

## Description of an HIV-1 BC Recombinant Virus Identified in a Pediatric Patient in the City of São Paulo

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**This case report refers to a 10-year-old HIV-1 infected patient, who was found to harbor a BC recombinant virus. This child lives in São Paulo and was infected by the mother-to-child route. Phylogenetic analyses revealed that this mosaic virus shares common breakpoints in the polymerase region with the recently published CRF31\_BC.**

**Key-Words:** HIV-1, AIDS, children, circulating recombinant forms.

In 2007, 2.5 million children (0-15 years old) were living with HIV [1]. The Brazilian Ministry of Health reported that 589 new pediatric cases of AIDS were notified by June 2007 [2]. HIV-1 shows a high genetic diversity and is classified into three groups: M, O and N. The vast majority of strains found worldwide are classified into the M group, which includes nine subtypes (A, B, C, D, F, G, H, J and K) and at least 43 circulating recombinant forms (CRFs) [3,4]. In Brazil, at the beginning of the HIV epidemic only HIV-1 subtype B was observed. In 1994, a study identified co-circulation of a low proportion of HIV-1 F [5]. In the same study, the first recombinant virus circulating in Brazil, an HIV-1 BF, was observed [6]. Today, HIV-1 subtype B predominates, with co-circulation of HIV-1 F and C [7,8]. Recombinant viruses are responsible for approximately 15% of all HIV-1 infections [8]. However, a high frequency of HIV-1 subtype C has been documented in the southern part of the country [8-10]. A recent study showed that the Brazilian HIV-1 subtype C epidemic was the result of the introduction of a single founder strain of HIV-1 subtype C from Burundi, probably in the early 1980s [11].

Patient cells infected with HIV-1 that bear an RNA strand of one HIV-1 subtype and an RNA strand of another subtype are prone to make a recombinant proviral DNA during the reverse transcription process. The equally high frequency of subtype B and C viruses associated with that replication mechanism favors inter-subtype recombination events and different BC mosaics, including CRF31\_BC, have been described [10,12,13]. CRF31\_BC infections account for approximately 25% of the total HIV-1 viruses circulating in newly-infected individuals in Porto Alegre, the capital of the southernmost state [10,12]. Given the frequency of CRF31\_BC in southern Brazil, we decided to investigate the introduction of this recombinant virus in São Paulo.

The case reported here is a boy, born in February 1997. He was infected by the mother-to-child transmission route and abandoned in an HIV children's care house, where he was

adopted. He was assisted first at another institution (Instituto de Infectologia Emilio Ribas, São Paulo, SP), where there is a record of HIV genotypic resistance testing in 2001. At that time, the test revealed resistance to AZT,3TC,DDI,D4T, moderate resistance to AZT+3TC and Abacavir and sensitivity to Nevirapine and Efavirenz.

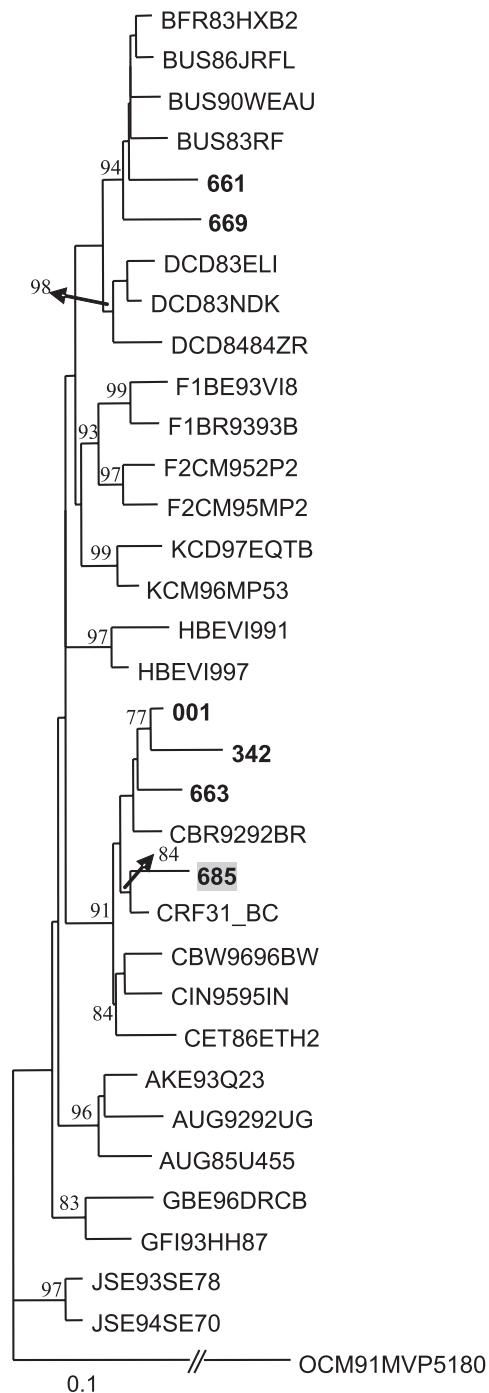
In 2003 the child was transferred to the outpatient clinic of the Instituto da Criança, Hospital das Clínicas, Faculdade de Medicina de São Paulo University (HC-FMUSP) Brazil, where he was maintained under a regimen of AZT+3TC/Amprenavir/Efavirenz up to May, 2007, at which time the CD4 count was 155 cells/ $\mu$ L and the viral load 5,891 copies/mL. In July 2007, a blood sample was sent to the Virology Laboratory of the Instituto de Medicina Tropical for genotyping (antiretroviral genotypic resistance) under the HIV-1 Genotyping National Network (RENAGENO, PN DST/Aids, Brazilian Ministry of Health). Five milliliters of blood were collected in an EDTA tube, which was centrifuged at 2,000 x g for 15 minutes. Plasma was collected and stored at -70°C, until use. HIV-1 protease (PR) and partial reverse transcriptase (RT) regions were sequenced with the ViroSeq™ Genotyping System kit (Celera Diagnostics, California, USA), according to manufacturer's instructions. Purified products were sequenced in an automated ABI Prism ABI 3100 Genetic Analyzer (Applied Biosystems, California, USA). All sequencing chromatograms obtained were assembled automatically (<http://www.ial.sp.gov.br/ci-bin/HIV/submissao>) for subtype analysis. Strong evidence of resistance was found against all transcriptase inhibitors (NNRTI and NRTIs) except for TDF + 3TC and also against PIs, except for LPV/r and APV/r.

HIV-1 subtype was investigated with the NCBI website and REGA subtyping tool [14] and confirmed by phylogenetic analyses, as shown below. The sequence generated by the automated edition, a set of reference strains representative of all HIV-1 clades and the CRF31\_BC reference strain, available in the Los Alamos HIV-1 database ([www.hiv.lanl.gov](http://www.hiv.lanl.gov)), were aligned using Clustal W. In the phylogenetic analysis, we included *pol* fragments from two HIV-1 clade B and three HIV-1 clade C isolates, previously analyzed in our laboratory. Alignment was manually corrected using BioEdit software (Ibis Therapeutics, USA). Phylogenetic analyses were performed with PAUP4b10 [15]. Neighbor Joining and Maximum Likelihood trees were constructed based on nucleotide substitution models determined by Modeltest v3.7 [16]. One thousand bootstrap replicates were used to assess

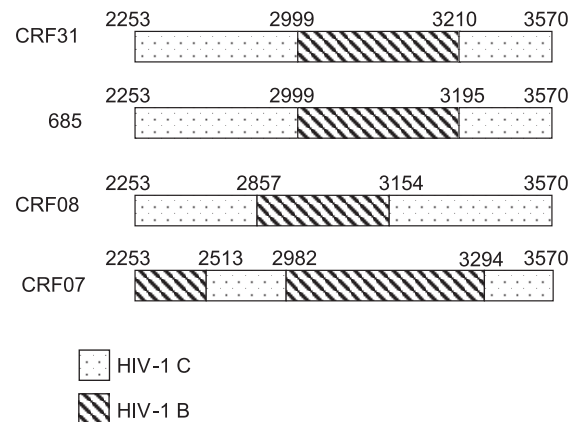
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**Figure 1.** Phylogenetic analysis of the HIV-1 *pol* region in São Paulo, Brazil. The tree was obtained using neighbor joining and a TVM+I+G model. Reference HIV-1 clades were obtained from the Los Alamos Sequence database. Bootstrap values above 70% in key branches are depicted. The two HIV-1 clade B and the three HIV-1 clade C from that laboratory are in bold, named by their RENAGENO entry number. The HIV-1 study sequence is in bold and in a light-grey shaded box.



**Figure 2.** Schematic structure of the BC recombinant virus genome that we analyzed. Breakpoints were determined after re-analysis of the original sequences by the Simplot program.



the phylogenetic robustness of the clusters. The sequence OCM91MVP5180 (GenBank accession no. L20571) was used as an outgroup. The recombination pattern was determined by bootscanning analysis with SIMPLOT 2.5 software [17].

Subtype analyses screening of the index case showed a BC recombinant virus. In phylogenetic analysis, the sequence clustered with CRF31\_BC on a neighbor joining tree, supported by a bootstrap value of 84%, whereas the remaining clade B and C *pol* fragments grouped with the respective HIV-1 subtype references (Figure 1). Bootscanning analysis showed that the sample shares the same breakpoints in *pol* region as CRF31\_BC (Figures 2 and 3).

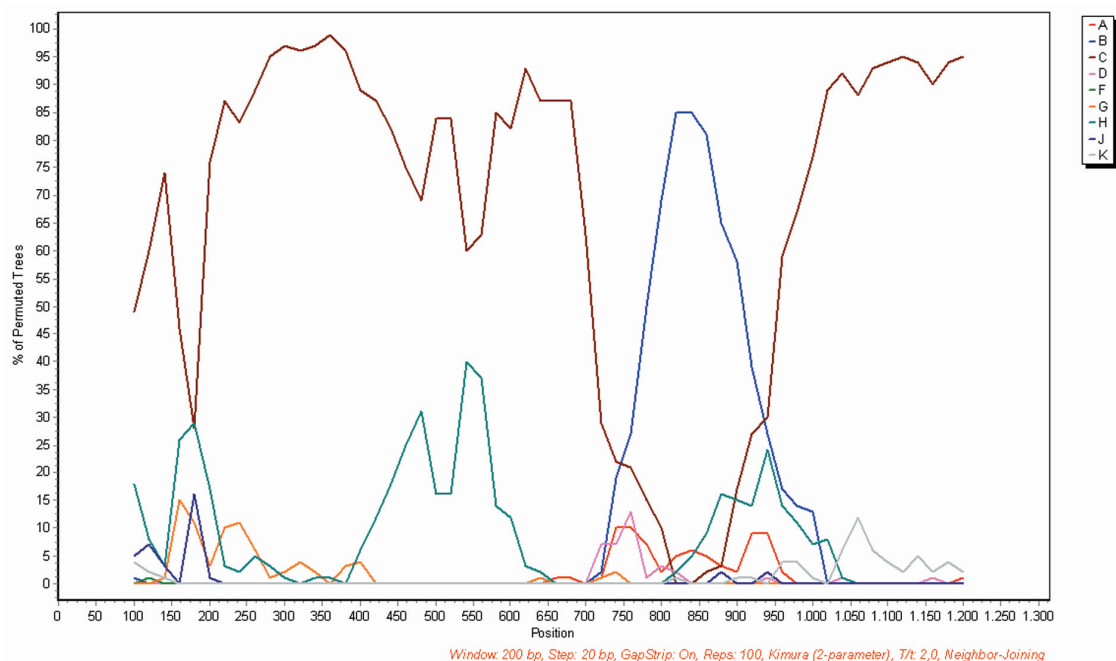
Phylogenetic analysis suggests that this child was infected by a BC recombinant virus. HIV-1 subtype C circulates at a low rate in São Paulo and Rio de Janeiro, being found in approximately 1% of HIV-1 infected individuals [18,19]. We believe that this low frequency of HIV-1 C diminished the chance for recombinant events between HIV-1 subtype B and C.

In São Paulo state, BF mosaics are frequent [6,20], but BC recombinants are very rare, especially in individuals infected more than 10 years ago, such as the child described here.

This fact may reflect that CRF31\_BC is emerging in our state, but we can not be sure that the mother was indeed infected in São Paulo. However, as the mode of transmission is known, this case indicates that this BC recombinant has been circulating in São Paulo since 10 years ago, much longer than previously thought.

Understanding the causes of the successful spread of CRF31\_BC in the southern part of the country, as well as monitoring the entry of this CRF into other regions of the country is important for public health strategies in Brazil, shedding light on aspects of virus fitness and niche competition between different HIV strains.

**Figure 3.** Bootscanning analysis of the partial *pol* region of the BC recombinant virus genome that we analyzed. Horizontal axes represent nucleotide position in this region, whereas vertical axes depict bootscanning values (%) that support the grouping of the isolate with each HIV-1 subtype.



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