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EVOLUTION OF GAG AND GP41 IN PATIENTS RECEIVING FIRST-LINE PI AND NNRTI REGIMENS.

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INTRODUCTION

HIV-1 can develop resistance to PR inhibitors (PIs) as a result of PR mutations that reduce PI binding affinity or compensate for the effects of PI binding site mutations. Such mutations occurred commonly when PIs were used without ritonavir-boosting (PI/r) or in incompletely suppressive ARV treatment regimens. However, PR mutations responsible for PI resistance are now uncommon following virological failure (VF) on an initial PI/r-containing regimen.

Two explanations have been proposed for the absence of PI-resistance PR mutations in patients with VF on an initial PI/r regimen: (1) PI-resistance mutations develop only in viruses repeatedly exposed to a narrow window of drug concentrations in which PI levels are high enough to exert selective drug pressure but low enough to allow virus replication; and (2) Mutations in *gag* and/or the cytoplasmic domain of *gp41* (*gp41-CD*) can also reduce PI susceptibility. However, ***gag* and/or *gp41-CD* mutations responsible for reduced PI susceptibility have not been consistently identified in individuals with VF on a PI/r regimens.**

METHODS

To identify gag and *gp41* mutations under selective PI pressure, we sequenced *gag* and/or *gp41* in 61 patients with VF on initial PI (n=41) or NNRTI (n=20) regimen. Of 41 PI-treated patients, we sequenced gag and gp41 in 11, gag alone in 13, and gp41 alone in 17 patients. Of 20 NNRTI-treated patients, we sequenced gag and gp41 in 13, gag alone in 3, and gp41 alone in 4 patients.

We calculated the proportions of nonsynonymous and synonymous mutations (dN/dS) in both genes and also attempted to identify sites displaying directional and/or diversifying selection using the Hyphy programs, MEDS and FEL, respectively.

We used *gag* (n=4,722) and *gp41* (n=4,008) polymorphism data from PI-naive patients in the LANL database to identify mutations displaying a high selection index. We defined the selection index as the log10 of the ratio of the prevalence of the pre-therapy amino acid divided by the prevalence of the post-therapy amino acid. Mutations with a high PI-selection index (>1.0) were defined as changes from a conserved amino acid at a position to an amino acid with a prevalence ten times less common acid at that position.

RESULTS

Description of patients and sequences

PI group: 38 patients received ATV/r for a median of 23 months; 3 received LPV/r. The median VL pre-therapy was 4.75; the median VL post-therapy was 4.0 ($p < 0.001$). All but 3 patients attained virological suppression some time during therapy. 9 developed >=1 PI (n=3) or NRTI (n=8) resistance-mutations.

NNRTI control group: 17 patients received EFV for a median of 17 months and 3 received NVP. The median VL pre-therapy was 4.85; the median VL post-therapy was 4.15 ($p = 0.001$). 15 developed >=1 NRTI (n=6) or NNRTI (n=15) resistance-associated mutations.

Phylogenetic analysis: All paired sequences from the 41 patients who received PIs and from the 20 control subjects clustered by individual in joint phylogenetic trees (**Figure 1**). All patients in the PI group and 18 in the NNRTI control group had subtype B sequences.

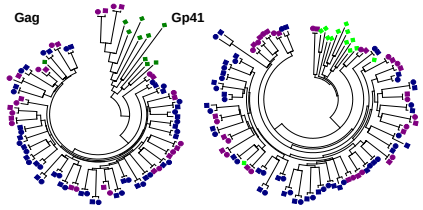


Figure 1. Phylogenetic clustering of Gag and Gp41 sequences from PI exposed (blue) and NNRTI exposed (purple) patients. Solid circles and squares represent baseline and failure samples, respectively. Solid diamonds represent different reference subtypes.

Comparison of pre- and post gag sequences

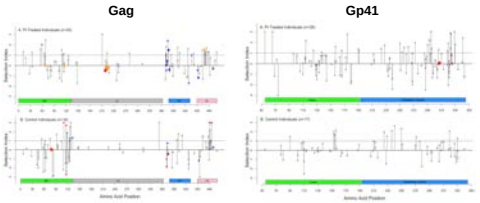
dN/dS: No difference in the median dN/dS in the complete gag (PIs 0.15 vs. NNRTIs 0.22; p=0.2), MA domain (PIs 0.20 vs. NNRTIs 0.39; p=0.4), or C-terminal domain (PIs 0.29 vs NNRTIs 0.40; p=1.0) between paired sequences from PI-treated and NNRTI-treated patients.

Individual positions: In the PI group, K59M, Q219H, F370Y, T371N displayed evidence for directional selection and positions 67, 115, 223, 468, 469, 474 displayed evidence for diversifying selection. In the NNRTI controls, Y79F, K110M, A/N371T, D480L displayed evidence for directional selection and positions 69, 116, 375, 484 displayed evidence for diversifying selection (**Figures 2**). There was no difference in the number of mutations with a high selective index between PI-treated and control patients (32/175 vs. 23/109 mutations; p=0.6). There was also no discernable difference in the number of positions with evidence for directional or diversifying selection between the two groups.

Comparison of pre- and post gag sequences (continued)

Protease cleavage site mutations: 12 patients in the PI group had 17 cleavage site mutations including 11 P2/NC, 2 PI/P6, and 2 P6/PR. 2 of these had a high selective index: I376T (I and T have prevalences of 80% and 0.3%, respectively) occurred in 1 patient and M378I (M and I have prevalences of 94% and 3%, respectively) occurred in 2 patients. 7 patients in the NNRTI control group had 9 cleavage site mutations including 1 MA/CA, 4 P2/NC, 1 P1/P6, and 3 P6/PR mutations (**Figures 2**). One of these, P485S, had a high selective index. One patient in the PI group had a PTAP insertion at codon 457.

Nucleotide ambiguities: In the PI group, 63 (36%) of 175 mutations were changes from a pre-therapy mixture of 2 amino acids to 1 of the possible resolutions of the mixture in the post-therapy sequence. Consistent with this observation, the median proportion of ambiguities pre-PI therapy was greater than post-PI therapy: 0.43% vs 0.04; p = 0.02). A similar but less marked evolution in nucleotide and amino acid mixtures was observed in the NNRTI group with the pre-therapy sequences having a higher proportion of nucleotide ambiguities compared with the post-therapy sequences: 0.13% vs. 0% (p=0.04).



Figures 2. Distribution of selection indexes in gag and gp41 in the PI and NNRTIs control groups. The height of each point is the selection index and the size of each point is proportional to the number of mutation occurrences. Positions exhibiting diversifying selection are colored orange. Amino acids exhibiting directional selection are colored red. Mutations at cleavage sites are colored blue. Mutations at cleavage sites that exhibit diversifying or directional selection are colored brown.

Comparison of pre- and post gp41 sequences

dN/dS: No difference between the median dN/dS in the complete gp41 (PIs 0.27 vs. NNRTIs 0.27; p=0.7) or cytoplasmic domain (PIs 0.36 vs. NNRTIs 0.3; p=0.6), between paired sequences from PI-treated and NNRTI-treated patients.

Individual positions: In the PI group, T307I and I/L325F displayed evidence for directional selection and positions 55, 101 displayed evidence for diversifying selection (**Figure 2**). In the NNRTI group, no mutation displayed directional selection and position 24 displayed evidence for diversifying selection. There was no difference in the number of mutations with a high selective index between PI-treated and control patients (30/1153 vs. 13/90; p=0.4). There was also no discernable difference in the number of positions with evidence for directional or diversifying selection between the PI and NNRTI groups.

CONCLUSIONS

This study is the first to analyze entire gag and gp41 sequences before and after PI/r therapy in which gag and gp41 changes are compared to those occurring in a control group with VF on an NNRTI-containing regimen.

We found in both the PI- and NNRTI-treated groups that many mutations developed in the gag MA and C-terminal domains and in the gp41 fusion and cytoplasmic domains. However, in neither gene, were there discernable differences in the overall numbers of mutations, number of mutations displaying evidence of directional selection, or numbers of mutations with a high selection index between the two treatment groups.

Many amino acid changes, particularly in gag, may reflect a reduction in quasispecies diversity following the bottleneck of treatment-induced virological suppression as evidenced by a significant decrease in the proportion of nucleotide ambiguities post-therapy. This observation underscores the usefulness of the NNRTI control group as many of the observed amino acid changes may have resulted from this bottleneck rather than in response to the specific treatment regimen received.

Several potentially PI-associated gag mutations occurred in the PI group including 17 cleavage site mutations, one PTAP duplication, and 4 codon 219 mutations (Q219H and H219Q) – a highly polymorphic position involved in cyclophilin A incorporation. Two cleavage site mutations – I376T in 1 patient and M378I in 2 patients – had a high selective index. However, patients in the NNRTI-control group also developed multiple cleavage site mutations.