Case Report/Relato de Caso

A first case of protease codon 35 amino acid insertion in a HIV-1 subtype B sequence detected in the Bauru Region, State of São Paulo, Brazil: case report

Primeiro caso de inserção de aminoácidos no codon 35 da protease em uma sequência de HIV-1 do subtipo B detectada na região de Bauru, Estado de São Paulo, Brasil: relato de caso

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ABSTRACT

Amino acid insertions in the protease have rarely been described in HIV-infected patients. One of these insertions has recently been described in codon 35, although its impact on resistance remains unknown. This study presents a case of an HIV variant with an insertion in codon 35 of the protease, described for the first time in Bauru, State of Sao Paulo, Brazil, circulating in a 38-year-old caucasian male with asymptomatic HIV infection since 1997. The variant isolated showed a codon 35 insertion of two amino acids in the protease: a threonine and an aspartic acid, resulting in the amino acid sequence E35E_TD.

Keywords: Codon 35 protease. HIV. Insertion.

RESUMO

Inserções de aminoácidos na protease têm sido raramente descritas em pacientes infectados pelo HIV. Uma destas inserções foi, recentemente, descrita no codon 35, embora seu impacto na resistência mantém-se pouco conhecido. Este trabalho apresenta um caso de uma variante viral com inserção no codon 35 da protease, descrita pela primeira vez em Bauru, São Paulo, Brasil, circulante em um homem, caucasiano, com 38 anos, o qual apresenta infecção assintomática pelo HIV desde 1997. A variante isolada mostrou uma inserção no codon 35 da protease de dois aminoácidos: uma treonina e um ácido aspártico, resultando na sequência de aminoácidos E35E_TD.

Palavras-chaves: Codon 35 da protease. HIV. Inserção.

INTRODUCTION

The principal objective of antiretroviral therapy in HIV infection is to suppress the plasma viral load to undetectable levels using combinations of drug classes[1], which slow the progression of the infection[2].

The antiretroviral drug selection pressure associated with a high mutation rate[3] has led to the emergence of resistant HIV variants. Resistant strains can result from amino acid changes or an insertion/deletion in the viral sequence, leading to an alteration in enzyme kinetics or antiretroviral drug accessibility[4].

Amino acid insertions in the protease (PR) have rarely been described in HIV-infected patients, occurring in 0.1 to 4.6% of patients naïve to or on treatment with protease inhibitors[5]. These insertions have been reported mainly in codons 17, 18, 22 to 25, 31 to 31, 35 to 38, 70, 71, 95 and 96[6].

Insertions in codon 35 of PR have recently been reported[7-9], but their impact on enzymatic activity, viral biology and resistance to protease inhibitors (PI) remains unknown[8]. These variants could show an advantage in proliferation in the presence of drug selection pressure[5,8,9].

The purpose of this study was to report a case of an HIV variant with an insertion in codon 35 of the protease, described for the first time in Brazil, which was identified circulating in an HIV-infected patient on treatment in Bauru, State of São Paulo.

CASE REPORT

The patient is a 38-year-old caucasian male with asymptomatic HIV infection since 1997 and no history of the conditions associated with AIDS. He had been previously treated with different drug combinations (**Table 1**), including didanosine, estavudine, zidovudine, lamivudine and efavirenz, while nowadays using lamivudive, tenofovir, atazanavir and ritonavir. The use of protease inhibitors was initiated in 1998 and continues up to the present, although their use was interrupted for four years (between 2001 and 2005). Since diagnosis, the patient never presented with a CD4 cell count lower than 200cells/mm^3 and the plasma viral load showed wide variation. After one therapeutic scheme that was not tolerated and two that led to failure, a drug resistance test (TRUGENE® HIV-1

TABLE 1 - Drugs combination used by the patient, showing the onset and end of each combination and the reasons for change.

Drugs combination	Onset	End	Reason for change
D4T/ddI/RTV	04/1998	11/2001	intolerance
D4T/ddI	11/2001	05/2004	therapeutic failure
AZT/3TC/EFV	05/2004	06/2005	therapeutic failure
3TC/TDF/ATV/r	06/2005	-	in current use

D4T: stavudine, ddI: didanosine, RTV: ritonavir, AZT: zidovudine, 3TC: lamivudine, EFV: efavirenz, TDF: tenofovir, ATV/r: atazanavir plus ritonavir.

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Received in 27/07/2010

Accepted in 24/01/2011

Genotyping Test on an OpenGene® DNA Sequencing System, Siemens Healthcare Diagnostics, Deerfield, IL , USA) was performed in the Molecular Biology Laboratory of the Blood Donor Center at the Botucatu School of Medicine, São Paulo State University (*Universidade Estadual Paulista*, UNESP), a laboratory of the Brazilian National Network for HIV-1 Genotyping (RENAGENO). At the time of writing this paper, the patient's CD4 cell count and the plasma viral load were 415 cells/mm^3 and 3.34 log, respectively.

The viral strain was subtype B (93% similarity in protease gene), according to the Viral Genotyping Tool available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). The sequence obtained showed a codon 35 insertion of two amino acids in the protease gene, a threonine, encoded by ACA and ACG, characterizing a dual viral population, and a single GAT sequence encoding aspartic acid, resulting in the sequence E35E_TD (**Figure 1**).

The mutation profile included the mutations I135T, D177E, Q197K, R211K and V245K in the reverse transcriptase gene and I13V, ins35TD, M46I, I62V, L63H, I64V and I72V in the protease gene, according to the Genotypic Resistance Interpretation Algorithm (HIVdb program) available at the Stanford University site (http://hivdb.stanford.edu/pages/algs/sierra_sequence.html).

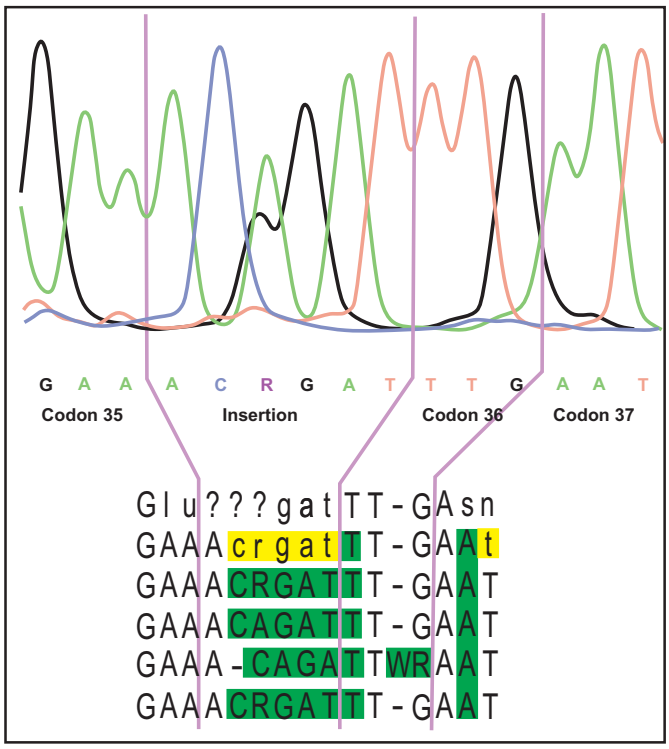

FIGURE 1 - Part of the electropherogram of the HIV-1 protease genomic sequence, showing the insertion of two amino acids between codon 35 and 36.

The first amino acid is encoded by ACR, the R represents dual population: A, in green and G, in black (see arrow) and; the other amino acid encoded by GAT.

DISCUSSION

Codon 35 of the protease has recently been described as a site at which insertions can occur. A two-amino acid insertion in protease codon 35, producing the sequence ETNLNL, circulating in a PI-naive patient and in his partner, was described in 2001. This strain has shown a normal replication capacity and susceptibility to protease inhibitors[7]. In our laboratory, of the 472 infected patients tested over a three-year period, only one showed an insertion in protease codon 35, suggesting that insertions in this region are rare. The sequence obtained in our laboratory (with ins35TD) was submitted to the Blastn algorithm (Blast Basic Local Alignment Search Tool) available at NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A 95% identity was determined, though no perfect alignment was obtained due to the presence of the insertion.

Various plasma viral load levels and CD4 T counts have been reported for viral variants with insertions in codon 35 of the protease gene, suggesting no relation with disease progression[10]. In this study, the isolated viral variant was detected using plasma viral RNA as the source, suggesting that this strain may be stable and manageable with HAART.

The presence of the insertions ins35G and ins35TN alone were not associated with decreased drug susceptibility. In the presence of the ins35TN and resistance mutations, the replication level was lower than when the resistance mutations were present with ins35G[8]. On the other hand, studies have shown that ins33L and ins35E can favor resistance to protease inhibitors[9]. The virus circulating in our patient did not exhibit a high resistance level to any protease inhibitor, but showed intermediate resistance to nelfinavir and potentially low-level resistance to atazanavir/r, fosamprenavir/r, indinavir/r and lopinavir/r. The mutation M46I decreases susceptibility to IDV/r, NFV, FPV/r, LPV/r, and ATV/r, when present with other mutations, suggesting that the low and intermediate resistance verified could be related to the presence of the M46I mutation. However, one study suggests that the ins35TD may have been selected during protease inhibitor therapy[5].

Viral and host conditions may be required for the emergence of insertions[5]. Our patient could not be evaluated before the initiation of therapy, and, thus, further studies of ins35TD are required to determine the factors that contribute to the selection of this variant.

FINANCIAL SUPPORT

Rede Nacional de Laboratórios de Genotipagem (RENAGENO), Departamento de DST/Aids e Hepatites Virais, Unidade de Laboratório and Ministério da Saúde.

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