MINIREVIEW

Insertions in the Human Immunodeficiency Virus Type 1 Protease and Reverse Transcriptase Genes: Clinical Impact and Molecular Mechanisms

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Treatment of human immunodeficiency virus (HIV) type 1 (HIV-1)-infected individuals with antiretroviral (ARV) drugs is highly effective in inhibiting viral replication, increasing both the duration and the quality of life (71). Regimens consisting of combinations of protease (PR), reverse transcriptase (RT), and fusion inhibitors are the current standard of care and typically reduce the circulating virus levels in previously untreated patients to below current limits of detection. Treatment failure, however, is not uncommon and is typically defined as a return to detectable levels of circulating virus, eventually resulting in decreases of CD4-cell counts and immune function and progression to AIDS.

The development of viral resistance to ARV drugs is a primary cause of treatment failure (24). Extensive genetic diversity is inherent in HIV because the highly error-prone RT enzyme creates a population of variants from which drug-resistant strains can be selected during treatment (49). Many individual point mutations (single amino acid changes) or groups of point mutations in the PR, RT, or gp41 gene have been associated in clinical studies with the failure of treatment with protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs), and fusion inhibitors, respectively (17, 24, 50, 52). HIV-1 strains isolated both from patients who have failed treatment and from laboratory strains modified by site-directed mutagenesis to possess specific mutations show reduced susceptibilities in vitro to one or more ARV drugs. Some point mutations are specific to individual drugs, while others confer cross-resistance to one or more drugs of the same class.

Over the past several years, strains with insertions of nucleotides in the PR and the RT genes (and that thus have extra amino acids in those proteins) have been found in patients who have failed ARV drug therapy. Numerous publications have examined the origins, prevalences, in vitro susceptibilities, and replication capacities of insert-containing strains, as well as the mechanisms by which the insertions affect drug resistance. This minireview focuses on the clinically related information regarding PR and RT insert-containing strains of HIV and briefly addresses the topics of mechanism and structure.

REVERSE TRANSCRIPTASE INSERTIONS

RT Codon 69 Inserts

Genotypic features. Strains possessing RT gene inserts are selected during NRTI therapy, as there are no reports of inserts in NRTI-naïve patients. The typical RT gene insert is composed of 6 extra nucleotides between codons 69 and 70 compared to the consensus HIV sequence (Table 1). These extra nucleotides result in a 2-amino-acid insert in the RT enzyme produced from the gene. Strains with these inserts almost always have a T-to-S point mutation at codon 69, leading to the accepted nomenclature of T69S+XX to describe the presence of these inserts. The most commonly inserted amino acids (XX) found in patient-derived strains are SG, SS, and SA, although at least 22 other combinations of codon 69 mutations and insert amino acids have been reported (40, 67). A small number of single amino acid inserts at this position have been reported, as have inserts of 5, 8, 11, and 15 amino acids (34, 39, 40, 62). At the nucleotide level, inserts appear to be duplications of neighboring genetic sequences (67), likely generated during reverse transcription by RT stalling and slippage (3, 20), although one report suggested that a 15-amino-acid insert was generated through intramolecular recombination (34).

Thymidine analog mutations (TAMs) are significantly associated with T69S+XX inserts, as approximately 95% of insert-containing strains also possess a T215Y/F mutation, 65% have an M41L mutation, and 52% have an L210W mutation (40). Uncommon variants at codon 67 are also frequently present (31, 39, 40, 59, 67). Nearly all other NRTI and NNRTI resistance mutations can be found in T69S+XX insert strains. While nearly all studies of T69S+XX inserts have been performed with clade B populations, inserts have also been found in clade C (18) and clade A (53) HIV-1 strains.

Susceptibility. Clinical T69S+XX insert-containing isolates show reduced susceptibilities to all NRTIs (12, 31, 34, 39, 62, 63, 67). The median fold changes in susceptibility for the 144 clinical isolates phenotyped in six different published studies are as follows: zidovudine (AZT), >500×; stavudine (d4T), 20×; didanosine (ddI), 5×; zalcitabine (ddC), 14×; lamivudine (3TC), >250×; abacavir (ABC), >10×; tenofovir (TFV), >10×. While nearly all T69S+XX insert strains contain other known drug resistance mutations, it is the interaction between TAMs, especially T215Y/F, and the insert that confers high-
level cross-resistance to the NRTI class. Site-directed mutagenesis studies have shown that placement of the T69S\slash H11001\_SG or T69S\slash H11001\_SA insert in a wild-type background reduces susceptibility to AZT, d4T, ddI, ddC, ABC, and 3TC 4- to 10-fold (31, 33, 38, 48, 67). Mutants created with these inserts plus the T215Y mutation have 10- to 20-fold reduced susceptibilities, and the presence of additional TAMs increases the fold change in susceptibility (31, 67). The T69S\slash H11001\_SS insert alone does not result in reduced susceptibility to AZT or d4T, but the addition of TAMs results in broad cross-resistance similar to that conferred by the other inserts (31, 38, 48). Comparisons of the susceptibilities conferred by the many different forms of T69S\slash H11001\_XX inserts have not been performed to determine if other inserts have restricted cross-resistance profiles, although this would be unnecessary, since most T69S\slash H11001\_XX insert strains contain multiple TAMs that likely ensure broad, high-level resistance. Changes in the content of the insert (e.g., T69S\slash H11001\_SS to T69S\slash H11001\_SG) have been reported in strains from patients monitored over time (8, 11, 31, 60). This may reflect selection of more resistant inserts in response to prolonged or modified therapy; however, the clinical significance is undefined.

NNRTI hypersusceptibility has recently been associated with TAMs (21, 42, 55, 64). Studies have shown that some, but not all, T69S\slash H11001\_XX insert strains are hypersusceptible to NNRTIs (39, 64). It has not been determined whether inserts participate in conferring NNRTI hypersusceptibility or whether the associated TAMs are responsible. Nevertheless, this finding has implications for the treatment of patients with insert strains.

**Prevalence.** Several studies have examined the prevalence of T69S\slash H11001\_XX inserts in cohorts from hospitals, clinics, and large reference laboratories (Table 2). Nine European studies (9, 12, 39, 44, 53, 59, 60, 63, 70) reported that an average of 1.6% of treated patients from clinical and hospital populations harbored T69S\slash H11001\_XX insert strains (range, 0.5% to 3.0%). Those studies evaluated patients through various time frames between 1996 and 1999. Samples from the United States and Europe submitted to a reference laboratory for genotyping from July to December 2000 showed a 0.6% prevalence of T69S\slash H11001\_XX insert strains (19). A recent study from a U.S. reference laboratory examined 61,236 specimens submitted between 1998 and 2002 and showed that the prevalence of T69S\slash H11001\_XX insert strains decreased from 0.75% to 0.48% over that period (29). It is unclear whether this is a true decline in prevalence or a change in the population sampled, since over that period the testing guidelines changed, which resulted in isolates from more nontreated patients being genotyped. A subanalysis with only those isolates from patients with any drug resistance mutations showed a slightly higher prevalence, but it

### Table 1. Genotypes of most common 2-amino-acid RT and PR gene inserts

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype at the following codon in RT gene:</th>
<th>Genotype at the following codon in PR gene:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>67 68 69 Insert Insert 70 71 72</td>
<td>34 35 36 Insert Insert 37 38 39</td>
</tr>
<tr>
<td>Wild type</td>
<td>GAC AGT ACT AAA TGG AGA GAA GAA ATG AAT TTG CCA</td>
<td>GAA GAA ATG AAT TTG CCA</td>
</tr>
<tr>
<td>Nucleotide</td>
<td>D S T AAA K W R</td>
<td>E E M AAT TGG CCA</td>
</tr>
<tr>
<td>amino acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert</td>
<td>GAC AGT TCT AGT TCT AAA TGG AGA GAA GAA ATG AAT TTG CCA</td>
<td>GAA GAA ATG AAT TTG CCA</td>
</tr>
<tr>
<td>Nucleotide</td>
<td>D S S S K W R</td>
<td>E E M AAT TGG CCA</td>
</tr>
<tr>
<td>amino acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Boldface nucleotides and codons indicate inserts and associated features.

### Table 2. Prevalence of PR and RT gene inserts

<table>
<thead>
<tr>
<th>Insert</th>
<th>Region, cohort</th>
<th>No. of samples tested</th>
<th>Prevalence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT T69S+XX</td>
<td>Spain, clinic</td>
<td>475</td>
<td>0.8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>The Netherlands, clinic</td>
<td>228</td>
<td>3.0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>France, clinic</td>
<td>2,152</td>
<td>2.4</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>France, clinic</td>
<td>300</td>
<td>3.0</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>France, clinic</td>
<td>632</td>
<td>2.4</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>France, clinic</td>
<td>302</td>
<td>0.7</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Italy, clinic</td>
<td>3,595</td>
<td>0.9</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Europe, clinic</td>
<td>634</td>
<td>0.5</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Japan, hemophiliacs</td>
<td>748</td>
<td>1.1</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>United States and Europe, reference labs</td>
<td>5,000</td>
<td>0.6</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>United States, reference lab</td>
<td>1,045</td>
<td>1.0</td>
<td>67</td>
</tr>
<tr>
<td>RT codon 103</td>
<td>United States, reference lab</td>
<td>100,000</td>
<td>0.003</td>
<td>68</td>
</tr>
<tr>
<td>PR inserts</td>
<td>United States, reference lab</td>
<td>24,000</td>
<td>0.09</td>
<td>30</td>
</tr>
<tr>
<td>PR codon 35</td>
<td>Germany, clinic</td>
<td>1,000</td>
<td>0.1</td>
<td>56</td>
</tr>
<tr>
<td>PR codon 70</td>
<td>United States, reference lab</td>
<td>100,000</td>
<td>0.001</td>
<td>69</td>
</tr>
</tbody>
</table>
also showed a decrease over the time period from 1.0% to 0.72%. The transmission of an RT insert-containing strain has been reported in a study of newly infected individuals (10).

Overall, these studies indicate that approximately 1% of genotyped strains from patients from clinical practice harbor T69S+XX inserts. The slight decrease in the prevalence of these strains over time suggests that more recent treatment practices (e.g., the use of potent combinations) may not select for T69S+XX insert strains as efficiently as prior, weaker, practices (e.g., the use of potent combinations) may not select for these strains over time suggests that more recent treatment courses of monotherapy and simple combination regimens. In contrast, the Q151M mutation, which is selected only by potent NRTI combination therapy and which also confers multi-NRTI resistance, maintained a consistent prevalence of approximately 2.3% in the drug-resistant population (29). Additional studies over more recent time periods and with patients initiating therapy in the highly active antiretroviral therapy (HAART) era will be necessary to measure the continued impact of T69S+XX insert strains.

**Treatment history.** The NRTI treatment regimens that select for T69S+XX insert-containing strains can be identified from well-documented treatment history data. The treatment histories of 36 patients from 12 different publications show that initial AZT monotherapy, followed by combination therapy with AZT plus other nucleosides (usually ddI or ddC), is the most common course of treatment in patients with T69S+XX insert-containing strains (8, 9, 11, 12, 31, 35, 53, 57, 59, 62, 63, 67). Patients who have received at least 1 year of AZT monotherapy showed development of T69S+XX insert-containing strains as early as 6 months after they switched to combination therapy with AZT-ddC or AZT-ddI (67). Two patients developed insert strains while receiving ddI monotherapy (or ddI-hydroxyurea) that was instituted after more than a year of AZT monotherapy (11, 31). Another patient was shown to develop a T69S+XX insert strain on AZT-ddI combination therapy after 3 years of ddI monotherapy (67). Three instances in which patients who developed T69S+XX insert strains received only NRTI combination therapy (i.e., no stretch of AZT monotherapy) have been reported; however, the initial combinations contained AZT (31, 53, 59).

Thus, it is clear that while AZT treatment is most associated with the development of T69S+XX insert strains, treatment with other NRTIs, either subsequently or in combination with AZT, is also required. It must be considered, however, that nearly all of the patients with insert strains from published studies were treated during the era of NRTI monotherapy or early combination therapy. There are no published cases in which insert strains developed during an initial course of monotherapy with any NRTI. It is possible that insert strains are generated during long courses of AZT (or other NRTI) monotherapy, but these strains are outcompeted by quasispecies with other primary drug resistance mutations (e.g., TAMs) that confer sufficient resistance. Treatment with a different drug or combination of drugs would then provide an environment suitable for emergence of insert (and TAM)-containing strains that have sufficient cross-resistance to allow continued replication. While the prevalence of T69S+XX insert strains in the population has remained stable through 2003 (29), it remains to be seen whether new approaches to antiretroviral therapy, namely, triple or quadruple HAART as an initial regimen, continue to select for T69S+XX insert-containing strains from naïve patients.

**Treatment outcome.** A few reports have documented the treatment outcomes for patients with T69S+XX insert strains (4, 9, 39, 53). The patients studied had cycled through various NRTI regimens for several years. A reduction of the virus load was primarily achieved in patients who received intensification therapy with an NRTI or the introduction of a new class of drugs (an NNRTI and/or PI), although the follow-up times were relatively short in those studies. The use of NNRTIs in patients with T69S+XX insert strains may have an advantage because of the potential hypersusceptibilities exhibited by some of these strains (39, 64).

**Replication capacities.** Two laboratory studies have specifically evaluated the replication capacities of T69S+XX insert strains compared to those of wild-type strains (47, 48). Both studies have shown that recombinant isolates derived from clinical strains replicate slightly less efficiently (65 to 82%) than wild-type HIV-1 isolates. Larger decrements in replication capacity were seen in laboratory studies in which the inserts were removed from the clinical strain, the insert was placed alone or with T215Y in a wild-type background, or the T215Y mutation was removed from a T69S+XX insert-containing strain. These findings indicate that the genetic background (i.e., other mutations) in which the inserts evolve is important in not only contributing to drug resistance but also interacting with the inserts to improve replication competence.

**Mechanisms of resistance.** Two molecular mechanisms by which mutations confer reduced susceptibility to NRTIs have been described (54). Mutations can decrease the binding of inhibitors to the RT enzyme relative to the binding of the natural substrates and thus reduce their effectiveness. Examples of mutations that reduce nucleotide analog incorporation are M184V, L74V, and K65R. Mutations can also increase the rate by which chain-terminating inhibitors are removed from the growing DNA strand; once these are excised, reverse transcription of that strand can continue. Enzymes with multiple TAMs show significantly increased rates of excision of d4T and AZT but only minor excision increases of other nucleoside analogs (15).

Several studies have examined the molecular mechanism by which T69S+XX inserts contribute to reduced susceptibility to NRTIs. Little effect on nucleotide discrimination has been found in studies of insert-containing enzymes (6, 33, 38). However, RT enzymes with inserts and TAMs have been shown to effectively excise AZT and d4T from terminated DNA strands at rates similar to or greater than those of enzymes with only TAMs (7, 38, 41, 65). The cooperation of T69S+XX inserts and TAMs in conferring this activity has been shown in both reconstruction experiments (in which mutations are added and tested in a particular sequence) and deconstruction experiments (in which mutations are removed). Additionally, RT enzymes with T69S+XX inserts and TAMs can unblock the DNA strands terminated by all of the nucleoside analogs (7, 41), thus providing the basis for reduced susceptibility to multiple NRTIs.

RT Codon 103 Insertions

**Genotypic features and prevalence.** Recently, HIV strains with insertions near codon 103 of the RT gene have been
observed in patients who have failed ART drug treatment (68). These inserts were composed of 3 or 6 nucleotides and at the nucleotide level appear to be duplications of a neighboring sequence. Each of the three strains reported had one or more NRTI mutations. Two strains possessed the Q151M complex of NRTI mutations, while the third had only the M184V mutation. The prevalence of these strains in a large U.S. reference laboratory database is 0.003% (68).

**Susceptibility.** Limited susceptibility testing of the codon 103 inserts showed resistance to efavirenz (EFV) and nevirapine (NVP). Resistance to the NRTI tested was consistent with the NRTI mutations present. Further analysis of clones derived from one patient’s quasispecies showed that a recombinant viral clone with the insert was approximately 50-fold less susceptible to EFV than a similar clone without the insert. Susceptibility to NVP was not substantially different between the clones. These results suggest that the codon 103 insert may contribute to reduced susceptibility to some NNRTIs.

### RT Gene Insertions and Enzyme Structure

Amino acid substitutions (mutations) primarily affect susceptibility to ARV drugs by changing the enzyme’s three-dimensional structure enough to reduce inhibitor binding or increase nucleotide analog excision without destroying enzyme activity. While no crystal structures of insert-containing RT enzymes have been reported, extrapolation from other RT enzyme models shows that T69S+XX inserts are located in the β3-β4 loop of the fingers region of the RT molecule (Fig. 1A). This loop extends over the active site, and mutations in the region are believed to affect the orientation of the RNA template as it passes through the enzyme. Inserts are most likely accommodated by extending the loop toward the outside of the molecule, thus exerting their effect on the template orientation without abolishing enzyme function.

Codon 103 inserts are located in the β5-β6 loop of the NNRTI binding pocket of RT, toward the backside of the active site (Fig. 1A). Molecular modeling studies have suggested that the extra amino acids that comprise these inserts are positioned near the tip of a hairpin loop such that the extra residues are extended toward the outside of the molecule (68).

### RT Gene Deletions

Deletions in the β3-β4 loop of the RT gene are another uncommon genetic feature identified in some ARV drug-experienced patients (26, 51, 58, 66). In contrast to RT gene inserts, which have extra nucleotides, RT genes with deletions have 3 fewer nucleotides that produce an enzyme with one less amino acid that is typically identified as codon 67. Seventeen unique patient-derived strains with codon 67 deletions have been reported in the literature, and their prevalence in ARV drug-treated patients is approximately 0.15% (39, 66). Deletion strains possessed an average of 4 NRTI and/or NNRTI drug resistance mutations (range, 1 to 10 mutations), often TAMs or the Q151M complex. Susceptibility testing showed that these patient-derived deletion strains had reduced susceptibilities to three to six NRTIs (26, 39, 51, 58, 66). Reports conflict on whether the deletion alone confers reduced susceptibility to NRTIs (26, 51, 66); however, this point is of minor significance since all deletion strains carry additional drug resistance mutations that engage in complex interactions that affect susceptibility to both NRTIs and NNRTIs (5, 27, 66).

### PROTEASE GENE INSERTIONS

#### Genotypic features.** In the protease gene, a heterogeneous group of inserts ranging from 3 to 18 nucleotides has been identified in patient-derived HIV-1 strains near codons 18, 25, 36, 70, and 95 of the PR gene (16, 30, 56, 69). The genotype of the most common PR gene insert is shown in Table 1. The exact positions of the PR inserts are variable, especially those near codon 36. At the nucleotide and amino acid levels, most PR insert strains appear to be duplications of neighboring genetic sequence. This is most evident in the larger PR inserts, where inserts of 5 or more amino acids near codons 36 and 95 are an exact duplication of the neighboring sequence. These inserts are likely generated by the RT stalling and slippage that is also believed to generate RT inserts (3, 20). Approximately two-thirds of the reported PR inserts have one or more major PR gene mutations, most frequently, I84V and L90M.

#### Susceptibility.** PR insert strains without major PR resistance mutations have been shown to be fully susceptible to all PIs (30, 56). PR insert strains with major PR resistance mutations show reduced susceptibility to PI; however, the range and magnitude of resistance are consistent with the PR resistance mutations present. In addition, significant changes in susceptibility were not found in viral constructs from which the inserts were removed by site-directed mutagenesis (30). Thus, it appears that the PR inserts do not contribute directly to PI resistance.

#### Prevalence and treatment history.** PR insert strains appeared in about 0.1% of patients whose samples were submitted to a large reference laboratory between 1999 and 2001 (29, 30). A similar frequency was shown in a European clinical cohort (56). The treatment histories of three patients with PR insert strains have been reported. One patient developed a strain with a 2-amino-acid insert near codon 36 after 8 months of indinavir treatment followed by 16 months of nelfinavir treatment (30), while another patient developed a strain with a 2-amino-acid insert near codon 18 after 1 year of indinavir treatment (37). Neither patient showed evidence of these insert strains prior to PI therapy. One patient from a separate study had a strain with a 2-amino-acid insert near codon 36 before being treated with any PI (56). The transmission of a PR insert-containing strain has been reported in a study of newly infected individuals (16).

#### Replication capacity.** The replication capacity of PR insert strains was evaluated by comparing them to both wild-type HIV strains and molecular constructs of the patient-derived strains from which the inserts were removed (30). In all comparisons, the intact patient-derived recombinant viral strains grew more efficiently than the paired strain from which the insert was removed but grew less efficiently overall than the wild-type control strain. Thus, it appears that interaction between inserts and other mutations and polymorphisms that were coselected or that coevolved with the insert are important for maintenance of the activities of insert-containing enzymes.

#### Enzyme structure.** No crystal structures of insert-containing PR enzymes have been reported. However, models of non-
FIG. 1. Locations of inserts in the protein structures of RT (A) and PR (B) enzymes. (The RT structure is adapted from reference 10a with permission of the publisher.)
insert-containing PR enzymes indicate that PR inserts are primarily located at or near externally exposed turns or loops of the molecule. These positions are more likely to accommodate extra amino acids by extending these residues toward the outside of the molecule. This orientation prevents extreme modification of the overall protein structure that could destroy enzyme function. The inserts near codon 25 are immediately adjacent to residues that compose the active site, and while these inserts have been isolated from patient-derived strains, no studies have assessed their impact on enzyme function.

CONCLUSIONS

Characterization of genetic variants of HIV-1 strains isolated from patients is important for obtaining an understanding of viral pathogenesis and improving antiretroviral therapy. While point mutations predominate as indicators of genetic variation in HIV-1, insertions and deletions have been reported in structural or regulatory HIV genes such as LTR (23), gag (25), env (28), and vif (1). Insertions in the PR and RT genes of HIV-1 are quite unique, as insertions are rarely found in other enzymatic proteins of HIV or other human viral pathogens. Although the prevalence of PR and RT gene inserts is relatively low compared to that of point mutations, RT gene inserts are involved in conferring resistance to multiple ARV drugs and pose a significant problem for patients infected with these strains. In contrast, the varied spectrum of PR gene inserts is less common than that of T69S+XX inserts, and PR gene inserts do not appear to overtly affect PI susceptibility but may be involved in restoring enzyme function damaged by the acquisition of drug resistance mutations. RT and PR inserts have been shown to be transmissible between individuals and are thus a part of the serious public health problem caused by the spread of drug-resistant strains.

It is unclear why inserts are selected during antiretroviral therapy in only a small proportion of patients receiving similar courses of ARV drugs. The same information is lacking with other mutation patterns, such as the Q151M mutation versus TAMs in patients receiving combination NRTI therapy. The underlying genetic variation of the PR and RT genes (e.g., polymorphisms) may drive preferential selection; however, no particular genetic features that are associated with the development of particular resistance mutations have been identified. Other virus-related factors such as the inherent replication rate, overall population diversity, and the above-mentioned genetic interaction may influence the preferential selection of insert strains. In addition, host-related factors, such as drug metabolism and immune system recognition of particular epitopes, may also influence strain selection. Further studies of these factors are necessary and important to elucidate the conditions by which certain genetic features in HIV strains are selected.

Most studies of drug resistance mutations (and this review of inserts) have focused on the susceptibilities and replication capacities of specific enzymes targeted by PI and RT inhibitors. Several reports, however, have shown that changes in the PI substrate, at gag cleavage and noncleavage sites, can improve the replication capacities and the in vitro processivities of PR enzymes (2, 13, 36, 43, 46, 72). Recently, it has been shown that insertions in the gag gene can also mediate this effect (61). NRTI susceptibility may also be influenced by inserts in the gag region (45).

The fact that insertions and deletions that affect replication capacity and drug susceptibility have been found only in the gag, PR, and RT genes may be partially due to the intensity by which these genes are studied. The HIV integrase gene is a new target for antiviral drugs, and a few inhibitors are in clinical trials. To date, several point mutations have been selected in vitro and confer reduced susceptibility to integrase inhibitors (14, 22, 32). However, as with the PR and RT inserts, it may require the analysis of hundreds or thousands of strains from integrase inhibitor-treated patients to determine whether inserts in this gene could contribute to virologic or clinical failure. In addition, it is possible that point mutations, inserts, and deletions in other structural and regulatory genes that enhance the overall rate of viral replication can influence the efficacy of ARV drug therapy. Expansion of genetic analysis to other genes in well-characterized ARV drug-treated patients is needed to evaluate this hypothesis.

Unique genetic variants illustrate the complex dynamics of HIV biology. The ability to generate variants that have not only different sequences (point mutations) but also various lengths (insertions) demonstrates the magnitude of genetic diversity generated during HIV replication. The capacity of the PR and RT proteins to accommodate structure perturbations as large as 15 amino acids, which remain functional and which provide reduced susceptibility to drugs, further shows the challenges facing effective ARV drug therapy. The continued study of genetic variants that affect ARV drug therapy provides essential information that will benefit the clinical outcomes of patients with HIV infection.

REFERENCES


