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HIV-1 Genetic Diversity and Transmitted Drug Resistance in Antiretroviral Treatment-Naive Individuals from Amapá State, Northern Brazil

Lindomar dos Anjos Silva,[1] Flavia Divino,[2] Márlisson Octávio da Silva Rêgo,[1] Ivina Geselle Lima Lopes,[1] Cláudia Maria Nóbrega Costa,[1] Francis Christian da Silva Pereira,[1] Ivano de Filippis,[3] and Gonzalo Bello[2]

Abstract

The pattern of HIV-1 subtype distribution and prevalence of transmitted drug resistance mutations (TDRM) is heterogeneous across different Brazilian regions. Little information is available about the molecular epidemiologic profile in Northern Brazil. HIV-1 protease (PR) and reverse transcriptase (RT) sequences were obtained from 97 drug-naive HIV-1-infected individuals from Amapá, one of the most isolated Northern Brazilian states, for subtype determination and analysis of drug resistance mutations. The most prevalent HIV-1 clade observed in Amapá was subtype B (74%), followed by subtype F1 (14%), BF1 recombinants (8%), subtype C (1%), CRF31_BC (1%), and CRF02_AG (1%). Only one TDRM (K103N) was detected in a single patient from our study population. This study reveals that the HIV-1 epidemic in Amapá is characterized by a high level of genetic diversity comparable to that observed in major Brazilian cities, but a much lower rate of TDRM (1%).

According to estimations by the Brazilian Ministry of Health, about 700,000 people were living with HIV and approximately 300,000 HIV-infected individuals were on antiretroviral therapy (ART) in Brazil in 2013.[1] The AIDS Brazilian epidemic is mostly driven by HIV-1 subtypes B, F1, C, and recombinants forms among those subtypes.[1–5] Whereas the HIV-1 subtype distribution profile has remained relatively stable over time in Brazil, the widespread use of ART in the country since 1996 has led to a substantial increase in the emergence and transmission of HIV strains harboring resistance mutations to one or more antiretroviral agents. According to national-wide studies that characterized HIV-infected treatment-naive individuals from different Brazilian regions, the overall rate of transmitted drug resistance mutations (TDRM) increased from 6.6% to 12.2% over the past 10–15 years.[2–5]

The relative prevalence of different HIV-1 subtypes and TDRM could greatly vary across different Brazilian regions[2–5] and molecular epidemiological data in some areas away from the most populated urban centers are scarce. Amapá is one of the northernmost Brazilian states, bordered by French Guiana and Suriname to the north, the Atlantic Ocean to the east, and the Pará state to the south and west. Amapá is the second least populous Brazilian state with 734,995 inhabitants. The Amazon Rainforest occupies 90% of the total area of Amapá and its population is highly urbanized, being mostly (75%) concentrated in Macapá (state capital) and the neighboring city of Santana. By 2013, about 1,347 AIDS cases had been cumulatively reported in Amapá since the first identification of AIDS in the state in 1988.[1] The incidence rate of AIDS cases in Amapá has doubled between 2001 (9.2/100,000 inhabitants) and 2012 (20.2/100,000 inhabitants), and is currently similar to the mean incidence rate observed in the North region (21.0/100,000 inhabitants) and in Brazil (20.2/100,000 inhabitants).[1]

There is almost no information about the molecular epidemiological profile of the HIV epidemic in Amapá. The only study performed to date analyzed very short fragments (<300 nucleotides) of the envelope and/or protease genes from a small number of patients ($n \leq 35$) with no information about treatment status.[6] The objective of this study was to perform a comprehensive description of the HIV-1 subtypes and rate of TDRM detected among ART-naive patients from different regions of Amapá.

A total of 97 peripheral blood samples from HIV-1-infected persons living in different cities through the Amapá

[1]Laboratório Central de Saúde Publica do Amapá (LACEN-AP), Macapá, Brazil.
[2]Laboratório de AIDS & Imunologia Molecular, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.
[3]Instituto Nacional de Controle de Qualidade em Saúde, FIOCRUZ, Rio de Janeiro, Brazil.

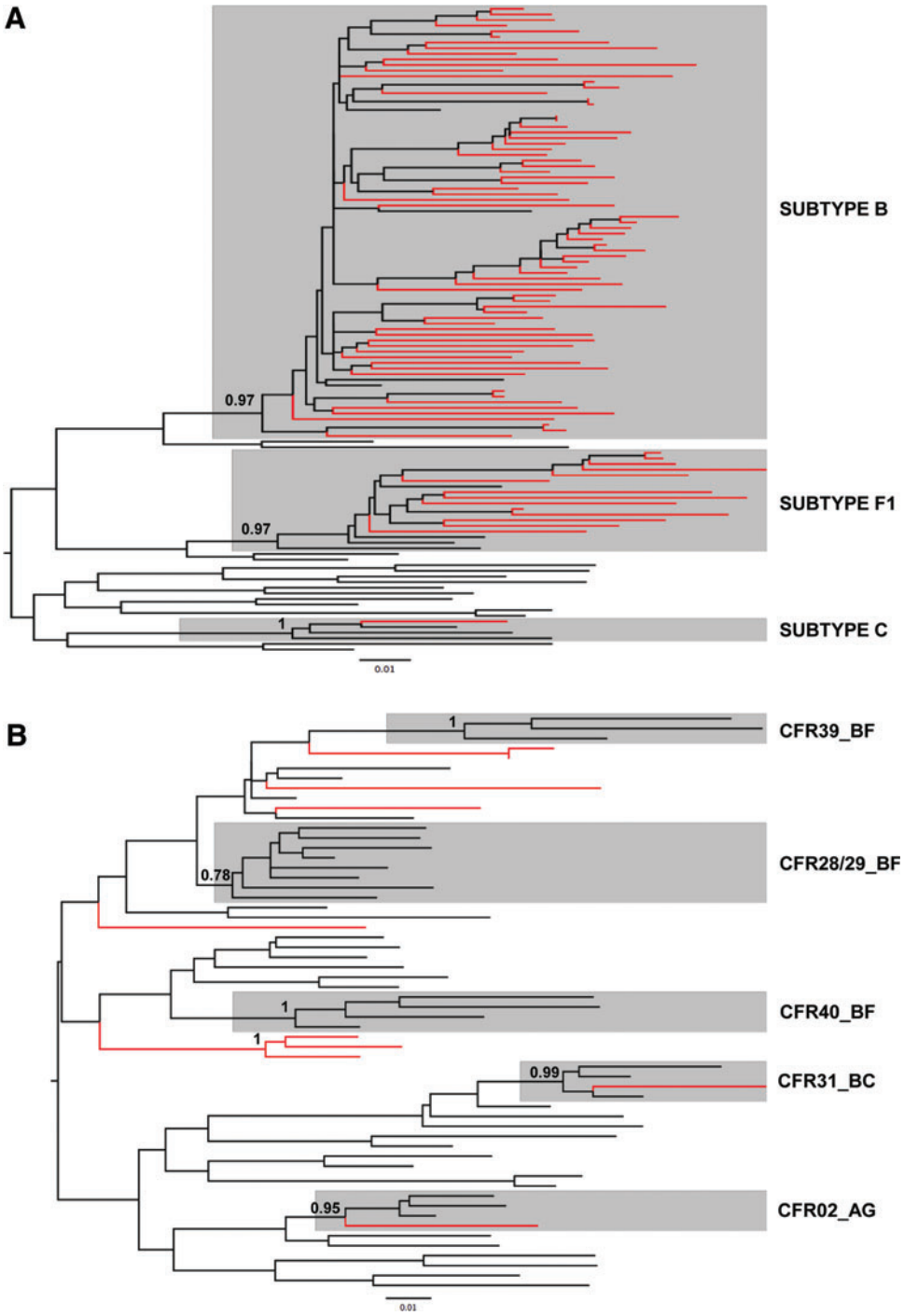

FIG. 1. Maximum likelihood phylogenetic tree of the *pol* region of HIV-1 isolates obtained from antiretroviral drug-naive patients from Amapá state. **(A)** HIV-1 sequences from Amapá classified as ''pure'' subtypes were combined with reference sequences of all HIV-1 group M subtypes (A–D, F–H, J, and K). *Shaded boxes* indicate the position of major HIV-1 subtypes circulating in Brazil. **(B)** HIV-1 sequences from Amapá classified as intersubtype recombinants (BF, BC, and AG) were combined with reference sequences of all HIV-1 group M subtypes, the CRF02_AG, and Brazilian CRF_BF (28, 29, 39, and 40) and CRF_BC (31) with recombination points at the *pol* gene fragment analyzed. *Shaded boxes* indicate the position of major Brazilian CRFs and the CRF02_AG clade. Sequences from Amapá are represented as *red branches*. The aLRT branch support was shown only at key nodes. Trees are rooted at midpoint and the branch lengths are drawn to scale with the bar at the bottom indicating nucleotide substitutions per site. Color images available online at www.liebertpub.com/aid

state were collected between 2013 and 2014. Patients attended the Public Health Central Laboratory (LACEN) in Macapá/Amapá, a reference unit from the Brazilian Ministry of Health that receives samples for monitoring of $CD4^+$ T cell count and plasma viral load. Inclusion criteria were patients with a recent or chronic diagnosis of HIV-1 infection of either sex, of any age range, and who never received ART.

Blood samples were stored at −70°C and later transported to the Laboratory of AIDS and Molecular Immunology (FIOCRUZ) in Rio de Janeiro for HIV subtype and resistance analyses. The study was approved by the Ethics Committee of Instituto Oswaldo Cruz (Number CAAE: 35785214.5 .1001.5248).

DNA was extracted from 200 μl of total blood and an HIV-1 *pol* fragment of about 1,100 nucleotides encompassing the entire protease (PR) and part of the reverse transcriptase (RT) (nucleotides 2253–3272 relative to the HXB2 clone) and was amplified and sequenced as previously described.[7] HIV-1 genetic subtypes were initially determined with the REGA HIV-1 Subtyping Tool 3.0 software[8] and later confirmed by maximum-likelihood (ML) phylogenetic and bootscan analyses with HIV-1 reference sequences from the Los Alamos

HIV database (ww.hiv.lanl.gov). ML trees were reconstructed with the PhyML 3.0 program,[9] using the SPR branch-swapping algorithm for heuristic tree search and the approximate likelihood-ratio test (aLRT) to estimate the reliability of the obtained tree topology. Bootscan analyses were performed with SimPlot 3.5.1 software,[10] based on 100 resamplings with a sliding window of 250 nucleotides moving in steps of 10 bases. TDRM to the nucleoside/nonnucleoside reverse transcriptase inhibitors (NRTI/NNRTI) and protease inhibitors (PI) were analyzed using the Calibrated Population Resistance (CPR) tool available through the Stanford University HIV Drug Resistance Database (http://cpr.stanford.edu/cpr.cgi).[11]

Around one-third (76/97) of the HIV-infected patients analyzed in this study lived in the city of Macapá (state capital) and the neighboring city of Santana, whereas the remaining patients (21/97) came from eight different small interior cities located throughout the state. Nine of the 10 most populated cities of the Amapá state were represented in our sample. Most patients analyzed were males (64%) and had a median age of 33 years (15–72 years range), with similar values for both males (31 years, 15–72 years range) and females (34 years, 17–56 years range). The median of CD4 T cell counts for our study population was 370 cells/μl and was distributed as follow: 35% below 200 cells/μl, 31% between 201 and 500 cells/μl, and 34% over 500 cells/μl. The median RNA plasma viral load was 12,138 copies/ml distributed in the following way: 15% below 1,000 copies/ml, 31% between 1,000 and 10,000 copies/ml, 29% between 10,001 and 100,000 copies/ml, and 25% over 100,000 copies/ml.

The most prevalent HIV-1 clade in our sample was subtype B ($n=72$, 74%), followed by subtype F1 ($n=14$, 14%) and subtype C ($n=1$, 1%) (Fig. 1A). The remaining viruses ($n=10$, 10%) were classified as intersubtype recombinant clades. Most recombinant viruses ($n=8$, 8%) displayed a BF1 recombinant structure and were distributed in five independent lineages of between one and three sequences each, none of which branched with the BF1 circulating recombinant forms (CRFs_BF) previously described in Brazil (Fig. 1B). We also identified one BC recombinant (1%) and one AG recombinant (1%) that were classified as CRF31_BC and CRF02_AG by REGA analysis. Consistent with this classification, the BC and AG recombinants branched with high support with the CRF31_BC and CRF02_AG reference strains, respectively (Fig. 1B). HIV-1 clades B, F1, and BF1 were detected in the major cities (Macapá and Santana) as well as in the small interior ones. The subtype C and the CRF02_AG-like variants were detected in the capital city Macapá, whereas the CRF31_BC-like strain was detected at the northernmost Brazilian city, Oiapoque, located in the border with French Guiana.

The viral diversity here detected in Amapá is much higher than that previously reported for this state by Machado *et al.* (2009), which described only the circulation of subtypes B (97%) and F1 (3%) in a population of HIV-infected patients recruited in 2002. Such inconsistent observations among studies may have resulted from differences in the population size and HIV-1 gene fragments analyzed or may reflect an increasing trend of HIV-1 diversity in Amapá over time. The HIV-1 molecular diversity in Amapá is comparable to that found in many major Brazilian metropolitan centers in which the epidemic is dominated by subtype B, F1, and BF1 recombinants.[2–5] This study also confirms the circulation in the northernmost Brazilian region of HIV-1 subtype C and CRF31_BC that prevails in the southernmost Brazilian states,[2–4] and of the CRF02_AG that is highly prevalent in West and Central Africa, but was rarely detected in Brazil. The HIV-1 complexity detected is consistent with multiple independent introductions of HIV-1 strains into Amapá from other Brazilian states and probably other countries.

Notably, only one TDRM to NNRTI (K103N) was detected in a single patient, whereas no TDRM to NRTI or PI were detected in the study population. Two out of 97 HIV-1 *pol* sequences analyzed were excluded due to the presence of >2 APOBEC3GF-mediated G to A mutations. The extremely low levels of TDRM (1%) observed in Amapá clearly contrast with the most recent surveys that showed an intermediate level (5–20%) of TDRM in cities of medium and large size from the Southeast (Minas Gerais, Rio de Janeiro and São Paulo), North (Pará and Tocantins), Northeast (Recife and Piauí), and Central-West (Brasilia, Goiânia, and Mato Grosso) Brazilian states.[3–5,12–15] The low rate of TDRM observed in Amapá might be associated with a low frequency of virological failure among treated patients, or may reflect a low access to ART or a later introduction of ART in areas located far from the major Brazilian urban centers.

In conclusion, the results from this study support the development of a complex HIV-1 molecular pattern in Amapá, one of the northernmost Brazil states, characterized by the cocirculation of subtypes B, F1, C and diverse recombinant forms, including subtypes B/F1, B/C, and A/G. Despite the relative small size of the HIV-infected population, the low spatial connectivity with other Brazilian states, and the great distance to the major epicenters of the epidemic, the overall genetic complexity of the HIV-1 epidemic in Amapá is comparable to that described in major Brazilian cities. The HIV epidemic in Amapá is also characterized by one of the lowest rates of TDRM (1%) described to date in the country, which does not support recommendations for pretreatment genotypic tests in naive patients. This study reinforces the importance of continuous monitoring of HIV-1 subtypes and transmission of drug-resistant strains in different Brazilian regions in order to obtain a clearer picture of the spatial heterogeneity of the HIV epidemic in this huge country.

Sequence Data

Sequences were deposited in GenBank under accession numbers KT737274 to KT737370.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Gonzalo Bello
Laboratório de AIDS & Imunologia Molecular
Instituto Oswaldo Cruz
FIOCRUZ
Av. Brasil 4365
21045-900 Rio de Janeiro, RJ
Brazil

E-mail: gbello@ioc.fiocruz.br
gbellobr@gmail.com