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HIV-1 genetic diversity and drug resistance among treatment naïve patients from Southern Brazil: An association of HIV-1 subtypes with exposure categories

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ABSTRACT

Background: The AIDS epidemic in Southern Brazil has unique features, showing co-circulation of HIV-1 subtypes C, B and recombinant forms. Florianópolis has the second highest AIDS incidence among Brazilian capitals, but limited information is available about HIV molecular epidemiology and prevalence of primary drug resistance.
Objectives: To investigate the molecular epidemiology of HIV-1 in Florianópolis and to describe the prevalence of primary HIV-1 drug resistance mutations (DMRs).
Study design: Epidemiological and clinical data from 82 untreated patients from Florianópolis (2008–2009) were analyzed. The HIV-1 subtype at envelope, protease, reverse transcriptase and integrase regions were determined by phylogenetic and bootscaning analyses and the drug resistance profile were analyzed at the Stanford HIV Drug Resistance Database.
Results: The most frequent HIV-1 genetic form was subtype C (65.8%) followed by mosaics BC (18.3%), subtype B (13.4%), subtype F1 (1.2%) and BCF1 recombinant (1.2%). HIV-1 subtype C and BC recombinants were much more frequent in the heterosexual exposure category, whereas subtype B was more common in the MSM exposure category. DRMs were seen in 11% of the sequences, 2.4% of them were related to PI, 5% to NRTI, 3.6% to NNRTI and 1.2% was related to INTI.
Conclusions: The present study confirms the high prevalence of subtype C and BC recombinants in Santa Catarina State and revealed a significant difference in the subtype distribution among distinct virus exposure categories. This study also shows a relative high prevalence of protease/reverse transcriptase primary drug resistance mutations and corroborates the usefulness of the integrase inhibitors in southern Brazil.

1. Background

The HIV/AIDS pandemic has a major impact in Brazil, being the most affected Country in Latin America with over 600,000 people infected.[1] The Brazilian HIV-1 epidemic can be divided in two distinct scenarios. While subtype B prevails in most of the Country, with a secondary occurrence of subtype F1 and BF1 recombinants,[2–9] Southern Brazil presents a distinct pattern with high frequency of subtypes C, B and BC recombinants, following a lower proportion of subtype F1 and BF1 recombinants,[10–17] comprising 19% of the infected individuals in the Country.

The universal access of Brazilian HIV-positive patients to free-of-cost licensed antiretroviral drugs has substantially reduced the AIDS-related mortality.[1] The effectiveness of antiretroviral therapy, however, may be limited by the increasing of resistance to antiretroviral drugs and by transmission of such drug-resistant viruses to drug-naïve patients. National surveys suggest that primary HIV resistance in Brazil is generally low, ranging from 6% to 8%.[2,18,19] However, very high prevalence of HIV primary drug resistance (18–37%) were observed in some localities, such as the City of Santos (Southeastern region)[20,21] and the State of Bahia (Northeastern region),[22] indicating important differences across country regions.

Most of the molecular epidemiology studies on HIV in the Southern region were carried out in the State of Rio Grande do Sul, but

Abbreviations: AIDS, acquired immune deficiency syndrome; ARV, antiretroviral; CPR, calibrated population resistance tool; CRF, circulating recombinant form; DRMs, drug resistance mutations; INT, integrase; INTI, integrase inhibitors; HIV, human immunodeficiency virus; MSM, men who have sex with men; NJ, neighbor-joining; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PCR, polymerase chain reaction; PI, protease inhibitors; PR, protease; RT, reverse transcriptase; URF, unique recombinant form.

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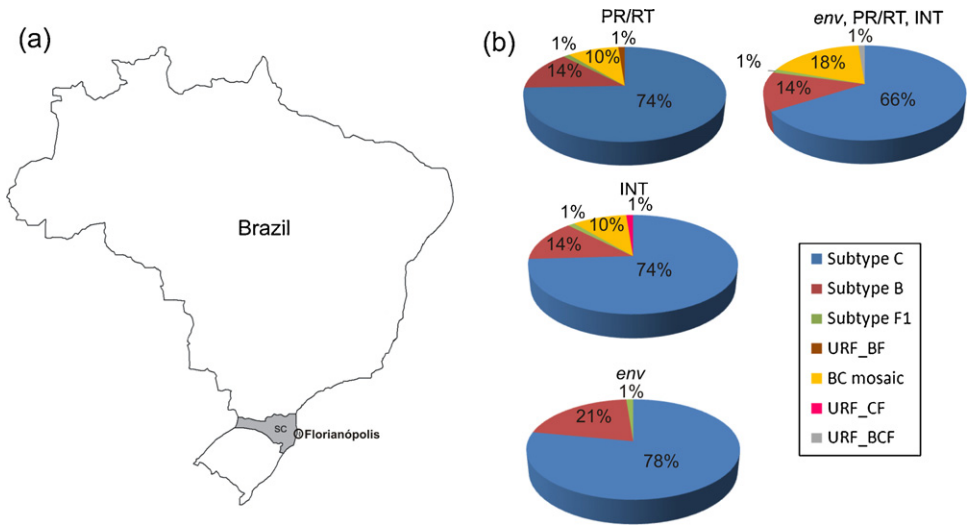

Fig. 1. Geographical location of Florianópolis and frequency of HIV-1 subtypes at the different genomic regions analyzed. (a) Map of Brazil highlighting the state of Santa Catarina (SC) and the metropolitan region of Florianópolis. (b) HIV-1 subtypes were determined by phylogenetic and bootscaning analyses, as described in Section 3.

limited data concerning the prevalence of HIV-1 subtypes and HIV primary drug resistance in the other southern states (Santa Catarina and Paraná) is available. Noteworthy, Santa Catarina State ranks third on AIDS incidence rate in Brazil (29.6 new cases per 100,000 inhabitants in 2008), while its capital, Florianópolis city, occupies the second position in the ranking of capitals (53.7 new cases per 100,000 inhabitants in 2008).[1] Moreover, Florianópolis city is a very important tourist pole in Southern Brazil, receiving people from other South American countries, mainly Argentina and Uruguay, which could have implications on the distribution of HIV subtypes and recombinant forms.

2. Objectives

This study describes the HIV-1 genetic diversity among different exposure categories and the prevalence of HIV primary drug resistance mutations based on sequence analyses of protease, reverse transcriptase, integrase and envelope genes among naïve patients from Florianópolis metropolitan area, Santa Catarina, Brazil.

3. Study design

3.1. Patients

Blood samples were collected from 82 HIV-infected patients followed up at Hospital Homero de Miranda Gomes located in metropolitan region of Florianópolis, Santa Catarina, Brazil. The individuals were selected from May-2008 to February-2009 and inclusion criteria included age over 18-year old, no sign or symptom of AIDS and no previous antiretroviral therapy. The Universidade Federal de Santa Catarina Ethics Committee approved the study and informed consent was obtained from all volunteers.

3.2. Extraction, amplification and sequencing of HIV-1 DNA

PBMC was isolated from 4 ml of blood by the Histopaque (Sigma–Aldrich, St. Louis, MO, USA) density gradient method and genomic DNA was extracted using a QIAmpl Blood Kit (Qiagen Inc., Chatsworth, CA, USA). HIV-1 protease (PR), reverse transcriptase (RT), integrase (INT) and envelope (*env*) genes were

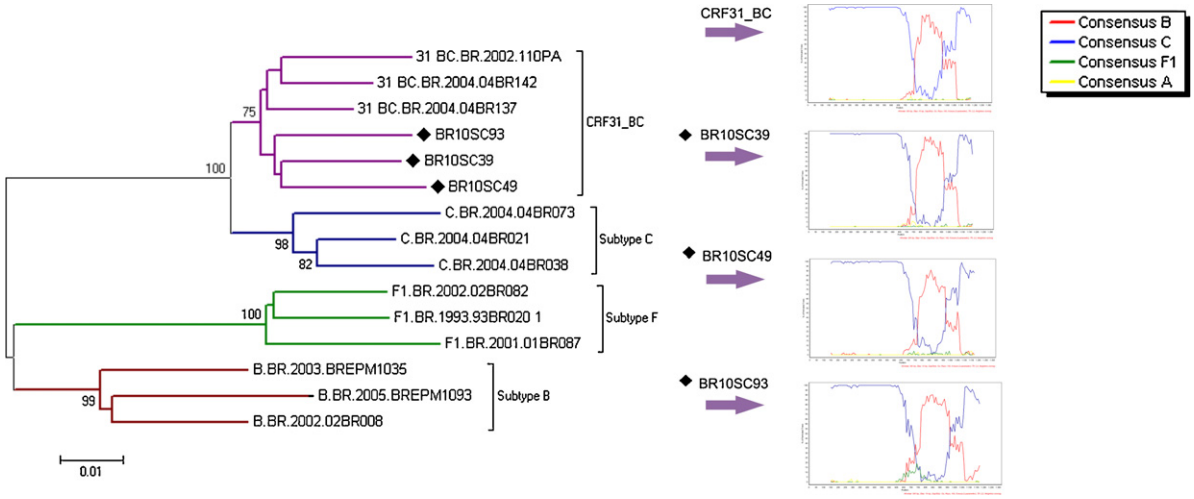

Fig. 2. Phylogenetic analysis of CRF31_BC-like sequences. (a) Neighbor-joining phylogenetic tree of PR/RT region (2316–3316 nt relative to HXB2), including only the CRF31_BC-like sequences from Santa Catarina (black diamonds) and subtype reference sequences. Reliability was tested with 1000 replicates bootstrapped, values considering significant (above 70%) are shown. (b) Bootscaning patterns corresponding to a CRF31_BC reference sequence and the three sequences from Santa Catarina classified as CRF31_BC like. Reference sequences were extracted from HIV Los Alamos Database.