5-1-2009

A novel codon insert in protease of clade B HIV type 1.

Parris S Jordan
University of California, San Diego

Art Poon
University of California, San Diego

Joseph Eron
University of North Carolina at Chapel Hill

Kathleen Squires
Division of Infectious Diseases and Environmental Medicine, Thomas Jefferson University

Caroline Ignacio
University of California, San Diego

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 25, Number 5, 2009

DOI: 10.1089/aid.2008.0310

Sequence Note

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

Parris S. Jordan,[1] Art Poon,[1] Joseph Eron,[2] Kathleen Squires,[3] Caroline Ignacio,[1]
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 drug[3] 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 insertions 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.

[1]University of California, San Diego, La Jolla, California 92093.
[2]University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.
[3]Thomas Jefferson University, Philadelphia, Pennsylvania 19107.
[4]Veterans Affairs San Diego Healthcare System, San Diego, California 92161.

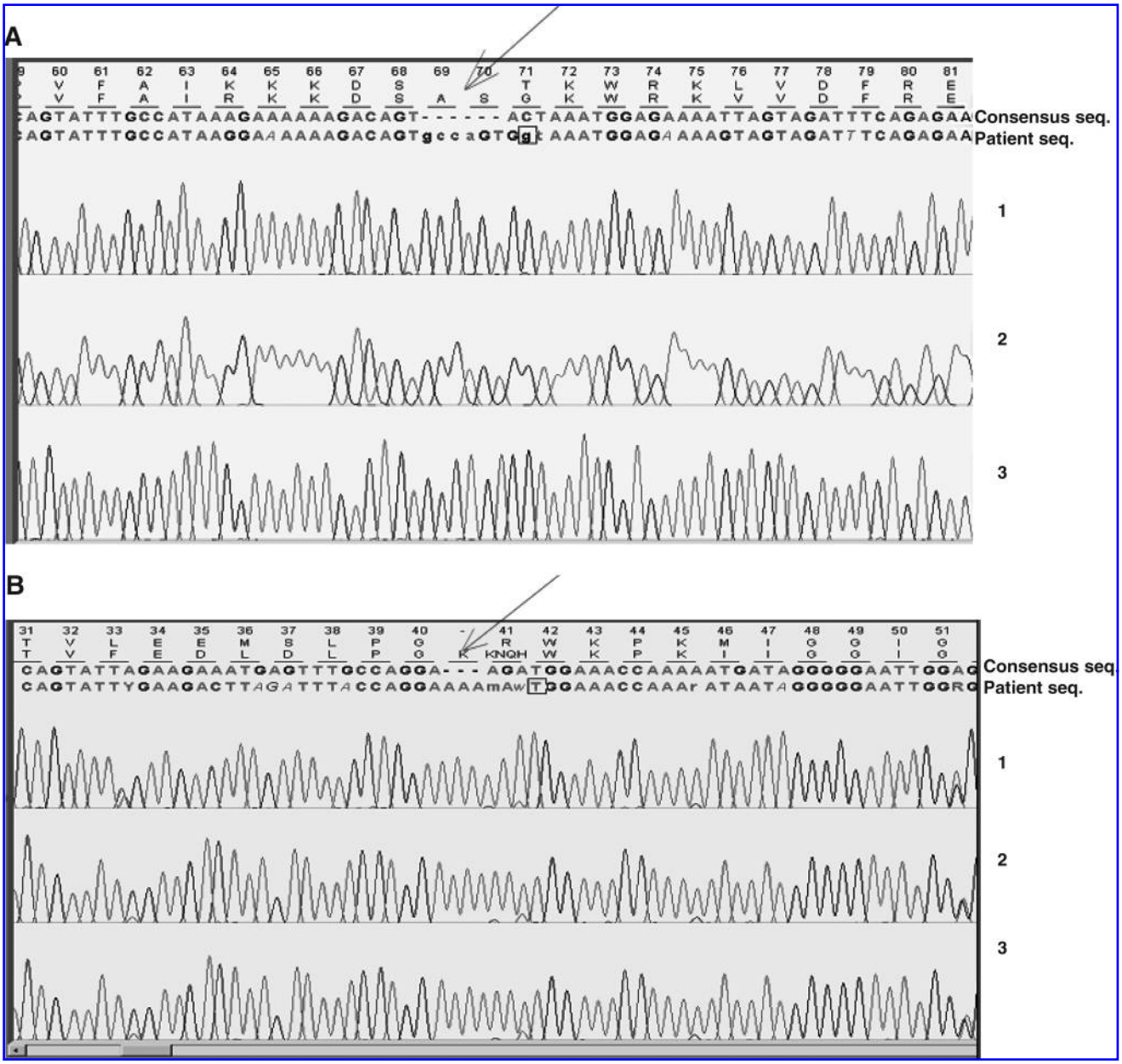

FIG. 1. (**A**) Electropherogram of the two codon RT insert. The arrow highlights the position of the additional bases. The consensus sequence is located on top of the patient sequence. Also shown are the three different primers (labeled 1, 2, and 3) that provided coverage for this coding region. (**B**)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

Acknowledgments

This work was supported by Grants AI69432 (ACTG), AI043638 (AIEDRP), MH62512 (HNRC), MH083552 (Clade), AI077304 (Dual Infection), AI36214 (the Viral Pathogenesis Core of the UCSD Center for AIDS Research), AI047745 (Dynamics), AI74621 (Transmission), AI069464 (Quintiles Clinical Trial), and AI69423 and AI50410 from the National Institutes of Health and the California HIV/AIDS Research Program RN07-SD-702. We would particularly like to thank 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.

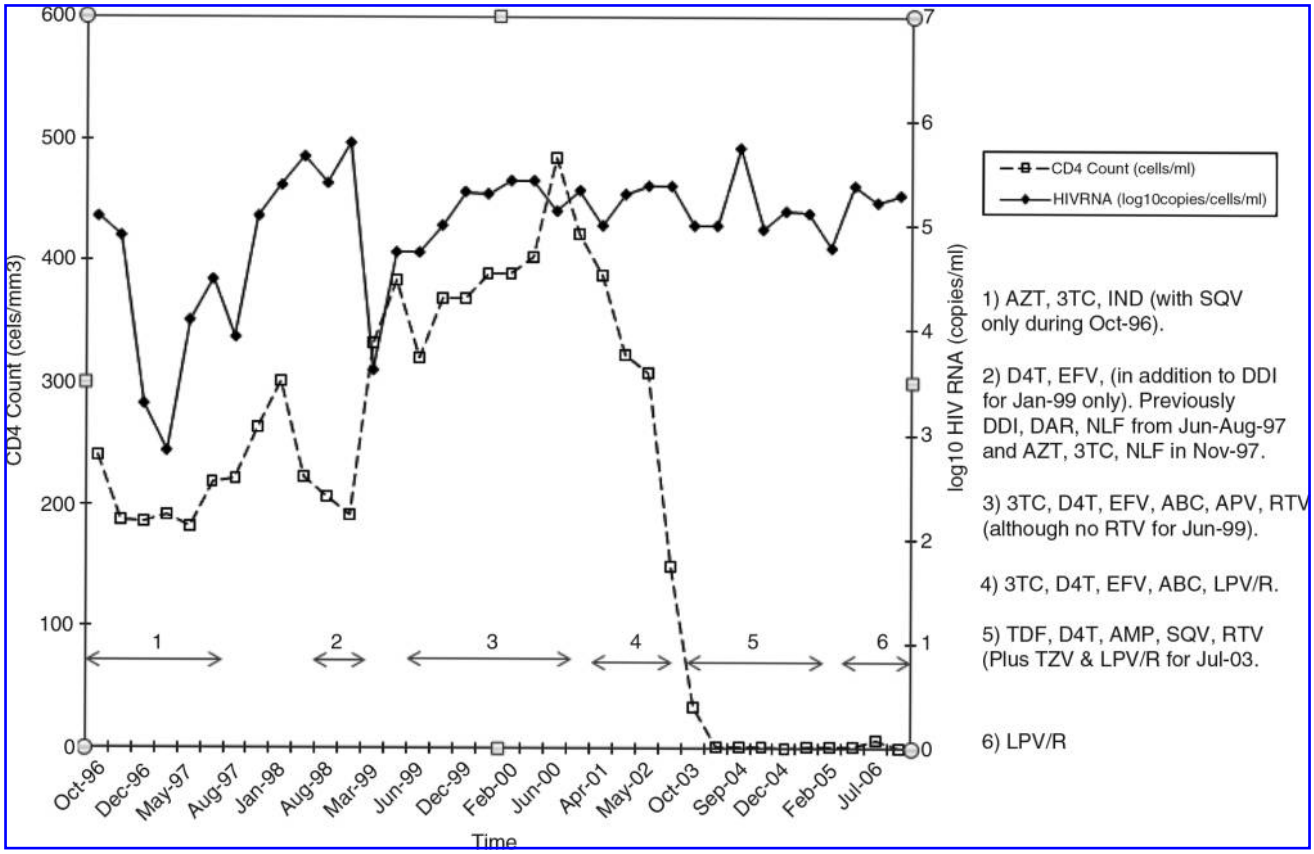

FIG. 2. Patient medication history, CD4 counts, and HIV viral load. Black diamonds are viral loads, open squares are CD4 counts, and double-headed arrows cover the dates of prescribed antiretroviral therapy [AZT, zidovudine; 3TC, lamivudine; IND, indinavir; SQV, saquinavir; D4T, stavudine; EFV, efavirez;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

Table 1. Phenotypic Data October 2003 Using the Monogram Phenosense Assay

Abbreviation	*Medication name*	*Fold change in IC_{50}: Phenosense*
NRTIs		
AZT, ZDV	Zidovudine	26.0
3TC	Lamivudine	>Max
D4T	Stavudine	5.2
DDI	Didianosine	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

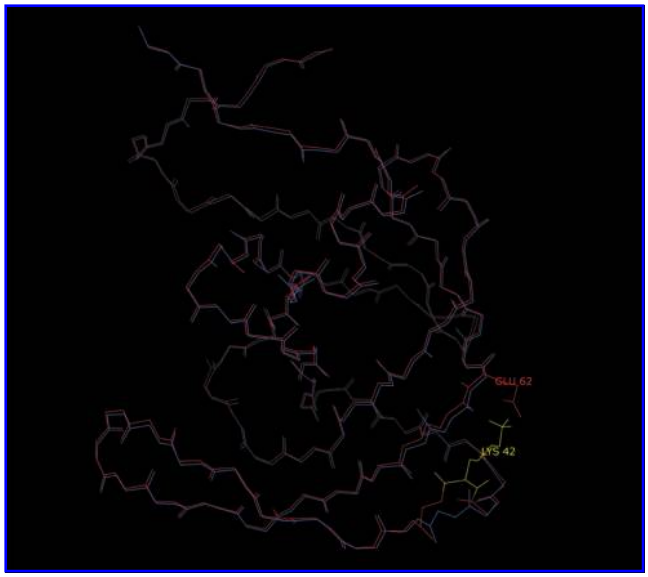

FIG. 3. Energy minimized computer images of a PR protein. The codon insert (red) is superimposed on a PR protein without the insert (blue). The resulting amino acid insert is highlighted yellow.

References

1. Hammer SM, Eron JJ Jr, Reiss P, *et al.*: Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. JAMA 2008;300:555–570.
2. Larder BA, Darby G, and Richman DD: HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science 1989;243:1731–1734.
3. Richman DD: HIV chemotherapy. Nature 2001;410:995–1101.
4. Hirsch MS, Gunthard HF, Schapiro JM, *et al.*: Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. Clin Infect Dis 2008;47:266–285.
5. Winters MA and Thomas MC: Insertion in the human immunodeficiency virus type 1 protease and reverse transcriptase genes: Clinical impact and molecular mechanisms. Antimicrobial Agents and Chemotherapy 2005;49:2575–2582.
6. Harrison GP, Mayo MS, Hunter E, and Lever AM: Pausing of reverse transciptase on retroviral RNA templates is influenced by secondary structures both 5′ and 3′ of catalytic site. Nucleic Acids Res 1998;26:3433–3442.
7. Winters MA, Coolley KL, Girard YA, *et al.*: A 6 base-pair insertion in the reverse transcriptase gene of HIV-1 confers resistance to multiple nucleoside inhibitors. J Clin Invest 1998;102:1769–1775.
8. Kim EY, Winters MA, Kagan RM, and Merigan TC: Functional correlates of insertion mutations in the protease gene of human immunodeficiency virus type 1 isolates from patients. J Virol 2001;75:11227–11233.
9. Kozisek M, Saskova KG, Rezacova P, *et al.*: Nintey-nine is not enough: Molecular characterization of inhibito-resistant human immunodeficiencey virus type 1 protease mutants with insertions in the flap region. J Virol 2008;82:5869–5878.

Address reprint requests to:
Davey Smith
University of California San Diego
9500 Gilman Dr. (MC-0679)
La Jolla, California 92093

E-mail: davey@ucsd.edu

This article has been cited by:

1. Annemarie M.J. Wensing, Noortje M. van Maarseveen, Monique Nijhuis. 2010. Fifteen years of HIV Protease Inhibitors: raising the barrier to resistance. *Antiviral Research* **85**:1, 59-74. [CrossRef]