

# Baseline Genetic Drug Resistance Analysis of South African HIV-1 Subtype C Proteases

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## Summary

Forty HIV-1 subtype C protease sequences from drug naive South African individuals were examined for amino acid mutations associated with resistance to protease inhibitors. Single and multiple accessory resistance mutations and polymorphisms were frequently detected in this study sample. These viruses would be expected to be susceptible to currently available protease inhibitors. However, it is important to assess the significance of multiple accessory mutations in the development of resistance once drug pressure is applied. In-vitro assays addressing this, will be the focus of future experiments.

## Background

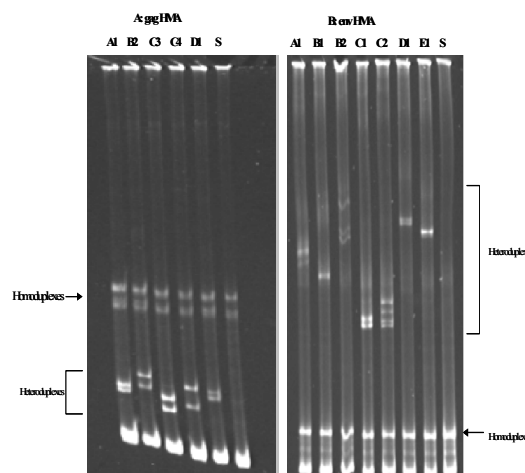
Baseline data on HIV-1 genetic drug resistance mutations in South Africa is limited. Information in this regard may assist in deciding whether or not to perform resistance studies prior to the initiation of antiretroviral therapy. We have examined the protease region of 40 classified HIV-1 subtype C primary isolates obtained from drug-naive patients from Gauteng and Limpopo Provinces of South Africa for mutations that could signify resistance to protease inhibitors based on subtype B data.

## Methods

Viral RNA was isolated from patients' plasma using the Qiagen viral RNA kit. Subtyping was determined by *gag* and *env* heteroduplex mobility analyses (HMA). The protease gene coding for amino acids 1-99 was amplified in a nested RT-PCR using consensus HIV-1 subtype C primers. Predicted amino acid sequences were examined for changes associated with drug resistance in relation to HIV-1 subtype B. Phylogenetic analysis of the protease gene regions was done to confirm the HMA profiles.

## Results

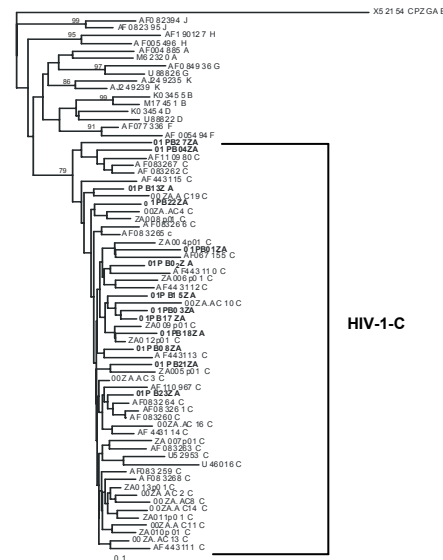
All samples were designated as subtype C based on *gag* and *env* heteroduplex mobility analyses (Figure 1), and phylogenetic analysis of the protease sequences (Figure 2). No mutations characteristic of primary resistance to protease inhibitors were detected. However amino acid changes M36I and I93L, which are known secondary drug resistance mutations in subtype B infections where each noted in 36/40 (90%) of these subtype C sequences. A combination of K20R and M36I known to contribute to indinavir and ritonavir resistance were detected in 10/40 (25%) of the isolates. The frequency of other observed amino acid substitutions associated with secondary drug resistance are shown in Figure 3.



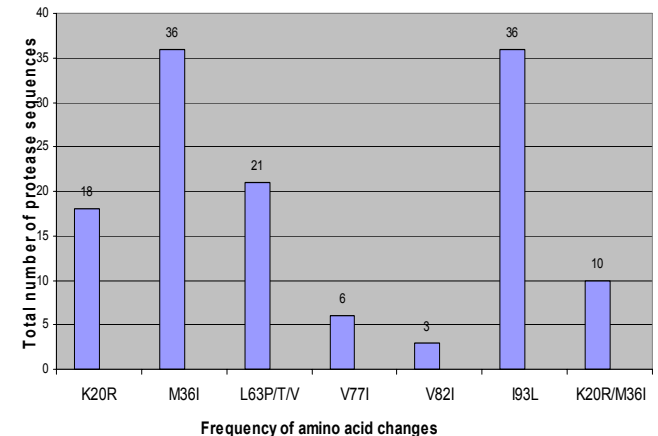
**Figure 1: A representative profile of *gag* (A) and *env* (B) HMA analyses.** Mobilities of heteroduplexes formed between the amplified *gag* product of 01PB15ZA and reference strains. **A:** The fastest mobility is shown by the heteroduplex between the unknown sample 01PB15ZA and the reference strain C3 (UG286 - Uganda). Lane S is the sample hybridized unto itself. **B:** The fastest heteroduplex is equally shown between the sample and a reference strain C1 (MA959 - Malawi). Sample 01PB15ZA was therefore designated HIV-1 subtype C based on the *gag* and *env* regions.

## Conclusions

- Although this analysis revealed several potential secondary resistance mutations, it would still be expected that many or most of the sequenced viruses would be susceptible to currently available protease inhibitors.
- However, the impact of single and multiple secondary resistance mutations and polymorphism on the propensity to develop resistance once drug pressure is applied is unknown.
- There is therefore need to evaluate apparent polymorphisms and continuous surveillance among patients failing anti-retroviral therapies in South Africa to determine the resistance patterns among subtype C viruses, the predominant variant responsible for the southern African epidemic.
- We are presently building observed potential drug resistance mutations into an HIV-1 subtype C molecular clone to evaluate their significance in the evolution of resistance to protease inhibitors.



**Figure 2: Phylogenetic analysis of protease sequences.** Protease sequences were aligned using ClustalX with reference HIV-1 subtype C protease sequences described in the Genbank from Southern Africa and other HIV-1 subtypes. A phylogenetic tree was constructed by the neighbour joining method and viewed with TreeView. The test protease sequences are shown clustered with subtype C reference sequences thus confirming their HIV-1 subtype C designation.



**Figure 3: Drug resistant associated amino acid changes in drug naive South African HIV-1-C proteases**