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Sequence Note

A Novel Codon Insert in Protease of Clade B HIV Type 1

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Douglas D. Richman,^{1,4} and Davey M. Smith^{1,4}

Abstract

A novel combination of three codon inserts in the *pol* coding region of HIV-1 RNA was identified in a highly antiretroviral experienced study subject with HIV-1 infection. A one codon insert was observed in the protease region between codon 40 and 41 simultaneously with a two codon insert present in the reverse transcriptase region at codon 69.

THE OBJECTIVE OF HIV TREATMENT is to suppress viral replication, and currently more than 20 different drugs have been approved to treat HIV.¹ Shortly after antiretroviral drugs were in clinical trial, drug resistance-associated mutations were first described.² HIV drug resistance has since been described for every active drug³ and drug resistance testing has been incorporated as part of standard clinical management and clinical trial design.⁴ In the course of a clinical study to assess the treatment of protease-resistant virus we discovered a previously unreported three base insert in HIV-1 clade B protease (PR) simultaneously with a six base insert in reverse transcriptase (RT) (GenBank accession number FJ159426).

HIV RNA was extracted, reverse transcribed, and the polymerase (*pol*) gene was amplified according to the manufacturer's instructions using the ViroSeq HIV-1 Genotyping System Version 2.0 (Celera Diagnostics, Foster City, CA). Sequence data were analyzed using ViroSeq Version 2.6 Sequence Analysis Software (Celera Diagnostics).

The six nucleotide insert (Fig. 1A) has been described previously and is known as a T69S + XX insertion.⁵ These inserts usually have a "T"-to-"S" point mutation at codon 69 and then a two amino acid insertion added to the functional protein. The proposed mechanism of decreasing susceptibility to ART by this insertion is to stall or to cause the slippage of RT during reverse transcription.⁶ Virus isolates containing these inser-

tions have reduced susceptibility to all nucleoside and nucleotide RT inhibitors.⁷ Stalling or slippage has also been hypothesized to be the mechanism behind the generation of PR inserts. Several PR insert strains have been identified both with and without major PR resistance mutations, although these PR inserts have not been shown to directly contribute to decreased susceptibility to protease inhibitors (PI).⁸ The insert we describe (Fig. 1B) has not yet been evaluated with site-directed mutants with and without the associated PI resistance mutations for impact on PI susceptibility *in vitro*. This insert, however, was identified in the setting of extensive, prolonged, and intermittent ART pressure (Fig. 2). The antiretroviral susceptibility profiles of the viruses with these inserts using the Monogram Phenosense assay are shown in Table 1.

To model the function of the described PR insert, we generated superimposed computer models of PR with or without our specific insert (Fig. 3). The close proximity of the one codon insert to the functional binding cleft of the PR homodimer could impact drug susceptibility, since the morphology and nature of the binding cleft may be altered by the addition of amino acids extending near or into the binding site leading to decreased PI binding.⁹ Further *in vitro* characterization of this novel PR insert with and without corresponding PR mutations associated with decreased susceptibility to PI still need to be evaluated.

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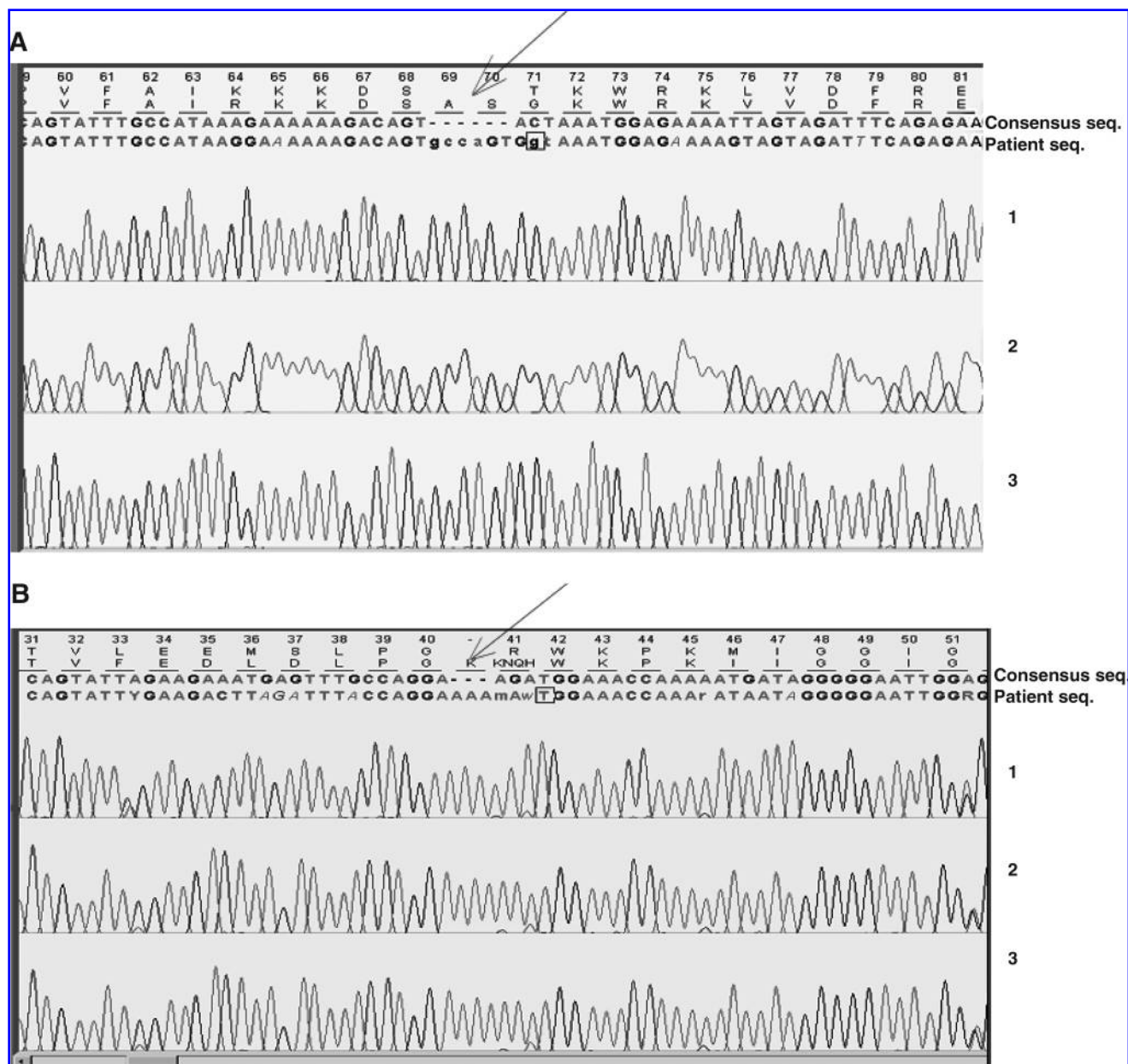


FIG. 1. (A) Electropherogram of the two codon RT insert. The arrow highlights the position of the additional bases. The consensus sequence is located on top of the patient sequence. Also shown are the three different primers (labeled 1, 2, and 3) that provided coverage for this coding region. (B) Electropherogram of the PR codon insert. The arrow highlights the position of the additional bases. The consensus sequence is located on top of the patient sequence and the three different primers (labeled 1, 2, and 3) that provided coverage for this region are shown.

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all of the participants and investigators involved in the ACTG 5126 trial. Written informed consent was obtained from all patients and the human experimentation guidelines of the U.S. Department of Health and Human Services and the individual institutions were followed in conducting this research. GenBank accession number: FJ159426.

Disclosure Statement

No competing financial interests exist.

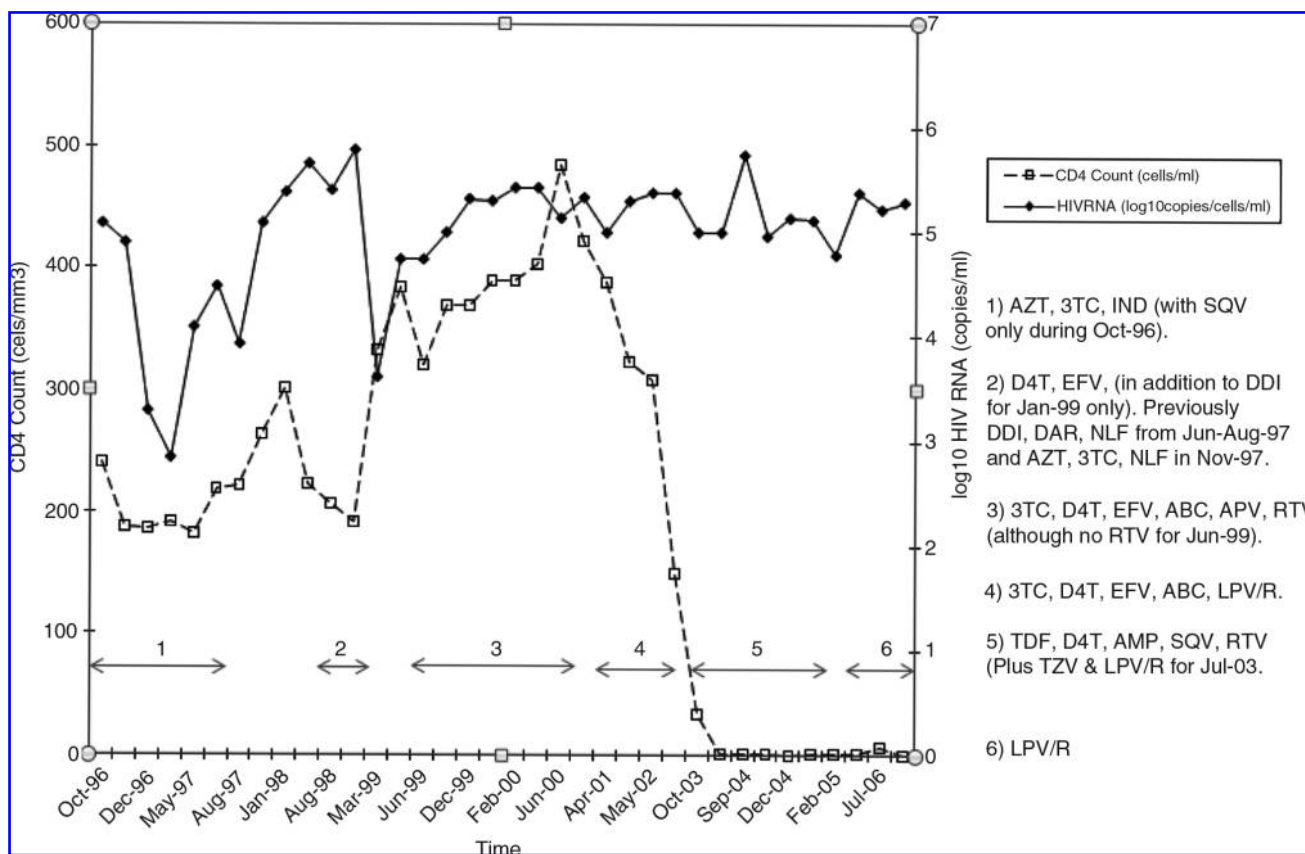


FIG. 2. Patient medication history, CD4 counts, and HIV viral load. Black diamonds are viral loads, open squares are CD4 counts, and double-headed arrows cover the dates of prescribed antiretroviral therapy [AZT, zidovudine; 3TC, lamivudine; IND, indinavir; SQV, saquinavir; D4T, stavudine; EFV, efavirez; DDI, didanosine; NLF, nelfinavir; ABC, abacavir; APV, amprenavir; RTV, ritonavir; LPV/R, kaletra (lopinavir + ritonavir); TDF, tenofovir; AMP, amprenavir; TZV, trizivir (zidovudine + lamivudine + abacavir)].

TABLE 1. PHENOTYPIC DATA OCTOBER 2003 USING THE MONOGRAM PHENOTYPIC ASSAY

Abbreviation	Medication name	Fold change in IC ₅₀ : Phenosense
NRTIs		
AZT, ZDV	Zidovudine	26.0
3TC	Lamivudine	>Max
D4T	Stavudine	5.2
DDI	Didanosine	4.4
ABC, ABV	Abacavir	25.0
DDC	Zalcitabine	479.0
TDF	Tenofovir	285.0
NNRTIs		
DLV	Delaviridine	>Max
EFV	Efavirez	>Max
NVP	Nevirapine	36.0
PIs		
LPV/r	Lopinavir	>Max
IDV	Indinavir	103.0
SQV	Saquinavir	29.0
RTV	Ritonovir	>Max
APV	Amprenavir	62.0
NFV	Nelfinavir	66.0

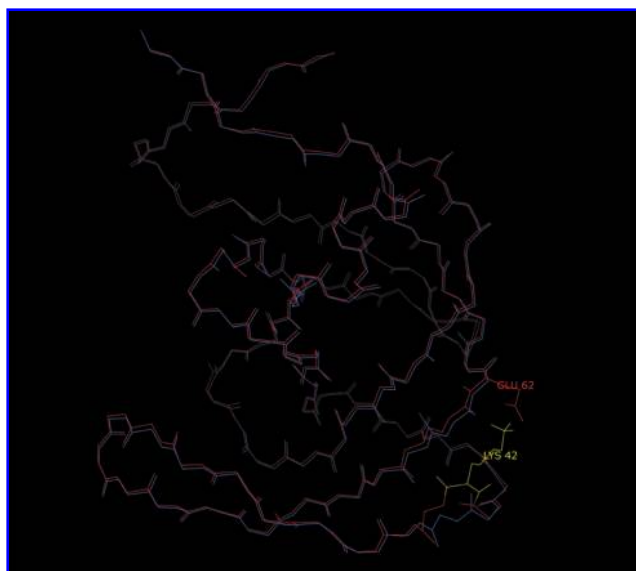


FIG. 3. Energy minimized computer images of a PR protein. The codon insert (red) is superimposed on a PR protein without the insert (blue). The resulting amino acid insert is highlighted yellow.

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