High Frequency of Recombinant Genomes in HIV Type 1 Samples from Brazilian Southeastern and Southern Regions

MONICK LINDENMEYER GUIMARÃES,1 ALINE DOS SANTOS MOREIRA,1 REGINA LOUREIRO,2 BERNARDO GALVÃO-CASTRO,3 THE BRAZILIAN NETWORK FOR HIV ISOLATION AND CHARACTERIZATION, and MARIZA GONÇALVES MORGADO1

ABSTRACT

We describe the genetic variability of HIV-1 subtypes and recombinant genomes in samples from southeastern and southern Brazilian regions. Phylogenetic analysis of a subset of 34 samples (8F, 7B, 7C, 2D, 1A, and 9 B’ variant) based on the DNA sequencing of the env gp120 and gp41, gag p17, and nef regions confirmed the presence of nine (26.5%) potentially HIV-1 recombinant genomes. From the eight C2–V3 gp120 subtype F samples, only two seem to be pure F. One of the samples, classified as B’ in the C2–V3 gp120 and as B in gp41 had the gag and nef regions clustering with subtype C. Two of seven C2–V3 subtype C samples presented distinct recombinant patterns as Bgag/Cenv/Bnef and Bgag/Cenv/Cnef. Putative recombinant breakpoints were obtained for three samples presenting discordant subtypes (F/B) between gp120 and gp41 env fragments showing that similar breakpoints could be observed between two unlinked samples (95BRRJ014 and 96BRRJ101). A higher degree of polymorphism was verified in the analysis of a subtype A sample (98BRRS058) in the C2–V3/gp41 env fragment. The intrasubtype C distance was found to be lower than that found for the other subtypes for all genomic regions. These data confirm that distinct HIV-1 subtypes and recombinant forms are actively participating in the Brazilian AIDS epidemic, and that the subtype C was introduced more recently into southern Brazil.

INTRODUCTION

The HIV/AIDS pandemic is highly heterogeneous and dynamic and composed of distinct microepidemics occurring in different regions of the world. As of September 2001, 222,356 AIDS cases have been reported to the Brazilian Ministry of Health and, on average, 597,443 people are estimated to be living with HIV/AIDS in the country.1

The human immunodeficiency virus type 1 (HIV-1) exhibits an extraordinary degree of genetic variability and has been classified, based on phylogenetic relationships, into groups, subtypes, subsubtypes, and circulating recombinant forms (CRFs). Three distinct groups were described: M (major), which contains the majority of the pandemic HIV-1 strains; group O (outlier), with very highly divergent strains,2 and group N (new or non-M non-O), found in Cameroon3 and strongly related to SIVCPZ from chimpanzees from this country.4,5 Based on phylogenetic relationships, the M group has been further divided into nine subtypes (A–D, F–H, J, and K) and, more recently, the F6 and A7 subtypes were divided into two subclades. Recombination events are responsible for the formation of HIV-1 mosaic genomes, with distinct subtype signatures in different genomic regions. Some of them, designated as CRFs, were obtained from apparently unlinked isolates with the same inter-subtype breakpoints and are actively participating in the global AIDS epidemic.8

Subtype B has been described as the predominant HIV-1 subtype in Brazil followed by F and C subtypes, respectively, occurring in high frequency in the southeastern and southern regions.9–13 Moreover, the V3 loop GWGR HIV-1 B’ variant and potential mosaic genomes involving the recombination or dual infections between B/F, B/C, and F/D subtypes have been previously described by our group and other groups.14–17 The presence of D and A subtypes has already been demonstrated as isolated cases in the country.18,19

The aim of this study was to evaluate HIV-1 samples, ob-

1Department of Immunology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil.
2Public Healthy Secretariat of RS State, Porto Alegre, RS, Brazil.
3Advanced Public Health Laboratory, Gonçalo Muniz Research Center, FIOCRUZ, Bahia, Brazil.

1261
tained from Brazilian regions known for bearing high frequencies of different HIV-1 subtypes, to identify and characterize the presence of recombinant intersubtype genomes.

MATERIALS AND METHODS

Virus strains

A set of 34 samples, previously subtyped by env-HMA,\textsuperscript{11,12,18} was obtained from two geographic regions of Brazil, characterized for the presence of more than one prevalent subtype. HIV-1 samples from southern Brazil are part of an ongoing study (R. Loureiro, unpublished data). For the discrimination of the GWGR-B\textsuperscript{t} subtype B variant, the FokI restriction fragment length polymorphism (RFPL) was carried out as previously described.\textsuperscript{14} For the present study eight samples from subtype F, seven from B, seven from C, two from D, one from A, and nine corresponding to the B\textsuperscript{t} Brazilian subtype B variant were selected for DNA sequencing and phylogenetic analysis based on the env C2–V3 gp120 and gp41 regions, gag p17, and nef region.

PCR amplification of the target sequences

DNA samples (\(\sim 1 \mu g\)) were PCR amplified by a nested protocol in a Perkin Elmer 480 or 9600 Thermal Cycle. PCR reactions were performed in a volume of 100 \(\mu l\) containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 200 mM of each dNTP, 20 pmol of each primer, 1.25 mM MgCl\(_2\) and 2.5 U Taq polymerase (Pharmacia Fine Chem., Sweden). The env C2–V3 PCR amplification was performed as previously described,\textsuperscript{20} using ED3/ED14 and ED31/ED33, respectively, as outer and inner primer sets. The gp41 PCR amplification was obtained by a nested protocol\textsuperscript{20} using ED31/MM1 (TGGTGAATATCCCTGGCTAA/HIV-1 HXB2 genome positions 8446 to 8365)\textsuperscript{21} and ES7/MM4 (CCTCTACTATCATATTGAA/HXB2 positions 8276 to 8295) as outer and inner primer sets. MM oligonucleotides were designed for this study. The gag p17 nested PCR amplification protocol\textsuperscript{1} was performed using GAG1/MM1 (first round) and ES7/MM4 (nested) primer sets to identify the putative recombination breakpoints among the gp120/gp41 discordant subtype samples. To cover the whole region, DNA sequencing was performed in both senses using primers ES7, ES8, ED12, ED14, ED31, and ED33 previously described\textsuperscript{20} and MM2 (GTCTGGGGCATCAAGACGCT/HBX2 7931 to 7950), MM4, and MM5.

Identification of recombinant viruses was performed using the Recombinant Identification Program (RIP) from the Los Alamos Database webpage (http://hiv-web.lanl.gov/) and confirmed by the SimPlot for Windows version 2.5 (http://www.med.jhu.edu/deptmed/sray/download/). Recombinant breakpoint positions were based on the HXB2 genome (GenBank accession number K03455).

RESULTS

To provide phylogenetic relationship inferences among subtype samples prevalent in two Brazilian regions and to test the presence of recombinant viruses, a subset of 34 HIV-1 samples, previously subtyped by env-HMA, was selected for this study.

The phylogenetic analyses of PCR-amplified fragments of the gag p17 (360 bp), env C2–V3 (300 bp), env gp41 (480), and nef (660 bp) regions are presented in Figure 1. Discordant subtyping among the four regions was observed for nine (26.5%) of the 34 samples. The one subtype B sample described in this study was also classified as B regardless of the region analyzed. The one subtype A sample described in this study also grouped with subtype A references in both C2–V3 and gp41 env regions. One of the samples from southern Brazil

Phylogenetic tree analysis

DNA sequences were aligned by using CLUSTAL W.\textsuperscript{26} The alignments were edited manually for minor adjustments. Phylogenetic tree constructions were performed using the neighbor-joining method\textsuperscript{27} with the Kimura two-parameter method for distance correction, and the reliability of the branching orders were determined by the 100 times bootstrap. Only bootstrap values above 70% were specified in the major subtype branches. The final trees were visualized using the TreeView program.\textsuperscript{28} The intrasubtype and intersubtype distances were calculated using the Kimura two-parameter correction with pairwise gap deletion based on the MEGA program.
(98BRRS004), classified as B0 in the C2–V3 gp120 and as B in gp41 had the gag and nef regions clustering with subtype C. Two of seven C2–V3 subtype C samples presented recombinant patterns as Bgag/Cenv/Bnef (98BRRS028) and Bgag/Cenv/Cnef (98BRRS035). Subtype F was highly variable exhibiting at least three distinct patterns. Indeed, from the eight samples subtyped as F based on the C2–V3 gp120, six seemed to be potentially recombinant viruses with genomes like Bgag/Fenv/Bnef (95BRMG005), Bgag/Fgp120/Bgp41/Bnef (96BRRJ101), Fgag/Fenv/Bnef (98BRRS037), Bgag/Fgp120/Bgp41 (95BRRJ014 and 95BRSP004), and Bgag/Fgp120 (98BRRS053). Moreover, nef sequencing did not give usable sequences for some HIV-1 samples, suggesting a high polymorphism of the nef-targeted sequences. These results are summarized in Table 1, which also includes the geographic origin of the HIV-1 samples as well as the year of sample collection.

Phylogenetic tree analysis of the gp120 C2–V3 region showed that almost all of our samples clustered consistently with the subtype reference strains, and the major subtype branches presented high bootstrap values (Fig. 1B). Nine representative B0 subtype B variant samples, bearing the GWGR sequence at the top of the V3 loop, clustered together in a separate group, although this clustering was not supported by a significant bootstrap value (>70%). One of the B0 samples (95BRSP001) was highly divergent, showing a 12 bp insertion at the top of the V3 loop in addition to several nucleotide substitutions. Sample 95BRRJ012, which presents the GFGR motif at the top of the V3 loop, also clustered together with the B0 subtype B variant, probably as a consequence of the great genetic similarity among them. Indeed, the GWG motif (TGG) was suggested to be an intermediate sequence to GFG motif (TTG or TTC).29 The remaining subtype B samples clustered in another branch, with the exception of sample 95BRRJ002, which appeared alone in one separate branch. Sub-

<table>
<thead>
<tr>
<th>Sample</th>
<th>State of origin</th>
<th>Date of collection</th>
<th>gag p-17 genotype</th>
<th>C2–V3 genotype</th>
<th>gp41 genotype</th>
<th>nef genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>95BRMG005</td>
<td>Minas Gerais</td>
<td>1995</td>
<td>B</td>
<td>F</td>
<td>F</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ002</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ005</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ006</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B0</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ008</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B0</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ009</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B0</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ011</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ012</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ013</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B0</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ014</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>F</td>
<td>B</td>
<td>WS</td>
</tr>
<tr>
<td>95BRRJ015</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ016</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B0</td>
<td>B</td>
<td>NA</td>
</tr>
<tr>
<td>95BRRJ017</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B0</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ019</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ020</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRRJ021</td>
<td>Rio de Janeiro</td>
<td>1995</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>95BRSP001</td>
<td>São Paulo</td>
<td>1995</td>
<td>B</td>
<td>B0</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>95BRSP004</td>
<td>São Paulo</td>
<td>1995</td>
<td>B</td>
<td>F</td>
<td>B</td>
<td>NA</td>
</tr>
<tr>
<td>95BRSP006</td>
<td>São Paulo</td>
<td>1995</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>96BRRJ100</td>
<td>Rio de Janeiro</td>
<td>1996</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>96BRRJ101</td>
<td>Rio de Janeiro</td>
<td>1996</td>
<td>B</td>
<td>F</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>98BRRJ045</td>
<td>Rio de Janeiro</td>
<td>1998</td>
<td>WS</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>98BRRS004</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>C</td>
<td>B0</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>98BRRS006</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>B</td>
<td>B0</td>
<td>B</td>
<td>WS</td>
</tr>
<tr>
<td>98BRRS010</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>NA</td>
</tr>
<tr>
<td>98BRRS011</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>98BRRS012</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>WS</td>
</tr>
<tr>
<td>98BRRS022</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>NA</td>
<td>C</td>
<td>C</td>
<td>WS</td>
</tr>
<tr>
<td>98BRRS028</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td>98BRRS034</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>NA</td>
<td>C</td>
<td>C</td>
<td>WS</td>
</tr>
<tr>
<td>98BRRS035</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>98BRRS037</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>B</td>
</tr>
<tr>
<td>98BRRS053</td>
<td>Rio Grande do Sul</td>
<td>1998</td>
<td>B</td>
<td>F</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Minas Gerais, Rio de Janeiro, and São Paulo are localized in southeastern Brazil whereas Rio Grande do Sul State is in the southern region. NA, not amplified; WS, without readable sequences.
type C sample 98BRRS028, a possible recombinant virus (B gag/C env/B nef), formed a separate single branch, closely related to the remaining C sample group.

Although the PCR amplification of subtype A sample 98BRRS058 was possible with all primer sets included in this study, usable sequences were obtained just for the gp120 and gp41 env regions. Phylogenetic analysis revealed that this sample appeared alone in one separate branch closely related to the subtype A reference strains in both regions (Fig. 1B and C). No significant bootstrap values (>70%) could be obtained for some of the major subtype branches in the phylogenetic analysis of the gag, C2–V3, gp41, and nef regions (Fig. 1). However, these values increased when highly divergent samples were excluded from the analysis. For instance, the bootstrap value obtained for the gp41 subtype F samples was 67%, increasing to 81% when the 95BRRM005 sample was excluded from the analysis (Fig. 1C). Moreover, subtype B samples 98BRRS028, 95BRRJ009, and 98BRRS037 were highly divergent in the nef region (Fig. 1D), and their inclusion in the phylogenetic tree analysis reduced the bootstrap value of the B branch from 83 to 64% and, except for sample 95BRRJ009, further SimPlot analysis of this region suggested a B/F recombinant profile (data not shown).

Amplification of the 1298-bp fragment encoding the env C2–V3/gp41 region was obtained for three samples (95BRRJ014, 95BRRS004, and 96BRRJ101) showing discordant gp120 and gp41 env regions. Phylogenetic analysis revealed that this sample appeared alone in one separate branch closely related to the subtype A reference strains in both regions (Fig. 1B and C).

The presence of several HIV-1 subtypes circulating in Brazil has already been described by our group and others.9–19 In this paper we have tried to improve the molecular analysis of HIV-1 samples obtained from two Brazilian regions, characterized by the presence of more than one prevalent subtype, to identify recombinant viruses. In fact, based on the phylogenetic analysis of env C2–V3 and gp41, gag p17, and nef regions, potentially HIV-1 recombinant viruses were found at a high rate (26.5%) among 34 randomly selected samples. The great majority (six of eight) of the potentially recombinant genomes were found among samples previously subtyped as F based on the env C2–V3 region. All of them were due to recombination between subtypes B and F. Subtype B and C recombinant viruses were also found in two of seven subtype C env samples from southern Brazil, with two distinct patterns (B gag/C env/B nef and B gag/C env/C nef).

In a previous study, with HIV-1 samples collected between 1990 and 1992, we were able to identify one B/F recombinant virus in 28 samples obtained from patients also infected in the southeastern region of Brazil.9,15 However, in that study only the C2–V3 gp120 envelope region was analyzed limiting the identification of the recombination events. Indeed, it was estimated that around 10% of HIV-1 circulating forms in the world correspond to recombinant viruses.31 With the extension of the analysis to other genomic regions, in addition to the envelope gene, as well as of full genome sequences, it became evident that the frequency of subtype recombinant viruses is quite important in the AIDS pandemic.32 Recent studies from Africa show that the proportion of samples with gag/env discordant subtypes can vary from less than 10% to up to 40%, according to the number of different subtypes found to be circulating in the region.33 In a study conducted in Manaus, Brazil, 45% of B/F recombinant viruses were identified.34 Similar data were also observed in samples from other South American countries (Argentina, Uruguay, and Bolivia), where no pure subtype F was found. In fact, all subtype F samples classified based on env HMA were found to be unique recombinant B/F forms or represented a new circulating recombinant form, CRF12.30 This same study supports the evidence that these B/F recombinants have been circulating since the beginning of the 1980s, showing that recombinant forms are viable and efficiently transmitted, as previously reported.15 Taken together, the available data concerning B/F recombinant viruses suggest that subtype F samples could be more susceptible for the recombination events, or we can also suppose that B/F recombination could improve virus infectiousness or increase the virus fitness, however biological studies should be made to confirm these hypotheses.

The three samples presenting discordant subtypes between gp120 and gp41 (95BRRJ014, 95BRRS004, and 96BRRJ101) were amplified in almost the full-length env C2–V3/gp41 fragment. The analyses of this fragment allow us to verify two similar mosaic genomes (F gp120 C2–C5/B C5–gp41ID/F gp41 ID-3) for samples 95BRRJ014 and 96BRRJ101. A distinct mosaic genome was found for sample 95BRRS004, which presents only one breakpoint localized in the C5 gp120 region. All three samples were classified as B in the gag p17 region, and sample 96BRRJ101 was also classified as B in the nef region, thus differing from the CRF12 samples that are classified as F in these regions. Indeed, this circulating recombinant form, recently described for HIV-1 samples from Argentina and Uruguay,30 is classified as subtype F in almost the entire gp160, with the exception of a small B segment located in the intracellular part of the gp41 protein (8483–8660), distinct from our samples, that is clearly F and B or B/F in the gp120 and gp41 regions (Fig. 2). Thus, further studies of full length sequencing of these unlinked Brazilian B/F recombinant viruses will be of paramount importance for the identification of new CRFs in South America.
FIG. 1. Phylogenetic analysis of 34 HIV-1 samples from southeast and south regions of Brazil and reference HIV-1 group M subtypes available in the GenBank. The phenograms obtained from the alignments of the gag p17 (360 bp), C2–V3 (300 bp) and gp41 (480 bp), and nef (660 bp) regions are represented, respectively, in A, B, C, and D. The sequence SIVCPZGAB was used as outgroup. The bootstrap values for 100 replicates are indicated in the major subtype branches. The scale bar indicates 10% nucleotide sequence divergence. HIV-1 samples analyzed in the present study are indicated in bold and those presenting discordant subtyping are underlined.
FIG. 2. C2V3–gp41 env recombinant B/F samples among Brazilian HIV-1 samples. (A) SimPlot analysis of the three Brazilian HIV-1 B/F recombinant samples and one representative CRF-12 virus compared to the subtype consensus reference sequences from Los Alamos Database. The search was made in windows of 200 bp, with a threshold of 90% for statistical confidence. Gaps were stripped. The x axis shows the nucleotide position along the alignment. The y axis indicates the similarity index between the HIV-1 subtype nucleotide sequences. (B) Schematic representation of the putative breakpoints within the C2–V3/gp41 region of recombinant B/F viruses based on RIP and SimPlot 2.5 program analyses. The breakpoint positions are based on the HXB2 genome. The arrows indicate the corresponding C2–V3 and gp41 env segments analyzed in Figure 1B and C.
The study of HIV-1 subtype C samples has been considered quite important for vaccine development. This subtype corresponded to 56% of the new HIV-1 infections in 1999 and seems to be spreading all over the world. Three circulating recombinant forms involving subtype C samples have already been described. Recombination between subtypes B and C has been identified among the HIV-1 samples obtained from southern Brazil described in the present study, with at least three distinct patterns (Bgag/Cenv/Bnef, Bgag/Cenv/Cnef, and Cgag/Benv/Cnef). In a previous study, a Cgag/Benv recombinant pattern was also detected among Brazilian HIV-1 samples collected in the southern region, in a setting of a UNAIDS initiative for HIV-1 subtyping in potential HIV-1 vaccine trial sites, suggesting that recombinant B/C genomes can be quite extensively spread in this region.

In contrast to the low number of subtype C samples already described for other Brazilian regions, a high prevalence of this subtype has been found in the south. The mean distance of 10.5% verified in the C2–V3 region among subtype C samples described in the present work supports the recent introduction of this subtype in the country, as previously proposed. In contrast, the highest distance diversity was observed within subtype B samples in all genomic regions included in the present analysis, suggesting that this subtype has been circulating in Brazil for a longer period of time, with multiple virus introductions.

The presence of HIV-1 subtypes D and A has been recently documented in Brazil. In this paper we described an imported case of subtype D infection in a sample from southeastern Brazil, found in a patient infected in Africa, which presented a high molecular distance when compared to the autochthonous Brazilian subtype D sample previously documented by our group. The HIV-1 subtype A sample analyzed in this study was identified in a molecular epidemiological survey carried out in southern Brazil, as presenting an indeterminate env HMA pattern, being further subtyped based on the phylogenetic analysis of the C2–V3 region. Moreover, the SimPlot analysis of the 1290-bp fragment covering the env C2–V3/gp41 region showed three distinct segments that did not match specifically with any of the known subtypes. This might explain the isolated branching of this sample near the subtype A reference samples in the

![Graph showing similarity percentage in HIV-1 subtype C and other subtypes](image)
phylogenetic tree analysis of this fragment. The presence of this subtype in Brazil was firstly described in an HIV-1 sample from Rio de Janeiro based on the analysis of the \textit{gag} p24 region.\textsuperscript{19} The RT-pol region was also analyzed in this paper, but it could not be assigned to any specific subtype group. Taken together, these “subtype A” samples, identified in two distant Brazilian regions, could represent new subtype A variants to be explored in further analysis. The potential role of these subtypes and recombinant genomes in different aspects of the AIDS epidemics is still a matter of investigation.

**SEQUENCE DATA**

GenBank accession numbers: AY071929–AY071952 (\textit{nef} gene), AY071953–AY071967 (C2–V3 \textit{env} gene), AY071968–AY071996 (\textit{gag} gene), AY01997–AY072000 (GP160 \textit{env} gene), AF463420–AF463458 (gp41 \textit{env} gene), AF062422 (95BRMG005,C2–V3), AF062424 (95BRMRJ014, C2–V3), AF062425 (95BRMRJ021), AF060953 (95BRMRJ002), AF060955–AF060962 (95BRMRJ005–95BRMRJ13), AF060963–AF060967 (95BRMRJ015–95BRMRJ020), AF062425 (95BRMRJ021), AF000238 (96BRMRJ100).

**ACKNOWLEDGMENTS**

The HIV-1 \textit{env} Subtyping Kit was kindly provided by the NIH AIDS Research and Reference Reagent Program and UNAIDS. M.L. Guimarães was personally supported by a grant from CAPES. Supported by the National Coordination of STD/AIDS/UNDP/World Bank (UNAIDS), PIAF/FIOCRUZ/Ministry of Health, FAPERJ, and the Brazilian Research Council (CNPq). Fiocruz is a UNAIDS Collaborative Centre. The Brazilian Network for HIV Isolation and Characterization: L.F.M. Brigido, D. Greco, and M. I. Linhares de Carvalho.

**REFERENCES**


Address reprint requests to:
Mariza G. Morgado
Laboratory of AIDS and Molecular Immunology
Department of Immunology
Oswaldo Cruz Institute/FIOCRUZ
Av Brasil 4365
Manguinhos Rio de Janeiro RJ, Brazil CEP 21045-900

E-mail: mmorgado@ioc.fiocruz.br