRF01_AE (19%) and 56 in CRF021_AG (23%) are polymorphic in sequences from drug naïve persons. Intersubtype prevalence of RT polymorphic residues was similar. For subtypes A, B, D, F, CRF01_AE and CRF02_AG, the protease was significantly more polymorphic than the RT ($p < 0.02$, Chi Square test).

Polymorphism in non-B protease and RT positions, which are associated with subtype B nucleoside RT inhibitor (NRTI) resistance[33, 37] (Table 2) include codons 69 (6% in subtype F), 75 (2% in CRF01_AE) and 118 (5, 2 and 6% in subtypes A, B and D, respectively). Mutations at position 69 (T69D/N/S/A) have been reported with exposure to all NRTIs, and contribute to NRTI resistance[44,45]. Within subtype B viruses rare insertions at this position have been described as imparting multi-nucleoside resistance in 1-2% of highly NRTI experienced individuals[46-49]. This insertion has only recently been reported in non-subtype B viruses[50]. Mutations at position 75 (V75T/I) are commonly selected, *in vitro*, by serial passage of subtype B virus in sub-inhibitory concentrations of stavudine[51], and are less frequently described *in vivo*, but can confer resistance to stavudine and didanosine[44]. Mutation V118I, together with E44D, causes intermediate lamivudine resistance[52,53], and has been recently suggested to confer resistance to multiple NRTIs[54].

Polymorphism at positions associated with subtype B non-nucleoside RT inhibitor (NNRTI) resistance is seen at codons 98 (in subtypes B, C, G), 106 (in subtypes G, CRF01_AE and CRF02_AG) and 179 (polymorphic in subtypes B, C, D, F, G, CRF02_AG, and is the consensus position in subtype A) (Table 2, Fig. 3B). A98G, V106A and V179D are residues in the NNRTI binding region of the p66 subunit associated with different levels of resistance to the NNRTIs[33,37].

In addition to this inter-subtype diversity, comparison of non-B RT consensus sequences to the subtype B RT consensus (http://hiv-web.lanl.gov) shows differences in overall 23 residues, of which 1 is in a position related to subtype B drug resistance (Fig. 3B). The functional consequences of RT polymorphism in distinct HIV-1 subtypes at positions related and not related to drug resistance are not known, and in the RT have been only sporadically addressed.

The potential impact of protease and RT non-subtype B polymorphism

The importance of protease and RT sequence differences among non-subtype B viruses for therapeutic success in non-B infected patients is not known. The higher frequency of protease polymorphism in non-B isolates, including protease positions 20, 36, 63, 82 and 93 as mentioned above, has raised concern that PI treatment of non-subtype B infected persons could be less effective than that of subtype B[15,16,18,55]. In the case of RT inhibitors, the relevance of polymorphism at positions associated with subtype B NRTI (codons 69, 75 and 118) and NNRTI (codons 98, 106, 179) resistance on the susceptibility of non-subtype B viruses has not been widely addressed, but could play a role in causing distinct degrees of susceptibility to these drug classes.

Genetic protease and RT variation may result in important differences between HIV-1 subtypes in the efficacy of antiretroviral therapy and in the evolution of drug resistance. Drug susceptibility can be measured through biochemical, phenotypic or genotypic assays and assessed as part of the clinical outcome of drug treatment. For each of these measures, we consider the data that bears on differences between subtypes to explore the role of intersubtype polymorphism in: 1) enzymatic properties of protease and RT; 2) phenotypic susceptibility to antiretroviral drugs; 3) evolution of subtype specific genotypic patterns of drug resistance; and 4) response to antiretroviral therapy among non-B infected persons.

Biochemical impact of non-subtype B polymorphism

The enzymatic efficiency of protease and RT has a potential role in explaining drug resistance differences among subtypes. Velázquez-Campoy, et al.[56] have investigated the catalytic efficiency and inhibition of subtype B proteases into which mutations commonly observed in subtypes A and C were engineered, sharing mutations M36I, R41K, H69K, L89M, with the addition of I13V, E35D and R57K for subtype A. They characterized the enzymatic activity in the presence of four PIs (saquinavir, indinavir, nelfinavir and ritonavir). Using calorimetry, these A and C like proteases had catalytic efficiency 1.5 to 11 fold higher than the comparator subtype B protease in the presence of PIs. They further report that with engineered drug resistance mutations (V82F/I84V), the fitness of these A and C like proteases can be increased up to 1000-fold higher compared to wild-type subtype B protease in the presence of the studied inhibitors[57]. They concluded that from a thermodynamic standpoint, the combined effects of the drug-resistant mutations and the natural (subtype specific) amino acid polymorphism are additive. Polymorphism need not be in the active site to influence the binding of inhibitors, and they may amplify the effects of drug-resistant mutations. This approach to inter-subtype genotypic differences supports the assumption that differences in enzymatic activity may be attributed to baseline polymorphism in non-B proteases.

Phenotypic resistance of non-subtype B viruses

Phenotypic drug-susceptibility assays measure the drug concentration that is required to inhibit HIV-1 replication by 50% (IC_{50}), and compares this with the concentration of drug required to inhibit a wild type (generally subtype B) HIV-1

Table 2. *Polymorphism at drug resistance positions in protease and RT from untreated persons*

Subtype	RT	Protease
A	V118I	L10I/V, K20R/I, L63C/V/P/T/S/A, A71Y, V77I, I93L
B	A98S,V118I, V179I/D/E	L10I/V/F, K20R, M36I, L63P/S/A/H/T/Q/C, A71T/V, V77I, I93L
C	A98S, V179I	K20R, L63P/T/V/S, V82I
D	V118I, V179I	L10V/I, K20R, L63P/Q/S/T, A71T, V77I, L93I
F	T69I, V179I	L10V/I, M46I, V77I, V82I, I93L
G	A98S/G, V106I, V179E	L10I, M46L, L63P, V77I, I93L
CRF01_AE	V75L, V106I, V179I/D	L10V/I, K20R, L63P/T, V77I, I93L
CRF02_AG	V106I, V179I	L10V/I, L63P/S/A/F/I, V77I, V82I, I93L/M

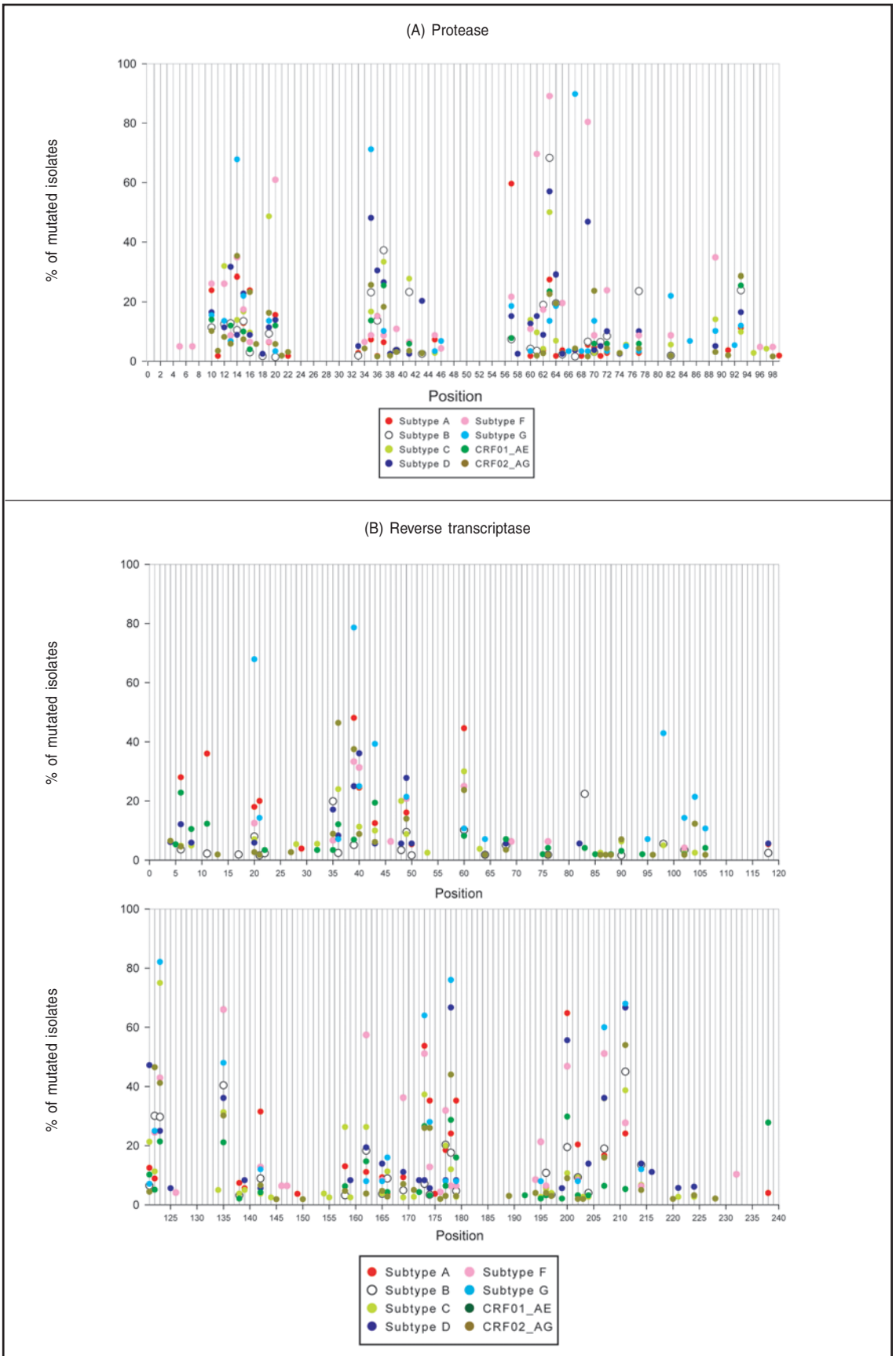

Figure 2. *HIV-1 protease and RT polymorphism. Comparison of protease and RT sequences from B (empty circles) and non-B (colored circles) infected untreated persons with the consensus sequence of the matching subtype. Single occurrences of mutations were excluded. Protease sequences were available from 110 subtype A, 1129 subtype B, 87 subtype C, 76 subtype D, 49 subtype F, 60 subtype G, 53 CRF01_AE and 257 CRF02_AG infected persons. RT sequences were available from 57 subtype A, 583 subtype B, 87 subtype C, 38 subtype D, 51 subtype F, 28 subtype G, 101 CRF01_AE and 114 CRF02_AG infected persons.*

virus. Several studies have demonstrated largely comparable *in vitro* susceptibility of non-subtype B viruses to most antiretroviral drugs in a variety of phenotypic assays[11,12,58-60].

The exception to this generalization may be the evidence suggesting that the V82I protease mutation in non-subtype B viruses may alter susceptibility to PIs. *In vitro* experiments by Kaplan, et al.[61], which examined the rate of selection of PI resistance in cell culture in the presence of an experimental PI (A-77003), demonstrated variants with reduced sensitivity to PIs that included the V82I and V32I mutations. Another study in subtype B by Maguire, et al.[62] has reported the emergence of V82I in 2 patients failing an amprenavir-containing regimen, both of whom developed I50V, an amprenavir related mutation. In non-B subtypes, Descamps, et al.[63] described a >5-fold decrease in phenotypic susceptibility to ritonavir and saquinavir in two subtype G strains with the V82I and M36I mutations.

Other studies supporting potential reduced phenotypic susceptibility in non-B viruses found that subtype D isolates may have a tendency toward slightly lower susceptibility to antiretroviral drugs compared to other subtypes[64]; 2 of 14 subtype F isolates in Romania had borderline and significantly diminished susceptibility to an investigational NNRTI[65]; and finally, 2 of 99 CRF02_AG seroconverters in Abidjan, Cote d'Ivoire demonstrated a 6.9-fold reduction of susceptibility to nelfinavir and a 4.2-fold resistance to nevirapine[60].

Although the majority of phenotypic data point to similarities in susceptibility between B and non-B subtypes, there is increasing evidence that V82I and related polymorphism in protease may play a role in subtype specific susceptibility to some PIs.

Genotypic impact of non-subtype B polymorphism

A wider range of data from non-subtype B infected patients is available from genotypic testing, which for reasons of cost and availability are more widely used to assess drug resistance. In addition, genotypic tests may demonstrate mixtures at specific positions where minority populations of virus at levels of 20-30% are too low to affect drug susceptibility in a phenotypic assay. Genotypic tests also detect transitional mutations that do not cause resistance by themselves but indicate the recent presence of selective drug pressure[66, 67].

The PI selected evolution of resistance-related mutations in the protease may differ with subtype. Sequences from persons failing nelfinavir with subtypes C[55,68], G[69] and CRF01_AE[70] demonstrate evolution of resistance with the L90M mutation rather than the mutations preferentially acquired in subtype B infection, where the D30N mutation predominates. Similar findings have been observed in 5 patients from Brazil[71], where different resistance patterns between B and non-B isolates in the protease were suggested despite similar drug treatment.

These observations in non-B infection add to the plausibility that differences in drug resistance evolution pathways between HIV-1 subtypes exist,

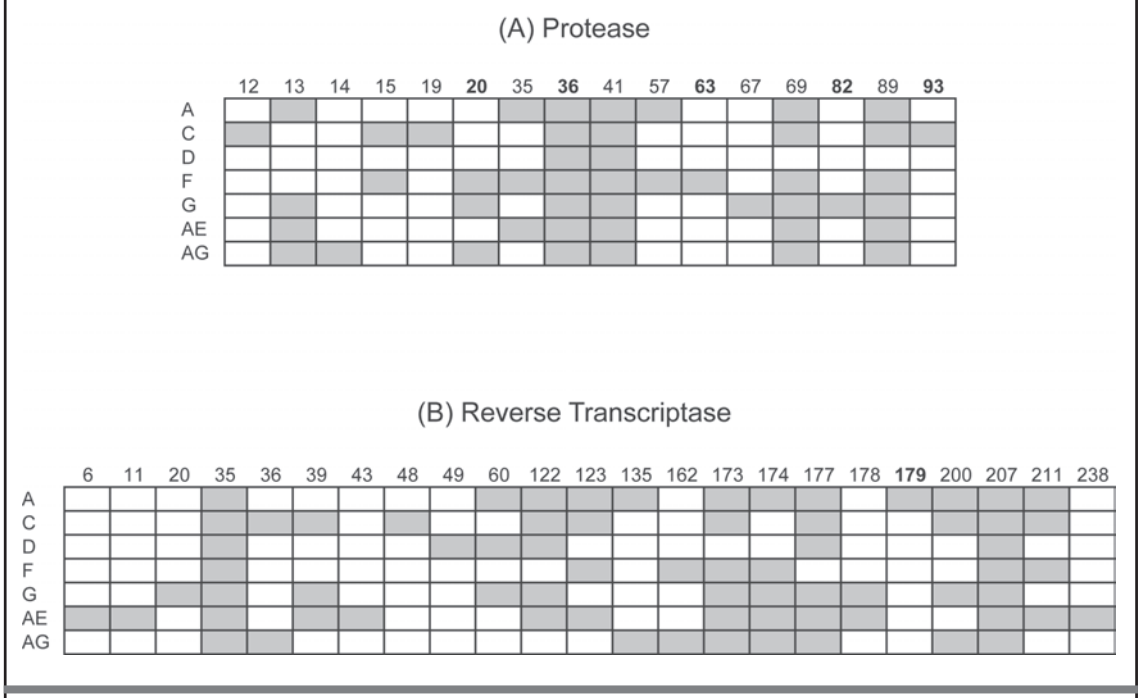

***Figure 3**. Non-B Protease and RT consensus positions which differ from consensus B. Consensus sequences of HIV-1 subtypes were taken from the Los Alamos Sequence Database (http://hiv-web.lanl.gov). Rows denote subtypes and circulating recombinant forms, and columns signify protein positions. Sites related to subtype B drug resistance are bold. White squares represent similar amino acids and gray squares represent different amino acids compared to the subtype B consensus. AE denotes CRF01_AE; AG denotes CRF02_AG*

and it is reasonable to postulate that baseline subtype-specific polymorphism plays a role in this evolution. However, larger data sets from well-characterized treatment regimens need to be collected and analyzed to validate these observations and hypotheses.

Clinical impact of non-subtype B polymorphism

There are few studies that directly compare the clinical outcome of antiretroviral treatment among patients with different subtypes, and hence phylogenetically different protease and RT genes. Data from studies of subtype B infection are conflicting, but may shed some light on the role of non-B polymorphism at positions related to drug resistance on treatment outcomes.

Subtype B protease polymorphism at positions associated with drug resistance, which exists prior to the emergence of a major or primary mutation, may alter the rate of development of resistance or the subsequent evolution of mutations and thus on the choice of antiviral therapy[72]. A recent, albeit controversial analysis of baseline polymorphism at drug-related positions was presented by Perno, et al.[73] from Italy. These authors assessed the role of protease and RT mutations in predicting virologic failure in 248 drug-*naïve* persons, who began a PI containing regimen. They found a statistically significant correlation, in a small number of subjects, between virologic failure after 24 weeks of PI based therapy and the number of protease mutations at baseline. Mutations at polymorphic protease codons 10 and 36 were identified as the strongest predictors of virologic failure, and patients with either mutation were at twice the risk of having virologic failure at week 24 compared to patients with neither.

In a similar study from Luxemburg[74] in 116 subtype B infected patients beginning a PI containing regimen, Servais, et al. found that a relatively poorer response to therapy was associated with high baseline protease polymorphism, and, to a lesser extent, with the presence of I93L and A71V/T at baseline. In drug experienced subtype B infected patients, there is a clearer association between mutation at protease positions 10, 36 and 93 and less favorable virologic response to ritonavir-saquinavir containing regimens[75,76]. In contrast, several smaller reports show little correlation between the number of amino acid differences from consensus and treatment efficacy in subtype B HIV-1[77,78].

In non-B subtypes, Frater, et al. studied 79 African patients starting antiretroviral therapy, and assessed therapeutic response and relationship between polymorphic codons at baseline and virologic response. They found that no single polymorphism had any impact on clinical outcome as measured by either virus load or CD4 cell counts[79].

In a larger study in Uganda, Weidle, et al.[80] reported similar virological and immunological responses to antiretroviral drugs in HIV-infected persons compared to those seen in North America and Europe. Similarly, Del Amo, et al.[81] examined differences in progression to AIDS and death between HIV-1-positive Africans and non-Africans in London, and found no differences in progression from HIV-1 infection to AIDS and from AIDS to death attributable to African ethnicity, or presumed infection with non-subtype B viruses. Studies of non-B infected patients in Canada[25], Sweden[82], and in the European PENTA-5 trial[23], failed to identify an association between HIV-1 subtype and virologic response or disease progression.

Studies comparing clinical outcome between persons infected with different HIV-1 subtypes receiving similar antiretroviral regimens are limited to small numbers of subjects where treatment did not include randomization to specific regimens based on subtype or sequence information. The current observations are potentially complicated by the variation among individuals infected with different HIV-1 subtypes. In any treatment center, infection with divergent specific HIV-1 subtypes may be associated with variable modes of acquisition of infection and with differences in compliance, nutrition, socio-economic status, and host genetic variables such as chemokine receptor alleles and HLA types. These, and potentially other subtle confounders in the assessment of disease progression and response to antiretroviral therapy, could obscure real differences in virus susceptibility and the potential for inter-subtype polymorphism to contribute to drug resistance.

Conclusion

Although HIV-1 antiretroviral therapy has been developed largely for treatment of subtype B isolates most clinical and virologic studies suggest, with certain exceptions, that differences in HIV-1 subtype alone, do not lead to striking differences in response to drug therapy. However, among some non-B subtypes, an increase in accessory PI mutations and mutations at significant residues may result in different clinical responses, and may change the subsequent breadth of resistance among those with virologic failure.

The biological consequences and the impact of both viral and host genetic variation and polymorphism on the success of drug therapy in persons infected with HIV-1 non-B subtypes remain to be defined. The potential impact of inter-subtype polymorphism on antiretroviral therapy in HIV-1 infection has yielded few conclusions. Although most clinical outcome and phenotypic reports indicate similarity between B and non-B isolates, a few studies point to potential differences. Genotypic and biochemical studies provide an explanation for potential inter-subtype differences in susceptibility; however, larger and more rigorous prospective studies are required to translate the observed inter-subtype differences in resistance patterns to more effective, long-term drug treatment strategies.

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