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Brief Communication

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Aline Aki Tanikawa[I,IV]

Sarita Fiorelli Dias Barreto[II,IV]

Rejane Maria Tommasini Grotto[III,IV]

Maria Inês de Moura Campos Pardini[III,IV]

Coexistence of HIV-1 variants with dipeptidic insertion in the reverse transcriptase gene

ABSTRACT

The aim of this communication was to describe the detection of the coexistence of HIV-1 variant with dipeptide insertion between codons 69 and 70 of reverse transcriptase. These variants were isolated from a 16-year-old male patient, undergoing treatment in the city of Marilia, SP, Southeastern Brazil. After confirmation of treatment failure, resistance to antiretroviral drugs testing was performed and two variants with the insertions of the aminoacids Ser-Gly/Ser-Ala at codon 69 of reverse transcriptase were detected, besides the T69S mutation. These insertions have low prevalence, have not been reported in situations of coexistence in Brazil and are related to multidrug resistance, which makes this epidemiological finding relevant.

DESCRIPTORS: HIV-1, genetics. HIV Reverse Transcriptase. Human Immunodeficiency Virus Proteins. Drug Resistance, Multiple, Viral.

[I] Programa de Pós-Graduação em Fisiopatologia em Clínica Médica. Faculdade de Medicina de Botucatu. Universidade Estadual Paulista. Botucatu, SP, Brasil

[II] Programa de Pós-Graduação em Pesquisa e Desenvolvimento: Biotecnologia Médica. Faculdade de Medicina de Botucatu. Universidade Estadual Paulista. Botucatu, SP, Brasil

[III] Departamento de Clínica Médica. Faculdade de Medicina de Botucatu. Universidade Estadual Paulista. Botucatu, SP, Brasil

[IV] Laboratório de Biologia Molecular. Faculdade de Medicina de Botucatu. Universidade Estadual Paulista. Botucatu, SP, Brasil

Correspondence:
Maria Inês de Moura Campos Pardini
Faculdade de Medicina de Botucatu
Laboratório de Biologia Molecular – Divisão Hemocentro
Distrito de Rubião Júnior, s/ n°
18618-970 Botucatu, SP, Brasil
E-mail: inespardini@gmail.com

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INTRODUCTION

The selective pressure of the antiretroviral therapy (ARV) scheme may result in the emergence of resistant variants, which pre-exist in a minority of HIV infected patients. Point mutation of one or two bases in the reverse transcriptase (RT) gene are linked to resistance to inhibitors of this enzyme. Moreover, there are reports that resistance to multiple nucleoside analogs can also be the result of several insertions/mutations present in the viruses.[1,5]

Prolonged use of ARV therapy with zidovudine, often together or followed by administration of other nucleoside inhibitors, is linked with the selection of variants with amino acid insertion between residues 69 and 70 of HIV-1 RT.[3,5]

The aim of this study was to describe the detection of coexisting HIV-1 variants with insertions of two amino acids between codons 69 and 70 of RT.

DESCRIPTION OF THE CASE

The patient was male, aged 16, dark skinned and with vertical exposure to HIV. The case was reported in 1996 and, since then, the viral load remained detectable and CD4+ levels low without, however, developing symptoms of Acquired Immune Deficiency Syndrome (AIDS). The lowest CD4+ lymphocyte count occurred in 2011, 234 cels/mm^3, and the viral load in that same year was 4.624 log. The administration of ARV drugs started in 2007 with the RT inhibitors zidovudine and didanosine, followed by the introduction of efavirenz in 2008. After the patient committed himself to adhering to treatment, antiretroviral resistance testing (TRUGENE® HIV-1 Genotyping Test on an OpenGene® DNA Sequencing System, Siemens Healthcare Diagnostics, Deerfield, IL, USA) was requested in 2011 and carried out in the Molecular Biology Laboratory of the Blood Transfusion Center at the Botucatu Medical School, *Universidade Estadual Paulista* (UNESP), part of the Ministry of Health Brazilian Network for HIV-1 Genotyping (RENAGENO).

The viral subtype F1 was characterized using the reverse transcriptase gene, according to analysis with the REGA HIV Subtyping tool.[a] The sequence obtained showed T69S mutation, following insertions of Ser-Gly (SG) / Ser-Ala (SA), which suggests the coexistence of viral variants with different amino acid insertions at codon 69, confirmed from sequences generated by forward and reverse primers, according to the specifications of the technique used. Analysis of the electropherogram (Figure) showed the nucleotide sequences AGT and GST simultaneously.

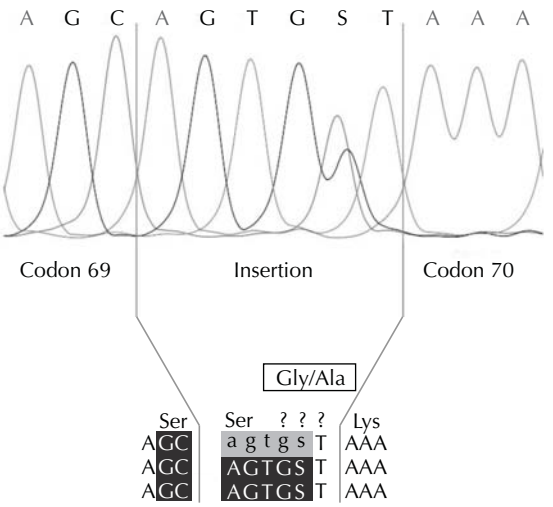

Figure. Part of the electropherogram of a genomic sequence of the HIV-1 reverse transcriptase region, demonstrating the insertion of two amino acids (Ser and Gly/Ala) between codons 69 and 70, which was confirmed based on sequences generated by forward and reverse primers, according to the specifications of the technique used (TRUGENE® hiv-1 Genotyping Test on OpenGene® DNA Sequencing System, Siemens Healthcare Diagnostics, Deerfield, IL, EUA).

The profile of mutation included A62V, K101P/Q, K103N, V106I, V179D/E, Y188L, T215Y mutations in the reverse transcriptase gene, as well as L10I and M36I mutations in the protease. No resistance to protease inhibitors was observed. In contrast, there was resistance to a variety of RT inhibitor drugs.

The sequence obtained was analyzed using the Blast Basic Local Alignment Search Tool (Blast) algorithm,[b] available from the National Center for Biotechnology Information (NCBI), with a similarity of 93%.

DISCUSSION

The insertion of two amino acids alone between residues 69 and 70 of HIV-1 RT does not confer significant resistance. However, when combined with substitutions, such as T215Y, M41L, A62V or L210W, it can lead to the emergence of resistant variants to multiple nucleoside analogs.[1,3,5]

Insertion mutations between codons 69 and 70 are present, with a low prevalence (approximately 0.5% to 2.4%),[2,3,4] in nucleoside analog reverse transcriptase inhibitors-experienced individuals, the most common being insertion of serine-serine, serine-arginine and

[a] REGA HIV Subtyping Tool [cited 2012 Nov 19]. Available from: http://www.bioafrica.net/rega-genotype/html/subtypinghiv.html
[b] National Center for Biotechnology Information (NCBI). Basic Local Alignment Search Tool (BLAST); 2012 [cited 2012 Nov 20]. Available from: http://blast.ncbi.nlm.nih.gov/Blast.cgi

serine-glycine. Such rearrangements are located in the structure of the β3-β4 hairpin loop of HIV-1 RT and are susceptible to interaction with the nucleotide binding process.[2]

In 1999, Larder et al[1] reported that the 69Ser-(Ser-Ser) mutation, by itself, only showed significant decrease in susceptibility to 3TC. However, Winters et al[5] (1998) reported that the 69Ser-(Ser-Ala) or 69Ser-(Ser-Gly) mutation, even without other genotypic mutations, would confer resistance to multiple drugs.

Detection of the single substitution, Thr69Ser, suggests that the mutation may develop before insertion of the amino acid. However, the specific mechanism required for the occurrence of the insertion is not known.[1]

In Brazil, there are no reports which show the presence of HIV-1 variants with insertion of dipeptides between codons 69 and 70 of the RT in coexistence, which makes the finding relevant from an epidemiological point of view, since such insertion is related to resistance to multiple nucleoside analogs and little is known about its prevalence or clinical significance.

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The authors declare that there are no conflicts of interests.