

**POSTER 53****WITHIN-HOST COEVOLUTION OF GAG P453L AND PROTEASE D30N/N88D DEMONSTRATES VIROLOGICAL ADVANTAGE IN A HIGHLY PROTEASE INHIBITOR-EXPOSED HIV-1 CASE**

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HIV-1 with protease inhibitor (PI)-resistant mutations often demonstrates lower replication capacity than that of wild-type HIV-1. However, the acquisition of mutations in Gag, the viral protease (PR) substrate, recovers viral replication capacity. This phenomenon is known as co-evolution of the PR and Gag. To better understand the mechanism of Gag and PR coevolution in drug-resistance acquisition, a drug-resistance case was analyzed by both bioinformatics and virological methods. We especially considered the quality of sequence data and analytical accuracy by introducing single-genome sequencing (SGS) (Palmer et al., 2005) and Spidermonkey Bayesian graphical models (BGM) (Poon et al., 2008), respectively. Plasma samples were collected eight times from a HIV-1 (subtype B)-infected patient who had received anti-HIV treatments, and 129 HIV-1 Gag-PR linkage sequences were analyzed by SGS. The resulting sequences were analyzed by Spidermonkey BGM, and eight pairs were identified as significantly coevolving. Among these, we focused on associations between Gag-P453L and PR-D30N/N88D because D30N<sup>PR</sup> and N88D<sup>PR</sup> are well-known nelfinavir-resistant mutations, and P453L<sup>Gag</sup> is the P5' position of the p1/p6 cleavage-site mutation. To confirm whether P453L<sup>Gag</sup>/D30N<sup>PR</sup>/N88D<sup>PR</sup> from patient-derived gag-protease has a virological advantage, six types of recombinant clones were constructed on a NL4-3 backbone, and each clone was evaluated for viral replication kinetics and drug susceptibilities. The results indicated that P453L<sup>Gag</sup> has the potential to improve replication capacity of viruses with D30N<sup>PR</sup>/N88D<sup>PR</sup>, but has little effect on nelfinavir susceptibility. In conclusion, we successfully determined significantly coevolving sites by both bioinformatics and virological methods, and viruses that acquired these co-mutations distinctly showed increased fitness. The two new methods employed in this study appear to be promising for detecting coevolution between/within the HIV gene under highly active antiretroviral therapy.