Early selection of resistance-associated mutations in HIV-1 RT C-terminal domains across different subtypes: role of the genetic barrier to resistance

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Objectives: Interpretation of drug resistance mutation (DRM) has been based solely on HIV-1 subtype B. Reverse transcriptase (RT) C-terminal domains have been disregarded in resistance interpretation, as their clinical relevance is still controversial. We determined the emergence of DRM in RT C-terminal domains of different HIV-1 subtypes, the genetic barrier for the acquisition of these DRM and their temporal appearance with ‘classical’ RT inhibitor (RTI) mutations.

Methods: HIV-1 RT sequences were obtained from information from 6087 treatment-naive and 3795 RTI-treated patients deposited in the Stanford HIV Resistance Database, including all major subtypes. DRM emergence was evaluated for subtype B, and was correlated with the number of DRM in the polymerase domain. Genetic barrier was calculated for each DRM studied and in each subtype.

Results: N348I, T369I and A360V were found at low prevalence in treatment-naive isolates of all subtypes. A371V was common to treatment-naive isolates. N348I was observed in all subtypes, while T369I was only selected in subtype C. A360V and T369V were selected by RTI treatment in several subtypes. A371V was selected in subtypes B and C, but is a signature in subtype A. RT C-terminal mutations were correlated with early drug resistance in subtype B. All subtypes have a low calculated genetic barrier towards C-terminal DRM acquisition, despite a few disparities having been observed.

Conclusions: C-terminal mutations were selected in all HIV-1 subtypes, while some represent subtype-specific signatures. The selection of C-terminal DRMs occurs early in RTI resistance failure in subtype B.

Keywords: connection, RNase H, genetic barrier, subtype, drug resistance

Introduction

Human immunodeficiency virus type 1 (HIV-1) genetic diversity has allowed virus classification into types, groups, subtypes, sub-subtypes and recombinant forms. The design of antiretroviral drugs, the mapping of drug resistance mutations (DRMs) and the development of genotype interpretation algorithms have been based on HIV-1 subtype B. However, this subtype represented only 10% of novel infections in 2007 and is prevalent only in developed countries. Recent interest in anti-HIV therapy efficacy and drug resistance development in patients infected with non-B subtypes is therefore paramount. Genetic differences between HIV-1 strains modify viral protein structures and therefore can impair drug binding and efficacy. Reverse transcriptase (RT) inhibitors (RTIs) of the non-nucleoside class, for example, are ineffective against HIV-2 and HIV-1 group O.

HIV RT is a heterodimer with two catalytic activities: polymerization of double-stranded DNA, conferred by its polymerase (POL) domain (codons 1–300), and cleavage of viral template RNA, catalysed by its RNase H (RNH) domain (codons 441–560). POL has been the target of two major classes of antiretroviral drugs, and therefore various DRMs have emerged in this domain. RT C-terminal domains were disregarded in genotyping assays until 2005, when mutations in RNH were shown to impair template RNA degradation during reverse transcription, conferring additional time for RTI excision. The importance of the connection domain (CN, codons 301–440) in resistance was evident with the demonstration that N348I and T369I/V confer resistance to
both RTI classes. Other DRM in CN and RNH do not directly decrease drug susceptibility, but increase resistance conferred by thymidine analogue mutations. The limited number of entire viral RT sequences from treated patients has prevented an assessment of the clinical relevance of DRM in RT C-terminal domains. Some studies showed reduced susceptibility to RTIs and/or treatment failure associated with definite CN DRM. One study suggested no correlation between therapeutic failure and CN DRM, while another showed a minor effect of those mutations in etravirine failure. Whether CN and RNH DRMs emerge early in treatment failure or only in multiply failed patients remains unknown.

Some RT CN DRMs appear to be polymorphic in non-B subtypes, further preventing elucidation of their role in resistance. Studies with non-B subtypes were performed with a limited number of viral isolates. Our objective was to determine the emergence of DRMs in RT C-terminal domains of different HIV-1 subtypes, estimating the genetic barrier to the acquisition of mutations and assessing their temporal emergence combined with POL mutations.

Materials and methods

HIV-1 RT C-terminal sequences were obtained from information from 6087 treatment-naive and 3795 RTI-treated patients deposited in the Stanford HIV Drug Resistance Database (accessed June 2013). Sequences of five HIV-1 subtypes and two major recombinant forms (A, n = 394 naive and 82 treated; B, n = 3055 and 3118; C, n = 1302 and 195; F, n = 154 and 48; G, n = 232; CRF01_AE, n = 281 and 182; and CRF02_AG, n = 669 and 170) were retrieved. All sequences contained entire or partial CN and/or RNH regions and included the POL domain.

The DRMs previously characterized in HIV-1 CN and RNH domains G335D, N348I, A360V, T369I/V, A371V, A376S, A400T, D488E, Q509L and Q547K were analysed. Differences in the proportions of G335D, N348I, A360V, T369I/V, A371V, A376S, A400T, D488E, Q509L and Q547K is similar across all HIV-1 forms. The RT CN polymorphisms G335D and A371V were abundant (60–80%) in treatment-naive sequences of several non-B subtypes (Table 1). The major NNRTI-related mutations N348I and T369I had a low frequency in treatment-naive sequences (<1% and <0.4%, respectively). However, T369V was present in 11% of treatment-naive subtype A and CRF02_AG sequences. A360V was also observed at low prevalence (<1%) in treatment-naive sequences of seven HIV-1 genetic forms, while the A371V mutation was abundant in most subtypes, except for B and C (3%). The RNH mutations D488E, I509L and Q547K were rare (0%–2%) in all HIV-1 forms.

All HIV-1 variants selected at least three different mutations in CN upon treatment exposure (Table 1). Selection of N348I was observed in all subtypes. In contrast, T369I was not selected under RTI treatment in any HIV-1 genetic form analysed, except for subtype C viruses of treatment group 2. A360V and T369V were selected by RTI treatment in subtypes A, B and CRF01_AE. A371V was selected in subtypes B and C, while in subtype A and related recombinant forms it appeared as a signature. G335D was not selected by RTIs in any HIV-1 subtype, but it slightly increased in subtype C upon treatment. A376S appeared correlated with treatment in subtypes A, B and CRF02_AG, while A400T was selected in subtype B and CRF02_AG. There was no evidence of DRM selection in the RNH domain.

We next analysed the emergence of CN mutations in subtype B viruses according to the number of POL domain mutations, and we observed that most of them increased in frequency (Figure 1a). CN mutations did not emerge alone, but their presence appeared early, correlated with drug resistance in POL. The proportion of N348I, T369V and A376S was higher with the combined use of both RTI classes (group 2), while A360V, A371V and A400T arose more frequently with exclusive exposure to NRTI (group 1).

The calculated genetic barrier showed that the acquisition of N348I, A371V, D488E, Q509L and Q547K is similar across all HIV-1 genetic forms (Figure 1b). For the acquisition of G335D, 68% of subtype F isolates needed two changes (score of 3.5) in contrast to the remaining subtypes, which needed only one change (score of 1.0). Isolates carrying T369 needed only one transition (ts) to acquire 369I, as well as those carrying A369 to acquire 369V. Emergence of 376S appeared less likely in subtype A isolates harbouring V376 (score of 3.5). Other subtypes presented A376 or T376 (78%–94%), needing only one ts to acquire 376S. Finally, 21% of subtype C isolates presented I400, which showed a higher barrier to acquiring 400T (two ts) than A400 (one ts).

Discussion

In the present work we showed for the first time that HIV-1 RT CN DRM can be selected in a wide range of HIV-1 genetic forms. Mutations in this domain have been previously reported, but have been restricted to individual HIV-1 subtypes.

HIV-1 non-B subtypes carry genomic polymorphisms that correspond to compensatory DRMs in subtype B. Herein, A371V was pointed out as a genetic signature of CRF01_AE and was present in a large proportion of subtype A and CRF02_AG isolates, as previously shown. However, it was rare in treatment-naive
### Table 1. Prevalence of individual DRMs at HIV-1 C-terminal RT domains in different subtypes according to patient treatment status

<table>
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<tr>
<th>DRM</th>
<th>A W</th>
<th>B W</th>
<th>C W</th>
<th>F W</th>
<th>CRF01_AE W</th>
<th>CRF02_AG W</th>
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<td>treated (2)^c</td>
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<td>treated (2)</td>
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<td>G353D</td>
<td>348/394^d</td>
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<td>36/44</td>
<td>67/3055</td>
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<td>21/262</td>
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<tr>
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<td>(82)</td>
<td>(2)</td>
<td>(2)</td>
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<td>(2)</td>
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<tr>
<td>N348I</td>
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<td>0/31</td>
<td>5/1612</td>
<td>3/34</td>
<td>19/149</td>
</tr>
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<td>(3)</td>
<td>(0.3)</td>
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<td>(13)</td>
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<tr>
<td>A360V</td>
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<td>0/31</td>
<td>14/1582</td>
<td>19/575</td>
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<td>(0)</td>
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<td>109/977</td>
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<td>(70)</td>
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<td>(29)</td>
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<td>NF</td>
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<td>12/695</td>
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<td>(0)</td>
<td>(2)</td>
<td>(0)</td>
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**Notes:**
- **NF:** not found.
- ^aFor subtype G, no RT C-terminal sequences from treated subjects were available at the Stanford HIV Drug Resistance Database.
- ^bTreated (1), treatment with NRTIs only.
- ^cTreated (2), treatment with NRTIs plus NNRTIs.
- ^dUnderlined values correspond to frequency of DRM that differs significantly (P < 0.05) between drug-naive sequences of each non-B subtype compared with subtype B.
- ^eNumbers in parentheses correspond to percentages of total sequences of each subtype carrying each specific mutation.
- ^fBold type indicates statistical significance between treatment naive versus treated for a given subtype (P < 0.05).
subtype B and C isolates. G335D and A400T also appeared to be polymorphisms, while six other DRM were rare in all HIV-1 forms studied: N348I, A360V, T369I, D488E, I509L and Q547K.

The estimated genetic barrier could partly explain differences in DRM emergence among distinct HIV-1 subtypes, but some disparities were observed. For example, T369V should be more easily selected in subtype A compared with other subtypes. Indeed, one-third of subtype A isolates exposed to both RTI classes presented T369V. In agreement with that, subtype B accumulated 369I, but subtype C and CRF01_AE selected for 369V, different from the expected outcome. A possible explanation for this phenomenon is the impact of 369V on the replicative capacity of distinct subtypes. Further studies are necessary to confirm this hypothesis.

Fitness cost could explain why N348I (score of 2.5) was more selected than T369I/V (score of 1 or 2) in subtype B independent of the RTI class used. Seven CN and RNH codons need only one ts to acquire the respective DRM, including G335D, A360V, T369I, 369V, A371V, A376S, A400T, and Q547K.

Figure 1. (a) Proportion of drug resistance mutations in HIV-1 RT connection domain in association with the acquisition of resistance mutation in the polymerase domain (as listed in Johnson et al.6) in HIV-1 sequences from patients treated only with NRTIs (treatment group 1; left panel), where only NRTI-related mutations were considered, or with both NRTI and NNRTIs (treatment group 2; right panel), where mutations to both NRTIs and NNRTIs were considered. Asterisks denote positive or negative correlation with resistance in the polymerase domain (P<0.05). (b) Calculated genetic barrier for drug resistance mutations at HIV-1 RT connection and RNase H domains of different subtypes. Transitions were scored as 1.0, while transversions were scored as 2.5. Horizontal bars across each RT codon and each subtype represent the percentage of isolates harbouring a given genetic barrier (x-axis), while the bar colours represent the total (sum) score for the specified amino acid change (y-axis) according to the inset colour scale (bottom). Y (C or T); R (A or G); N (A, C, T or G); H (A, C or T); mut, mutation.
A369V and A371V. However, our analysis of treatment-experienced patients revealed that G335D was not selected in subtype B, while 369I/V were rare in most subtypes; 400T was selected in subtype B under NRTI treatment and in CRF02_AG under NRTI/NNRTI treatment. Interestingly, the proportion of this mutation decreased in subtype B under NRTI/NNRTI treatment, a fact that requires further assessment. We also observed that three RNH DRM previously reported in vivo—D488E, Q509L and Q547K—were absent in our sequences.

N348I was shown to arise early in drug therapy failure, while A360V and A371V correlated with the number of thymidine analogue mutations. Here, we further extend the observation of early emergence to A360V, A371V, A376S and A400T in subtype B isolates under RTI exposure. Such early emergence can influence treatment efficacy and durability, and deserves attention.

In conclusion, we showed that some HIV-1 RT connection mutations are selected in all HIV-1 genetic forms, while others are present as genetic signatures of specific subtypes. The calculated genetic barrier highlighted, in general, a low barrier to resistance acquisition in the RT C-terminal domains and an early selection of these mutations during RTI failure.

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Transparency declarations
None to declare.

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