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SEQUENCE NOTE

HIV Type 1 Diversity from Newly Diagnosed Patients in Santos Metropolitan Area/Brazil

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Abstract

HIV-1 from infected subjects has been characterized in order to provide a more accurate view of the strains that are currently found in a given region. In this report, we focused on characterizing the *pol* gene diversity obtained from newly diagnosed patients in Santos metropolitan area, Brazil. This region is composed of nine cities and an international port. Analysis of the 33 samples revealed that 22 strains belonged to subtype B, 4 to subtype F, and 2 to subtype C; 5 strains were B/F recombinants. Our results demonstrated that 18.2% of samples were primary antiretroviral resistance genotypic mutations, with high-level resistance to reverse transcriptase inhibitors in both subtypes B and F and in recombinant forms B/F. Our data revealed that the primary antiretroviral resistance genotypic mutations should be carefully investigated in developing countries with widespread access to antiretrovirals, such as Brazil.

The Brazilian epidemic of human immunodeficiency virus type 1 (HIV-1) is complex with the presence of subtypes B, F, and C, and a multitude of recombinant genomes emerging from these subtypes.[1–8] We previously described two HIV-1 circulating recombinant forms in Santos, Brazil classifieds as CRF28_BF and CRF29_BF.[5] The Santos metropolitan area includes an international port and nine cities. It was the first region of Brazil to include HIV-1 antiretroviral therapy in HIV/AIDS programs in 1996. In this article we describe sequence analyses of the *pol* (protease and reverse transcriptase) gene in HIV-1 newly diagnosed individuals from the Santos metropolitan area. We report the genetic diversity and primary drug resistance mutations found in subtypes B and F and recombinants B/F.

We studied 33 samples from newly diagnosed patients enrolled in Brazilian HIV/AIDS programs. Samples were collected in 2006–2008, after informed consent was obtained. The population group studied included 33 unrelated individuals, 18 male and 15 female, with a mean age of 35 years and a mean viral load of 4.8 $\log_{10}$ copies/ml (b-DNA, Siemens). The DNA was extracted from the buffy coat using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's procedure. Amplification of the HIV-1 *pol* gene was carried out using nested polymerase chain reaction (PCR). Reagents and thermocycling profiles have been previously described.[3] Purified PCR products were sequenced bidirectionally with an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA). The reaction products were sequenced and analyzed using a model ABI 3100 automatic sequencer (Applied Biosystems). Sequences were corrected and assembled using the Sequencer 4.0 program (Genecodes, Ann Arbor, MI).

Phylogenetic analysis was conducted in the 1200-bp protease and reverse transcriptase regions of *pol*. Alignments were generated using Clustal X. Phylogenetic relationships were established using the neighbor-joining methods present in the PAUP evolutionary package (PAUP version 4.0b10). Trees were constructed in accordance with the HKY85 evolutionary method and the consistency of tree topologies was evaluated by bootstrapping. The GenBank accession numbers used in the comparative phylogenetic analysis are as follows: subtype A: AB098330 and AF004885; subtype B: M93258 and M38431; subtype C: AF286228 and U52953; subtype D: AF133821 and AF457090; subtype F: AY173957 and AF005494; subtype G: AF061640 and AY371121; subtype H: AF005496 and AF190128; subtype J: AF082394 and AF082395; subtype K: AJ249235 and AJ249239; and CPZGAB2: AF382828.

Analysis of recombination points was preliminary done by bootscanning using Simplot software, version 2.5. Breakpoints

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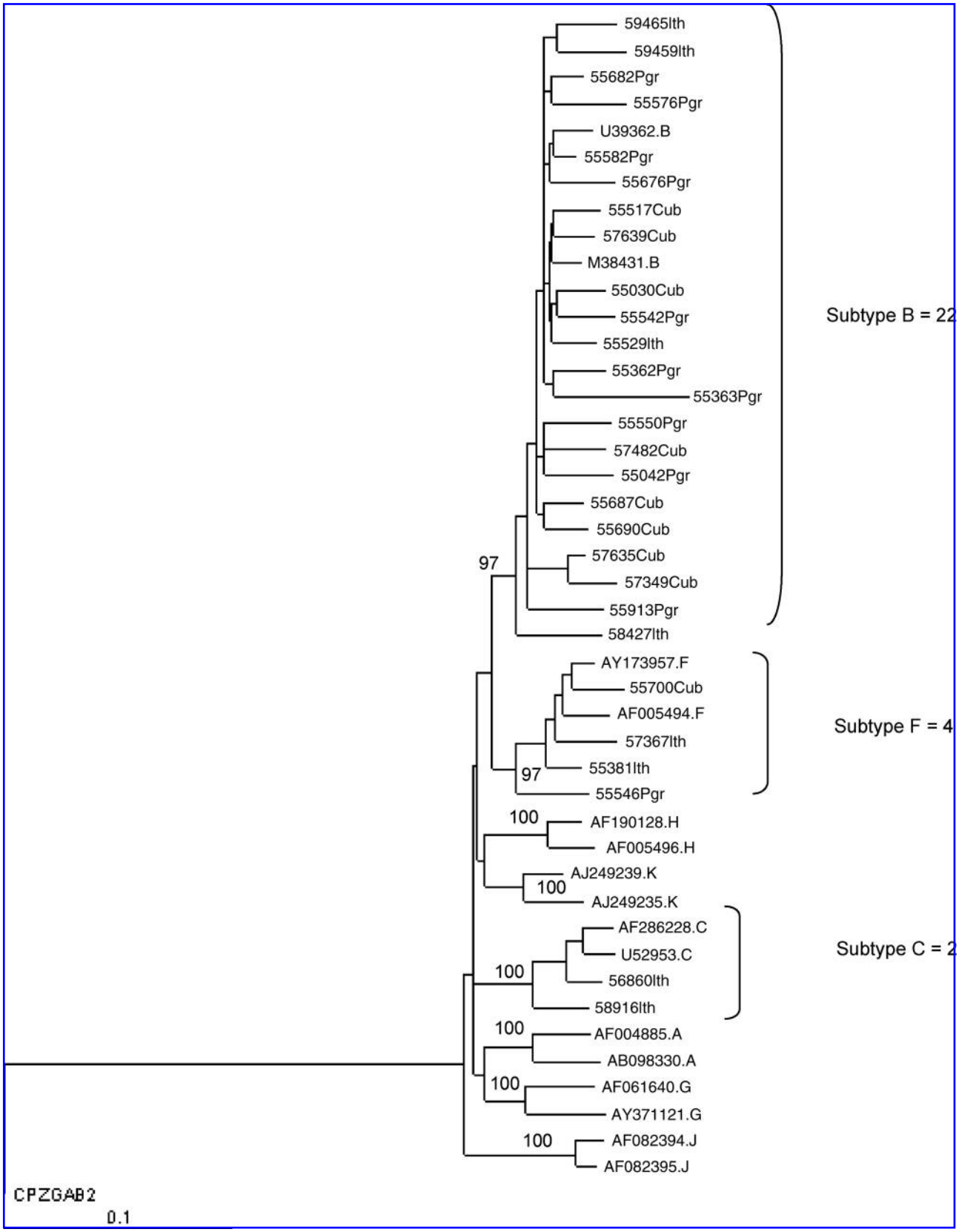

FIG. 1. Phylogenetic classification. The distinct HIV-1 is delineated, and the tree was generated by the neighbor-joining method in accordance with the HKY85 evolutionary method. The bootstrap values (100 reiterations) of major branches of the tree are shown. The following reference sequences were included, corresponding to subtypes A, B, C, D, F, G, H, J, and K. The CPZGAB *pol* sequence was used as an outgroup to root the tree.

FIG. 2. Bootscan analysis of HIV-1 *pol* sequences of two of five recombinant B/F viruses from Santos Metropolitan area Brazil shows the variety of recombinant forms found. The horizontal axis represents the nucleotide distance of the window from the 5′ end of the query sequence (nt 2253 in HXB2). The initial 297 bp corresponds to a protease (PR) gene and next 900 bp to reverse transcriptase (RT). The vertical axis represents the percentage of trees (using 100 bootstrap replicates) that supports branching with the consensus subtype reference sequence. A 250-nt window advanced in 20-nt increments was used. Sequences were gap-stripped, the transversion-to-transition ratio was set to 2.0, distances were calculated according to Kimura's two-parameter model, and trees were performed with the neighbor-joining algorithm. The bootscan plot of sample 55385Ith is representative of the CRF28_BF and CRF29_BF *pol* gene structure.

were mapped more precisely by the inspection of subtype signature nucleotides in alignment with a set of sequences of subtype reference isolates available on the Los Alamos National Laboratory HIV database.

The analyses of codon mutations were performed according to instructions on the Los Alamos database. Decisions about genotype resistance were guided by Stanford University HIV Drug Resistance Database program. GenBank accession numbers are: GQ294595–GQ294624.

According to the phylogenetic analysis of 33 *pol* sequences, 22 sequences were classified as subtype B, 4 as subtype F, and 2 as subtype C (Fig. 1). The remaining five sequences that did not cluster with any subtype were analyzed using the bootscan. The analysis revealed that these sequences were B/F recombinant forms in protease and/or reverse transcriptase regions of the *pol* gene. In bootscan analysis, the amplified *pol* fragment containing 1200 bp was compared to the *pol* fragment of subtype B, F, and C reference strains, which circulate in Brazil, and a subtype A strain as an outgroup (GenBank accession numbers: AF033819, AF005494, AF286228, and AB098330). Only sequences with bootstrap values above 80% throughout the analysis and in which a fragment of at least 250 bp was shown to belong to a discordant subtype were considered recombinant forms. Guided by the bootscan graphic output and visual inspection of sequence alignments, we were able to map breakpoints in recombinant sequences. Breakpoints corresponded to mid points in the sequence stretch between signature nucleotides of subtypes B and F. Recombinant breakpoints in three samples are the same as identified in CRF28_BF and CRF29_BF, position 2571 in relation to HXB2. A representative bootscan plot for this group is shown in Fig. 2, sample 55385Ith. Two samples were unique recombinant forms B/F; a bootscan plot is shown in Fig. 2, sample 55361Pgr.

We have examined the sequences for amino acid changes that are known to contribute to drug resistance, including critical and accessory amino acid substitutions. The analysis of the protease gene did not show any relevant codon mutation related to antiretroviral resistance; however, reverse transcriptase gene analysis showed six samples with drug resistance mutations from newly diagnosed patients. Sample 55042Pgr: mutation M184V; sample 55363Pgr: M184I; sample 57349Cub: A98G and Y181V; sample 58427Ith: K103N; all these samples belong to subtype B; samples 57367Ith with the mutation M230L belong to subtype F, and sample 55361Pgr: mutations K103N and M230L in recombinant B/F.

Monitoring the genetic diversity of HIV-1 in Brazil is important to understanding the molecular epidemiology and the spread of the epidemic. Because multiple HIV-1 subtypes circulate in Brazil, recombinant forms are emerging, as previously reported.[1–8] According to the current study, the major circulating HIV-1 subtypes in the Santos metropolitan area are B followed by recombinants B/F, subtype F, and subtype C (Figs. 1 and 2).

It is of note that there is a low number of pure subtype F strains. This is not a surprise, since other studies also reported the low frequency of pure subtype F as compared to recombinant B/F strains.[1–9] It is conceivable that after the initial introduction of a subtype F strain in Brazil, successive recombination events may be responsible for the reduction of this minority subtype in the context of heavily subtype B epidemics. Furthermore, well-adapted B/F recombinants, CRF28_BF and CRF29_BF, may be expanding in this specific geographic area.

We have examined the sequences for amino acid changes that are known to contribute to drug resistance from newly diagnosed patients. Although our casuist is not very large, we identified the antiretroviral resistance-related mutation in subtype B, subtype F, and recombinants B/F. Our results demonstrated that 18.2% of samples were primary antiretroviral resistance genotypic mutations, with a high-level resistance to the reverse transcriptase inhibitor in both subtypes B and F and in recombinant forms B/F. This high frequency of transmitted resistance in Brazil was also exhibited by Sucupira *et al*.[10]

Our data revealed that the primary antiretroviral resistance genotypic mutations should be carefully investigated in developing countries with widespread access to antiretrovirals, such as Brazil.

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Disclosure Statement

No competing financial interests exist.

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