

Identification of a mutation (A400T) in the connection domain of the reverse transcriptase associated to exposure and resistance to nucleoside reverse transcriptase inhibitors

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INTRODUCTION

Reverse transcriptase inhibitors (RTIs) resistance mutations have been identified in the RT polymerase domain (amino acid (AA) 1 to 318) and it has been recently suggested that mutations in the RNase H domain (AA 427 to 560) could significantly contribute to an increase of resistance to Nucleoside RTIs (NRTIs) (Brehm J et al, CROI 2006; Roquebert et al, J. Med. Virol 2007). However, there is few data concerning the RT connection domain (AA 319 to 426).

OBJECTIVES

- To compare *in vivo* the prevalence of RT mutations, particularly in the connection domain in naive and in RTI pre-treated patients.
- To determine some specific associations between RTI resistance mutations and mutations within the other RT domains.

PATIENTS AND METHODS

- HIV-1 RT sequences from 171 HIV-1 infected patients monitored at Pitié-Salpêtrière Hospital, Paris, France were analyzed.
- Among these 171 patients, 64 never received any antiretroviral treatment and the other 107 were RTI-pretreated patients harboring virological failure with a plasma HIV-1 RNA > 1000 copies/mL. For experienced patients, resistance genotypes were performed under therapy.
- The entire RT was amplified and sequenced into three fragments : from AA 1 to 243, from AA 244 to 426 and from AA 427 to 560.
- Genotyping HIV resistance testing was performed by automated population-based full sequence analysis (ABI system). Results of the genotypic analysis are reported as AA changes at positions along the reverse transcriptase (RT) gene compared with the wild type HIV-1 subtype B consensus sequence (<http://hivdb.stanford.edu/>).
- Using the entire RT sequence of each patients, isolates were subtyped by comparing their sequences with reference sequences of known subtypes in GenBank software.
- Statistical analysis : Fisher's exact test was used, applying the False Discovery Rate (FDR) method to take into account multiple testing, to compare frequency of mutations between naive and pretreated patients. Further investigations of the relationship among positions in the pretreated patients was performed by a principal component analysis.

RESULTS

- Characteristics of patients are presented in Table I. According to their treatment histories and among the pre-treated patients, 30/107 (28%) were only NRTI-experienced patients, whereas 77/107 (72%) were both NRTI and NNRTI-experienced patients.
- By evaluating the 560 AA in RT sequences, we identified mutations at 22 positions significantly associated (FDR<0.1) with RTI exposure (Table II). Among these positions, one mutation, the A400T, has never been previously reported to be associated with RTI exposure.

- Fig 1 illustrates, using correlation matrix, the associations between each of these 22 mutated positions that have been associated to RTI exposure in the present study. Positive correlations occurred between the mutation A400T and positions 103, 118, 190 and 215 (F but not Y). Statistically negative correlation occurred between A400T and position 65.
- A principal components analysis of the relative distance between the 22 RTI treatment-associated positions based on their degree of co-mutation in isolates from experienced patients is shown in [fig.2](#). A clustering seems to be shown between A400T and mutated positions 184, 103, 215, 75, 190, 208, 118, 101 and 74.
- The univariate analysis identified some factors that were associated to the A400T mutation (p<0.1): treatment experience, subtype B, ZDV, d4T, ABC or NVP exposure, at least one TAM, at least 4 NRTIs received in the past and NNRTI exposure.
- In multivariate analysis, 3 factors remained independently associated to the A400T mutation:
 - Subtype B (OR = 4.4, p = 0.029)
 - ABC exposure (OR = 3.1, p = 0.034)
 - NVP exposure (OR = 4.1, p = 0.014)

Figure 1 : Correlation matrix. This figure shows the correlation between each the 22 mutated positions. Positive correlations are in deep blue whereas negative correlations are in light blue. Correlations between A 400T and other mutated positions are in the last column boxed in pink.

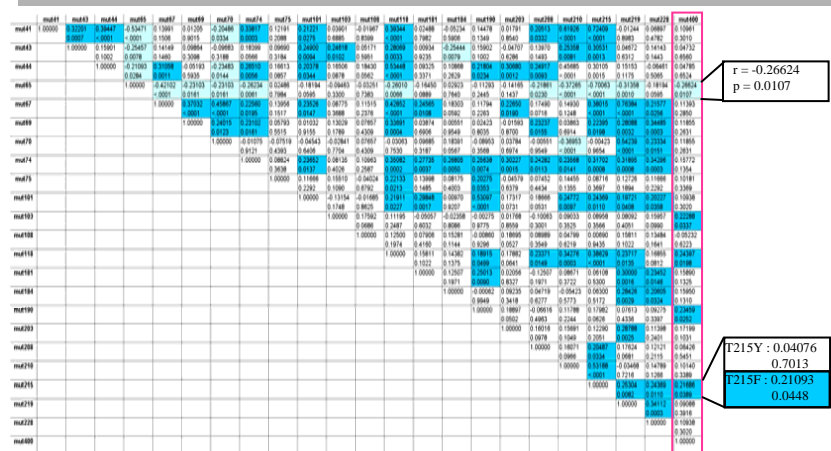


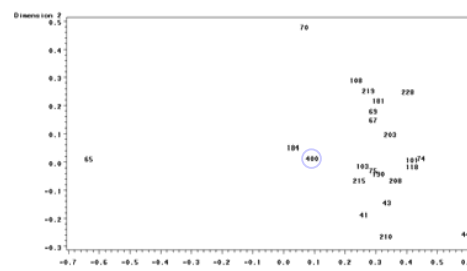
Table I : characteristics of patients

		patients n (%)
NRTI naive n = 64	B subtype	48 (75)
	number of NRTI received	2
	median = 5	5 (5)
	3	11 (10)
	4	18 (17)
NRTI experienced n = 107	B subtype	87 (81)
	number of NRTI received	2
	median = 5	5 (5)
	3	11 (10)
	4	18 (17)
Number of TAMs median = 2	0	27 (25)
	1	13 (12)
	2	26 (24)
	3	29 (27)
	4	12 (11)
NNRTI	naive	30 (28)
	experienced	77 (72)

Table II : Positions leading to a FDR < 10% between naive and pretreated patients

Mutated position	FDR	naive patients	pretreated patients
mut41	<0.001	0	53
mut67	<0.001	0	50
mut74	<0.001	0	28
mut118	<0.001	1	33
mut184	<0.001	0	82
mut215	<0.001	3	66
mut219	<0.001	3	44
mut70	0.0002	0	24
mut43	0.0003	2	27
mut210	0.0004	6	35
mut103	0.0005	0	21
mut65	0.0010	0	20
mut69	0.0010	2	24
mut190	0.0033	0	18
mut228	0.0033	0	19
mut181	0.0057	0	17
mut208	0.0101	0	16
mut44	0.0184	0	15
mut203	0.0343	3	20
mut75	0.0608	0	12
mut400	0.0810	49	74
mut101	0.0943	3	19
mut108	0.0965	0	11

Figure 2 : Principal component analysis. This figure shows the 22 positions that have been found as associated with RTI therapy in this study. The graph is a two-dimensional projection of the distances among the 22 positions. Positions that are closed together on the graph are those with a high degree of co-mutation in patients, whereas positions that are far apart are those with a low or negative degree of co-mutation.



CONCLUSIONS

- By evaluating the 560 AA in RT sequences, we identified mutations at 22 positions significantly associated (FDR<0.1) with RTI exposure. Among these 22 positions, 18 have been previously identified as RTI resistance mutations (IAS RTI resistance mutations list), 4 have been previously described as associated to RTI exposure (43, 203, 208 and 228) and 1 mutation, the A400T, has never been previously reported to be associated with RTI exposure.
- The A400T mutation is located in the RT connection domain and is positively correlated with mutations in position 103, 118, 190 and 215.
- The fact that A400T is associated with 215F but not 215Y suggests that the type of TAMs profile (II vs I) could have an influence on the selection of the mutation A400T or inversely.
- Multivariate analysis showed that A400T mutation was significantly associated to ABC and NVP exposure. There are other studies presented in this conference describing associations between connection domain mutations and NVP exposure (oral/posters # 90, 593, 594).
- A400T mutation is associated with specific RTI resistance mutations and with ABC or NVP exposure. Biochemical studies are now warranted to determine its own impact on the level of resistance to RTI.

215Y : 0.04076
0.7013
215F : 0.21093
0.0448