AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 23, Number 11, 2007, pp. 1434–1441

DOI: 10.1089/aid.2007.0121

Sequence Note

HIV-1 Strains Identified in Brazilian Blood Donors: Significant Prevalence of B/F1 Recombinants

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ABSTRACT

In the Brazilian HIV-1 epidemic subtypes B, C, and F1 are cocirculating in the high risk population groups, and there is a high prevalence of intersubtype recombinant forms. The dynamic nature of the HIV epidemic in Brazil led us to study HIV-1 subtypes present in HIV-infected blood donations collected from 2001 to 2003. Donations from 91 seropositive donors were evaluated. Genetic subtype was obtained for 88 specimens based on sequence analysis of *gag* p24, *pol* IN, and *env* gp41 IDR. HIV-1 subtype B was the predominant strain present in the donor population (73.9%). A significant prevalence of intersubtype recombinants of subtypes B and F1 was found (22.7%). Subtype C (1.1%) and F1 (2.3%) were rare. None of the B/F1 recombinants is CRF28_BF or CRF29_BF. The high level of unique B/F1 recombinant strains in this population demonstrates the dynamic and complex nature of the HIV epidemic in Brazil.

BRAZIL HAS A COMPLEX HIV-1 EPIDEMIC. The epidemic was initiated with the introduction of HIV-1 subtype B, likely originating from the United States, and established itself in the risk group of men having sex with men.[1] Subtype F1 was introduced into Brazil in the early 1980s and was primarily found among intravenous drug users and female prostitutes.[1] More recently, subtype C entered southern Brazil and is spreading from the state of Rio Grande Do Sul to the north and east.[2–4] Currently, subtypes B, C, and F1 are cocirculating in all risk groups with their prevalence differing only by geographic location.[1,3] The cocirculation of subtypes within high risk groups has resulted in a high prevalence of unique intersubtype recombinant forms (URF).[2–4] B/F1 recombinants are common where subtypes B and F1 cocirculate.[3,5–8] Similarly, B/C recombinants are emerging in the south and southeastern regions of the country.[9]

Although subtype B is the predominant strain found throughout Brazil, the epidemic is changing.[3] The prevalence of subtype C is now higher than the prevalence of subtype B in southern Brazil.[2–4] Subtype F1 is declining with B/F1 recombinants now more common than nonrecombinant F1.[3,5–8] The dynamic nature of the HIV epidemic in Brazil led us to evaluate HIV-1 subtypes present in HIV-infected blood donations. Blood donors represent a wider cross section of the population than selected high risk groups and thus may provide a broader overview of the HIV-1 strains circulating in Brazil.

HIV-seropositive plasma was collected from 91 blood donors in Brazil between 2001 and 2003 at three blood banks located in the cities of Salvador, Fortaleza, and Goiania. Salvador, the capital city of the State of Bahia, with 2.5 million inhabitants and Fortaleza, the capital city of the State of Ceará, with about 2.3 million inhabitants are located in the North East region of Brazil. The city of Goiania, the capital of Goias State, with 1.2 million inhabitants is located in the Central West region. The HIV-1 prevalence rates for these three cities are similar at approximately 0.5% of the adult population. The selection crite-

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ria for blood donation in Brazil are very stringent and any donor declaring a risk factor for acquisition of HIV infection is rejected. Thus, all positive patients were likely unaware of their serostatus and declared no known risk exposure to HIV during the screening interview. The study was conducted in conformance with national and local approval from the Institutional Committee on Ethics of Brazil in Research at Professor Edgard Santos University Hospital, Bahia, Brazil.

Brazilian regulations require all blood donations to be tested for HIV antibodies by at least two different enzyme immunoassay (EIA) methods (e.g., virus lysate- and recombinant peptide-based tests). Each test is performed in duplicate and if a sample is reactive in at least one test, it is retested using both assays. Donations with a reactive result upon retest are rejected. Plasma was separated from each HIV-reactive blood unit and sent to the Retrovirology Laboratory at the Federal University of Bahia Hospital where HIV seroreactivity was confirmed by Western blot.

HIV-1 viral load was obtained for 89 specimens using the RealTi*m*e HIV-1 assay (Abbott Molecular Inc., Des Plaines, IL) as previously described (Table 1).[10] For two specimens, 01BAB035 and 01CEB077, HIV-1 RNA was not detected despite the specimens being HIV antibody reactive. The viral loads for quantified specimens ranged from 68 to 5.37×10^5 copies per ml (1.83–5.73 $\log_{10}$ copies per ml) with a median value of 5.25×10^4 copies per ml (4.27 $\log_{10}$ copies per ml). No correlation could be ascertained between subtype and virus load because of the predominance of subtype B in our study population.

To determine the genetic subtype of the HIV-1 strain present in each specimen, three regions of the genome were independently amplified by reverse transciptase polymerase chain reaction (RT-PCR) and sequenced as described: *gag* p24 (851 nts in length), *pol* integrase (IN) (864 nts), and *env* gp41 immunodominant region (IDR) (597 nts).[10,11] For some of the specimens, smaller regions of *gag* p24 or *env* gp41 were amplified. Subtype classification was assigned based on phylogenetic analysis of each amplified sequence. Sequences derived from each genome region were aligned to HIV reference strains of known classification using Clustal W (MegAlign, Lasergene v5.06, DNASTAR, Madison, WI) and manually edited. The alignments were gap stripped and converted into Phylip format using Bioedit (v 7.0.4.1, T. Hall, North Carolina State University, Raleigh, NC). Alignments were subjected to phylogenetic analysis using PHYLIP software (v 3.5c for windows, J. Felsenstein, University of Washington, Seattle, WA) using Dnadist (Kimura two-parameter) to estimate genetic distances, Neighbor (neighbor-joining method) for phylogenetic relationships, and Seqboot for branch reproducibility. Trees were constructed using TreeView (v1.6.6, R.D.M. Page, University of Glasgow, UK). The SimPlot program (v 2.5, S. Ray, Johns Hopkins University, Baltimore, MD) was used to evaluate sequences for intersubtype recombination. Putative recombinant sequences were aligned with one reference sequence for each of the major HIV-1 group M subtypes (A, B, C, F1, and G); for subtype B, reference strain HXB2 or a strain from our Brazil specimen set was used and for subtypes C and F1, reference strains from Brazil were used. SimPlot and BootScan were run using a window of 200 nucleotides and step of 20 nucleotides. For FindSites, reference strains HXB2 (subtype B), 93BR020 (subtype F1), and 92BR025 (subtype C) were used.

Sequence amplification and analysis of *gag* p24 and *env* IDR regions were successful for 88 specimens; *pol* IN was successful for 85 specimens (Table 1). Amplification failed in all three regions for three specimens, 01BAB035, 01BAB064, and 01CEB077; two specimens had undetectable virus and 01BAB064 had a low viral load (68 copies per ml) by the RealTi*m*e HIV-1 assay. Amplification of pol IN failed for 01BAB084, 01BAB105, and 01GOB040. Phylogenetic trees derived from selected *gag* and *pol* sequences are shown in Fig. 1. Sixty-five specimens contained viruses that were subtype B across the genome regions that were amplified, one was subtype C, and two were subtype F1. Six viral isolates showed discordant subtype classification between gene regions; all were subtype F1 in *gag* and subtype B in *pol* and *env* [isolates indicated by an asterisk (*) in Fig. 1; isolate 39340 not shown in Fig. 1A]. Sequences that were basal in the subtype branch (for example, 182189 in Fig. 1B) or that fell between subtype branches (for example, 01BAB060 in Fig. 1B) were examined for intersubtype recombination using SimPlot. There were no recombinant sequences in the *gag* fragments. Within the pol integrase region, 11 sequences were identified as recombinants comprised of subtypes B and F1 (indicated by $<$ in Fig. 1B). There appear to be three subtype B/F1 recombinants in the *env* sequences; the recombination breakpoint could be defined for 153117-4. However, *env* sequences for 01CEB082 and 01GOB039 did not have definable breakpoints due to the short sequence lengths and the subtypes were assigned as unclassified (U).

In the Brazilian strains *pol* IN appears to be a hot spot for intersubtype recombination. Of 22 recombination breakpoints that were identified, 21 were present within 11 *pol* IN sequences. Seven different recombination patterns were observed for the *pol* IN sequences; Fig. 2 shows a schematic of the recombinant structures. The recombinant breakpoints were defined based on the results of FindSites analysis. Isolates 01BAB032 and 01BAB060 have the same structure. Isolates 01BAB034, 01BAB086, 01BAB101, and 01BAB102 have the same recombination pattern in *pol* IN; however, in *gag*, 01BAB034, 01BAB101, and 01BAB102 are subtype F1 and 01BAB086 is subtype B (Fig. 1A). Only isolates 01BAB034 and 01BAB102 branch together in *gag* and *env*. Many of the recombination breakpoints were shared between different isolates (Fig. 2). At some sites the crossover was always the same subtype switch, from B to F1 or F1 to B, suggesting there may be a selective advantage to the recombinant structure. At other sites both subtype switches were observed implying no structural preference. Other reports of B/F1 recombinants in Brazil also found the *pol* region to be a hot spot for recombination and found conservation of breakpoints.[7,12,13] These observations could indicate that B/F1 recombinant strains have undergone additional recombination with some breakpoints preserved in the progeny while other breakpoints are added or lost.[13] Alternatively, recombination is occurring repeatedly at the same or similar sites in *pol*.[7] Recombination occurs most frequently in genome regions with high sequence conservation and is 2-fold higher in *pol* than in *gag* and *env*.[14]

Table 2 summarizes the genotyping results for the viruses found in this Brazilian blood donor population. Subtype B was

TABLE 1. SPECIMEN DATA

Number	*Specimen ID*[a]	*Collection date*[b]	*Viral load*[c]	*Genetic subtype*[d]		
				gag *p24*	pol *IN*	env *IDR*
1	37811	Dec/2000	5.17	F1	F1	F1
2	38154	Jan/2001	4.26	B	B	B
3	39212	Mar/2001	5.03	B	B	B
4	39340	27 Mara/2001	4.17	F1	B	B
5	39462	4 Mar/2001	5.11	B	B	B
6	181615	Aug/2000	4.27	B	B	B
7	182189	Aug/2000	4.54	F1	B/F1	B
8	425622	Jul/2000	3.82	B	B	B
9	437895	10 Apr/2000	3.85	B	B	B
10	439200	16 Oct/2000	3.63	B	B	B
11	440163	Oct/2000	4.20	B	B	B
12	443592	Nov/2000	3.32	B	B	B
13	465249	Apr/2001	4.19	B	B	B
14	465302	Apr/2001	4.71	B	B	B
15	O13650060	Jul/2000	5.73	B	B	B
17	01BAB032	5 May/2001	4.16	F1	B/F1	B
18	01BAB034	6 May/2001	4.15	F1	B/F1	B
19	01BABA035	25 May/2001	ND	Neg	Neg	Neg
20	01BAB036	30 May 2001	3.34	B	B	B
21	01AB053	6 Jul/2001	3.67	B	B	B
22	01BAB054	11 Jul/2001	3.39	B	B	B
23	01BAB055	Sept/2000	2.66	F1	B	B
24	01BAB056	Sept/2000	3.37	B	B	B
25	01BAB057	Aug/2000	2.57	B	B	B
26	01BAB058	Jan/2001	4.21	B	B	B
27	01BAB060	Jul/2000	4.23	F1	B/F1	B
28	01BAB061	Sept/2000	4.34	B	B	B
29	01BAB062	Feb/2001	4.89	B	B	B
30	01BAB063	3 Aug/2001	4.48	F1	B	B
31	01BAB064	25 Jan/2002	1.83	Neg	Neg	Neg
32	01BAB066	29 Jan/2002	4.28	B	B	B
333	01BAB067	15 Feb/2002	4.54	B	B	B
34	01BAB068	15 Feb/2002	3.88	F1	B	B
35	01BAB075	19 Apr/2002	4.48	F1	B	B
36	01BAB076	5 Apr/2002	4.57	B	B	B
37	01BAB080	17 May/2002	5.05	B	B	B
38	01BAB084	3 Aug/2001	3.01	B	Neg	B
39	01BAB085	7 Jun/2002	3.90	F1	B	B
40	01BAB086	17 Jun/2002	3.90	B	B/F1	B
41	01BAB093	5 Jul/2002	4.42	B	B	B
42	01BAB094	28 Jun/2002	4.21	B	B	B
43	01BAB095	9 Aug/2002	4.61	B	B	B
44	01BAB096	30 Aug/2002	4.94	F1	B/F1	B
45	01BAB097	18 Oct/2002	4.23	B	B	B
46	01BAB098	18 Oct/2002	4.04	B	B/F1	B
47	01BAB099	18 Oct/2002	3.69	B	B	B
48	01BAB100	18 Oct/2002	3.61	B	B	B
49	01BAB101	Nov/2002	4.01	F1	B/F1	B
50	01BAB102	Nov/2002	4.01	F1	B/F1	B
51	01BAB103	Nov/2002	4.09	B	B	B
52	01BAB104	6 Sept/2002	4.14	B	B	B
53	01BAB105	19 Jul/2002	2.90	B	Neg	B
54	01BAB106	26 Mar/2002	3.77	B	B	B
55	01BAB107	Sept/2000	3.03	B	B	B
56	01BAB108	6 Sept/2002	4.01	B	B	B
57	01CB043	Mar/2002	4.46	B	B	B
58	01CEB044	Mar/2002	4.38	B	B	B
59	01CEB045	Mar/2002	4.51	B	B	B
60	01CEB047	Mar/2002	4.94	B	B	B
61	01CEB048	Mar/2002	4.64	B	B	B

(*continued*)

TABLE 1. SPECIMEN DATA (CONT'D)

Number	*Specimen ID*[a]	*Collection date*[b]	*Viral load*[c]	*Genetic subtype*[d]		
				gag *p24*	pol *IN*	env *IDR*
62	01CEB049	Mar/2002	4.32	B	B	B
63	01CEB050	Jan/2002	4.78	B	B	B
64	01CEB052	Jan/2002	4.05	B	B	B
65	01CEB069	5 Feb/2002	5.61	B	B	B
66	01CEB070	5 Feb/2002	4.10	B	B	B
67	01CEB071	5 Feb/2002	4.45	B	B	B
68	01CEB072	5 Feb/2002	4.30	B	B	B
69	01CEB073	5 Feb/2002	5.72	B	B	B
70	01CEB074	5 Feb/2002	5.04	B	B	B
71	01CEB077	21 May/2002	ND	Neg	Neg	Neg
72	01CEB078	21 May/2002	4.55	B	B	B
73	01CEB079	21 May/2002	5.05	F1	B/F1	F1
74	01CEB081	21 May/2002	5.04	B	B	B
75	01CEB082	21 May/2002	4.47	B	B	U
76	01CEB083	21 May/2002	4.76	B	B	B
77	01CEB087	2 Apr/2003	4.86	B	B	B
78	01CEB088	2 Apr/2003	3.21	B	B	B
79	01CEB089	2 Apr/2003	4.42	B	B	B
80	01CEB090	2 Apr/2003	4.40	F1	F1	F1
81	01CEB091	2 Apr/2003	4.93	B	B	B
82	01CEB092	2 Apr/2003	3.64	F1	F1	U
83	01BOG029	Mar/2002	4.52	B	B	B
84	01GOB030	Mar/2002	4.74	B	B	B
85	01GOB031	Mar/2002	4.64	B	B	B
86	01GOB034	Jun/2002	4.31	B	B	B
87	01GOB038	Aug/2003	5.21	B	B	B
88	01GOB039	Aug/2002	5.40	F1	B/F1	B
89	01GOB040	May/2002	2.36	B	Neg	B
90	01GOB041	May/2002	2.93	C	C	C
91	01GOB042	May/2002	3.67	B	B	B

[a]Collection site indicated by ID: BAB indicates Bahia; CEB indicates Ceará; GOB indicates Goias; specimens 1–16 were collected in Bahia.

[b]Day (if available), month/year.

[c]Viral load determined using a RealTime HIV-1 assay and expressed as $\log_{10}$ RNA copies per ml. ND indicates HIV-1 RNA was not detected.

[d]neg, specimen was RT-PCR negative.

the predominant strain found (65 isolates, 73.9%), followed by intersubtype recombinants of subtypes B and F1 (20 isolates, 22.7%). Subtypes C and F1 were present but rare (1.1% and 2.3%, respectively). For the B/F1 recombinant strains, 16 were subtype F1 in *gag* p24 whereas only one *pol* IN and one *env* sequence were F1 derived. In contrast, there were only four subtype B-derived *gag* p24 sequences as compared to 8 for *pol* IN and 16 for *env* IDR.

The results in this study are consistent with previous studies that show a high prevalence of subtype B in other population groups within Brazil.[3,6,8,12,15,16] In contrast, the frequencies of subtypes C and F1 were lower than some of the previous reports.[3–6] For subtype C, this may be due in part to the unequal geographic distribution of subtype C in the country with a higher prevalence in the south and decreasing prevalence moving into north and central Brazil.[2–4] The population in this study came from the northeast and central west regions. Previous reports may have overestimated the prevalence of subtype F1; reanalysis of F1 subtypes that had been classified based on one genome region often showed the viruses were B/F1 recombinants when additional genome regions were evaluated.[9] Based on the genome regions sequenced, we identified two pure F1 strains. Since CRF12_BF is F1 in all the regions evaluated in this study, we subsequently sequenced the *pol* protease and RT genes of the two putative F1 isolates. Phylogenetic analysis revealed that they are subtype F1 throughout this region and do not contain any of the recombination breakpoints found in CRF12_BF (data not shown).[17]

One of the unique features of the Brazilian HIV epidemic is the variety of intersubtype recombinants of subtypes B and F1 and the absence of a dominant B/F1 circulating recombinant form.[6,12] Even without full-genome sequences, 20 B/F1 recombinant strains were identified in our study population. Six isolates have a *gag*/*pol*/*env* recombination pattern of F1/B/B, consistent with CRF28_BF that was recently identified in Brazil.[13] However, none of the six isolates shares recombina-

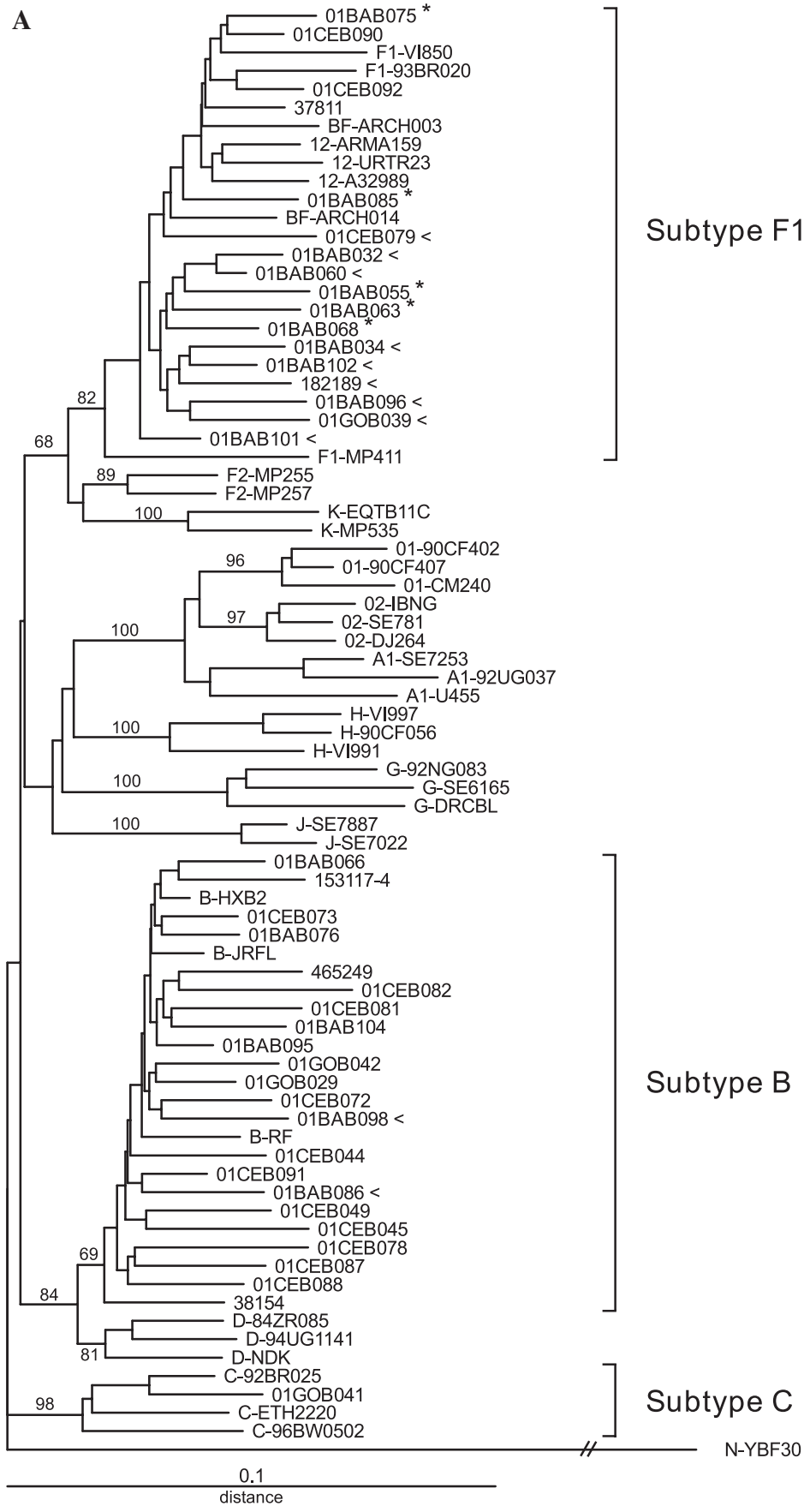

FIG. 1. Phylogenetic trees derived from *gag* p24 and *pol* IN sequences. Trees were constructed as described in the text. Group N isolate YBF30 was used as the outgroup. Bootstrap values ≥68% are shown for the major branches. (**A**) *gag* p24, sequence alignment was 620 nucleotides in length after gaps were stripped. (**B**) *pol* IN, sequence alignment was 836 nucleotides in length after gaps were stripped. The symbol * indicates isolates with discordant subtype classification between gene regions and < indicates isolates with recombinant *pol* IN sequences.

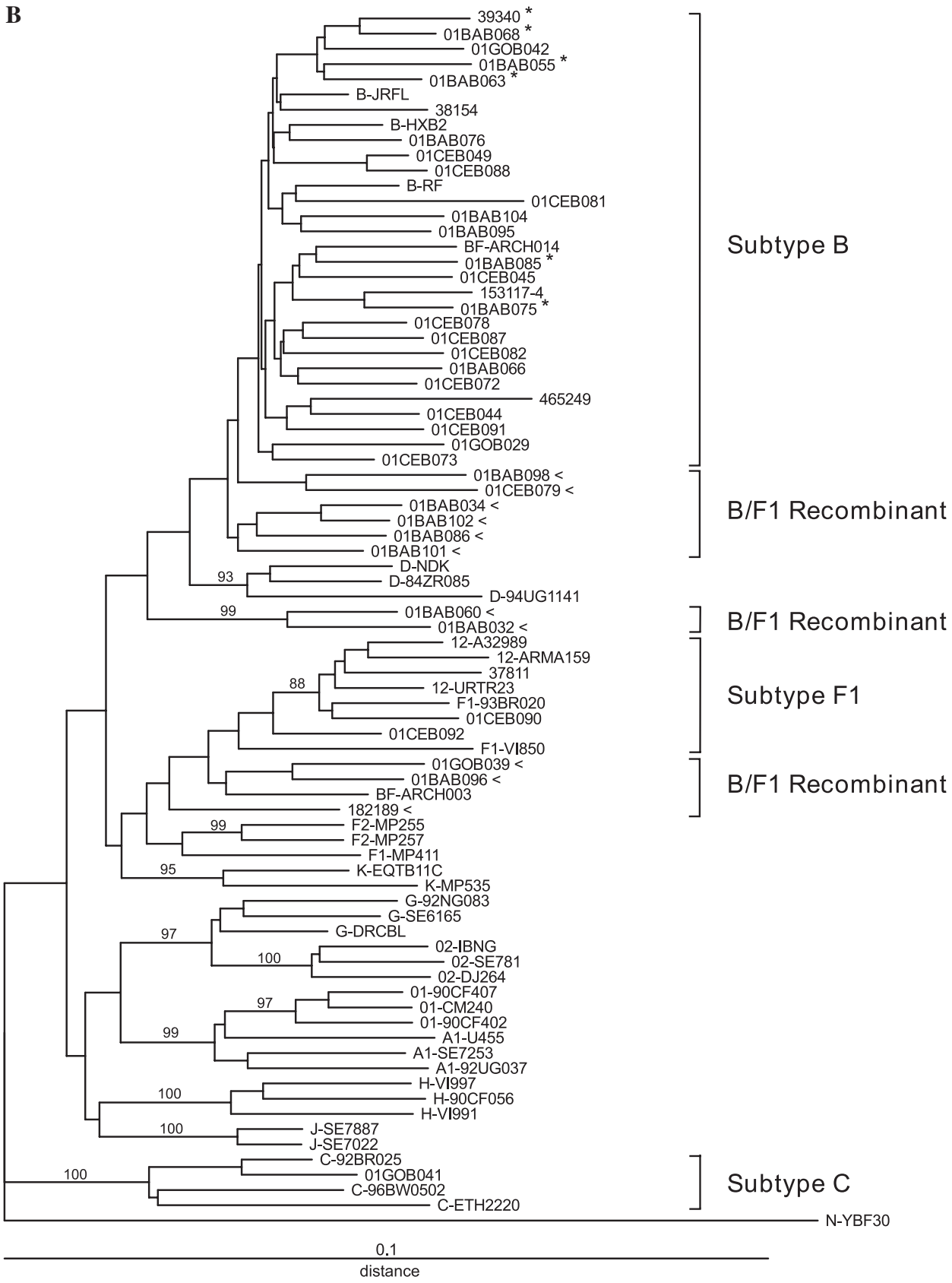

FIG. 1. *Continued.*

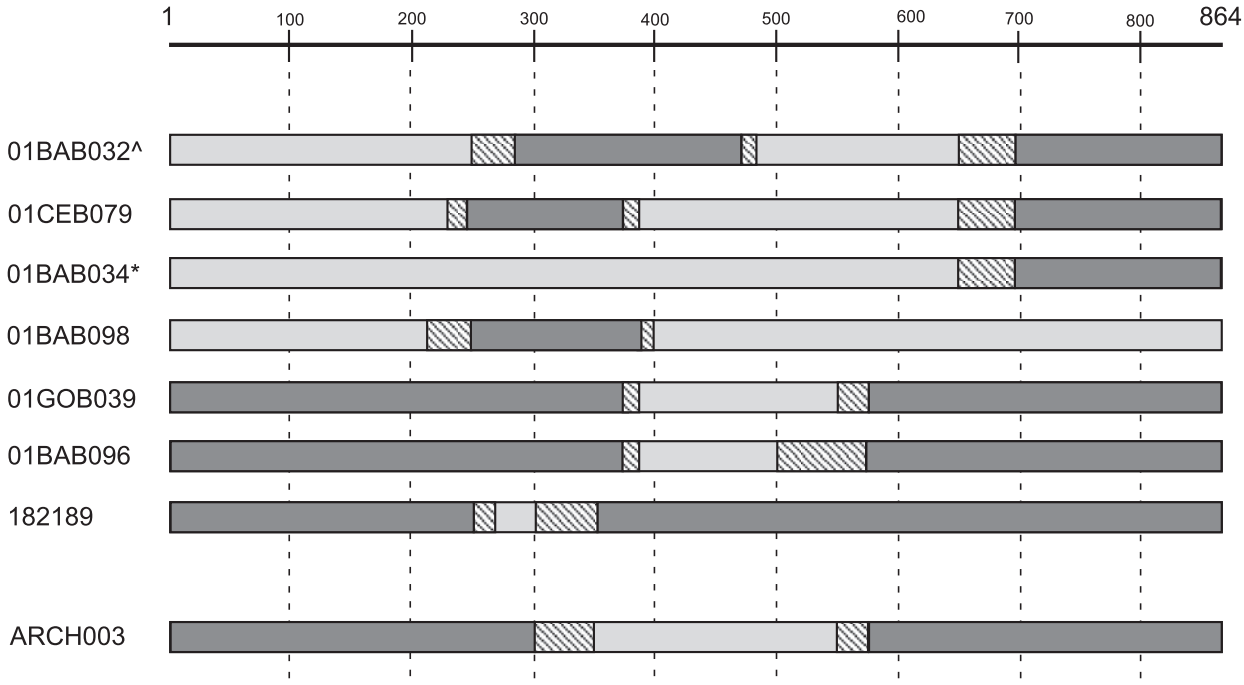

FIG. 2. Intersubtype recombination breakpoints within *pol* IN sequences. Nucleotide positions are numbered from the start of the IN coding region with position 1 equivalent to nucleotide 4230 in reference strain HXB2 (accession number M38432). The subtype B sequence is indicated by light colored solid boxes, subtype F1 by dark solid boxes, and crossover regions by hatched boxes. ^The pattern for 01BAB032 is also present in 01BAB060. *The pattern shown for 01BAB034 is also present in 01BAB086, 01BAB101, and 01BAB102. ARCH003 is a URF reference strain.

tion breakpoints with CRF28_BF references strains in *pol* protease-RT (data not shown). Three isolates with the F1/B/B pattern (39340, 01BAB063, and 01BAB068) consistently branch together in phylogenetic trees derived from each of the three genome regions and have the same recombination breakpoint within protease-RT (data not shown). Although this subcluster within the phylogenetic trees is not supported by bootstrap values, these three isolates may represent a CRF. Isolates 01BAB32 and 01BAB060 have a *gag/pol/env* pattern of F1/BF1/B and form a branch within each phylogenetic tree that is supported by bootstrap values and are candidates for a CRF. Thus of the 20 B/F1 recombinant strains, 5 have the potential to represent two novel CRFs and 15 appear to be URFs. None of the B/F1 recombinants has patterns consistent with Brazilian strains of CRF28_BF and CRF29_BF.[13]

The high prevalence of URFs and the apparently low prevalence of circulating recombinant forms (CRF) in Brazil are likely the result of the introduction of subtypes C and F1 into an established epidemic of HIV-1 subtype B infections. Cocirculation of more than one subtype within a high risk group that is repeatedly exposed led to a high occurrence of dual infections and resulted in ongoing recombination of pure subtypes and intersubtype recombinants.[12] In contrast, in Argentina, a B/F1 recombinant was introduced into a population with a low prevalence of HIV-1. Subsequent expansion of this B/F1 strain in the relatively naive population gave rise to CRF12_BF.[17] We did not observe any dual infections in the study population. However, the methods used here are biased for detection of the predominant strain present in the specimen and would not have detected a second virus present at much lower levels. Virolog-

TABLE 2. SUMMARY OF GENETIC SUBTYPES IN THE STUDY POPULATION

	Number of isolates			
Genetic subtype	*Overall (%)*	gag *p24*	pol *IN*	env *IDR*[a]
B	65 (73.9%)	69	70	81
C	1 (1.1%)	1	1	1
F1	2 (2.3%)	18	3	3
Intersubtype recombinant	20 (22.7%)	0	11	3
Total	88	88	85	88

[a]Two unclassified *env* sequences are counted as recombinants.

ical factors may also favor B/F1 recombination during coinfection, for example, viral fitness. Whatever the mechanism, the high level of unique B/F1 recombinant strains in the population demonstrates the dynamic and complex nature of the HIV epidemic in Brazil.

ACKNOWLEDGMENTS

We thank Dr. Shihai Huang at Abbott Molecular for assistance with the RealTi*m*e HIV-1 analysis, and Dr. Aurelino Santana, Salvador, Bahia and Dr. Francisco Braga, Fortaleza, Ceara for providing specimens collected at their blood banks. GenBank accession numbers for HIV-1 sequences are EF150546–EF150555 and EF150557–EF150631.

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