

# Molecular Epidemiology of HIV-1 in Venezuela: High Prevalence of HIV-1 Subtype B and Identification of a B/F Recombinant Infection

\*†Erika Castro, ‡Gloria Echeverría, ‡Leopoldo Deibis, §Beatríz González de Salmen,  
\*Aline Dos Santos Moreira, \*Monick L. Guimarães, ¶Francisco I. Bastos, and  
\*Mariza G. Morgado

\*Department of Immunology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil; †Non Governmental Organization, CRIATEII, Porlamar, Margarita Island, Venezuela; ‡Immunology Institute, Central University of Venezuela, Caracas, Venezuela; §STDs and AIDS Program, State Hospital “Luis Ortega,” Porlamar, Margarita Island, Venezuela; and ¶Department of Health Information, CICT, FIOCRUZ, Rio de Janeiro, Brazil

---

**Summary:** The authors assessed HIV-1 variability in two distinct areas of Venezuela (the capital Caracas and Margarita Island) through the analysis of blood specimens and clinical and epidemiologic data of 72 persons. Proviral DNA was evaluated through heteroduplex mobility assay (HMA) based on the envelope region. Additionally, FOK I restriction enzyme digestion assay was performed in all subtype B ED31/33 amplified products to check the presence of the typical Brazilian subtype B GWGR variant. Sequencing and phylogenetic analysis for C2-V3 region of gp120 was performed in selected cases. The vast majority of samples were found to belong to subtype B, with a North American/European RFLP profile. An F subtype HIV-1 based on the *env* region was identified for the first time in Venezuela. Genetic analyses of the protease and reverse transcriptase fragments of this sample depicted a recombinant B/F genetic profile. The discrimination capacity of HIV-1 subtypes using the primer set ED3/14-ED31/33 among the Caracas samples was found to be higher than for those from Margarita Island. The authors' results point to relevant differences between the samples of continental and Caribbean regions of Venezuela, requiring further evaluation of larger samples. **Key Words:** HIV-1 diversity in Venezuela—B/F recombinant virus in Venezuela—Molecular epidemiology—Heteroduplex mobility assay—Non-B HIV-1 subtypes in Latin America.

---

---

This study was supported by grants of the National Council for Scientific and Technological Research from Venezuela “CONICIT,” PAHO/Venezuela, and the Oswaldo Cruz Institute.

Results shown in this report are part of the PhD thesis of Erika Castro in the field of Tropical Medicine from the Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. Current address for Erika Castro: Instituto de Inmunología, Universidad Central de Venezuela, Ciudad Universitaria, Los Chaguaramos, Caracas, 1050-A, Venezuela; e-mail: ebatenjer@hotmail.com

Address correspondence and reprint requests to Mariza G. Morgado, Laboratory of AIDS & Molecular Immunology, Dept. of Immunology, Oswaldo Cruz Institute/Fiocruz–UNAIDS Collaborating Centre, Avenida Brasil 4365, Manguinhos, Rio de Janeiro, CEP 21045–900, RJ, Brazil; e-mail: mmorgado@ioc.fiocruz.br

Manuscript received February 5, 2002; accepted October 21, 2002.

Since the beginning of the AIDS epidemic, HIV-1 polymorphism has been an important issue for HIV/AIDS researchers worldwide. As a consequence of this effort to monitor HIV-1 diversity, 24 major genetic forms, including 9 distinct subtypes (A–D, F, G, H, J, and K) and 14 circulating recombinant forms (CRFs; CRF01\_AE, CRF02\_AG, CRF03\_AB, CRF04\_cpx, CRF05\_DF, CRF06\_cpx, CRF07\_BC, CRF08\_BC, CRF09\_?, CRF10\_CD, CRF11\_cpx, CRF12\_BF, CRF13\_cpx, and CRF14\_BG), have already been reported for the major group (1–3).

Data from South American countries are also included in such studies, showing predominantly the presence of

subtype B. Briefly, subtypes B, C, D, variant B' from subtype B, and the recombinant B/F and B/C forms have been identified in Brazil (4–13). Reports from other countries of the region have shown the existence of subtypes B, C, F, and the recombinant B/F form in Argentina (14,15); subtypes B and E in Uruguay (16,17); subtypes B and F in Bolivia (18); and subtype B in Colombia (19), Paraguay (20), Honduras (21), and Mexico (22). In addition to subtype B in Chile, a single case of subtype A infection has been reported (23). Venezuela's earliest research on HIV-1 polymorphism verified the presence of subtype B in a small number of samples (24,25) and, more recently, the occurrence of an F/B protease/reverse transcriptase recombinant strain in 1 person participating in an UNAIDS study (26).

We conducted a survey to analyze HIV-1 variability and epidemiologic characteristics of infected persons from Caracas and Margarita Island, the two localities with the highest morbidity rates for AIDS in Venezuela (27). This study also represents the first contribution toward the establishment of the Venezuelan National Program on HIV-1 Surveillance of Molecular Variability.

**METHODS**

**Patients and Methods**

The recruitment of patients and the collection of biologic sample were carried out from June 1998 to October 1999. Two independent

convenience samples of outpatients were enrolled in the study: one from Margarita Island (STDs Department of the State Hospital), and a second from Caracas (Retrovirus Reference Center of the Institute of Immunology). Only confirmed HIV-1-infected (by Western blot or immunofluorescence) patients were included in the study.

This study included 72 HIV-1-infected patients, 22 from Margarita Island and 50 from Caracas. The Caracas sample was a pool of persons living in 12 of the 23 states of the country seeking specialized care in the reference center, located in the capital. All volunteers answered a questionnaire addressing sociodemographic and behavioral data, signed informed consent, and allowed the peripheral extraction of 5 mL of whole blood.

**HIV-1 Subtype Characterization**

Five milliliters of ethylenediamine tetraacetic (EDTA) anticoagulated blood were obtained from each patient. The plasma was collected, and the remaining white cell layer (2–3 mL) was resuspended in 0.5% Saponin/0.4% NaCl (vol/vol), thoroughly vortexed, centrifuged (400g for 5 minutes at room temperature), and washed twice with phosphate-buffered saline (PBS) by centrifugation in the same conditions. Resulting pellets were stored at -20°C until proviral DNA extraction at FIOCRUZ, Brazil. Proviral DNA was extracted by the proteinase K-SDS/phenol/chloroform method as previously described (4).

A nested polymerase chain reaction (PCR) protocol was used to amplify the gp120 C2-C3 (ED31-ED33 primer set) using ED3-ED14 as outer primers (28,29). DNA samples that did not give amplified products using this protocol were amplified with other primer sets like ED5-ED12, ES7-ES8, or other combinations between them, according to the algorithm shown in Figure 1. PCR-negative samples for the *env* region primers were further analyzed with a multiplex PCR protocol (30), with modified *env* primers (ED5-ED12 [outer]/ED31-ED33 [inner] primers, in substitution of those described in the original reference), to check the positivity to HIV genome.

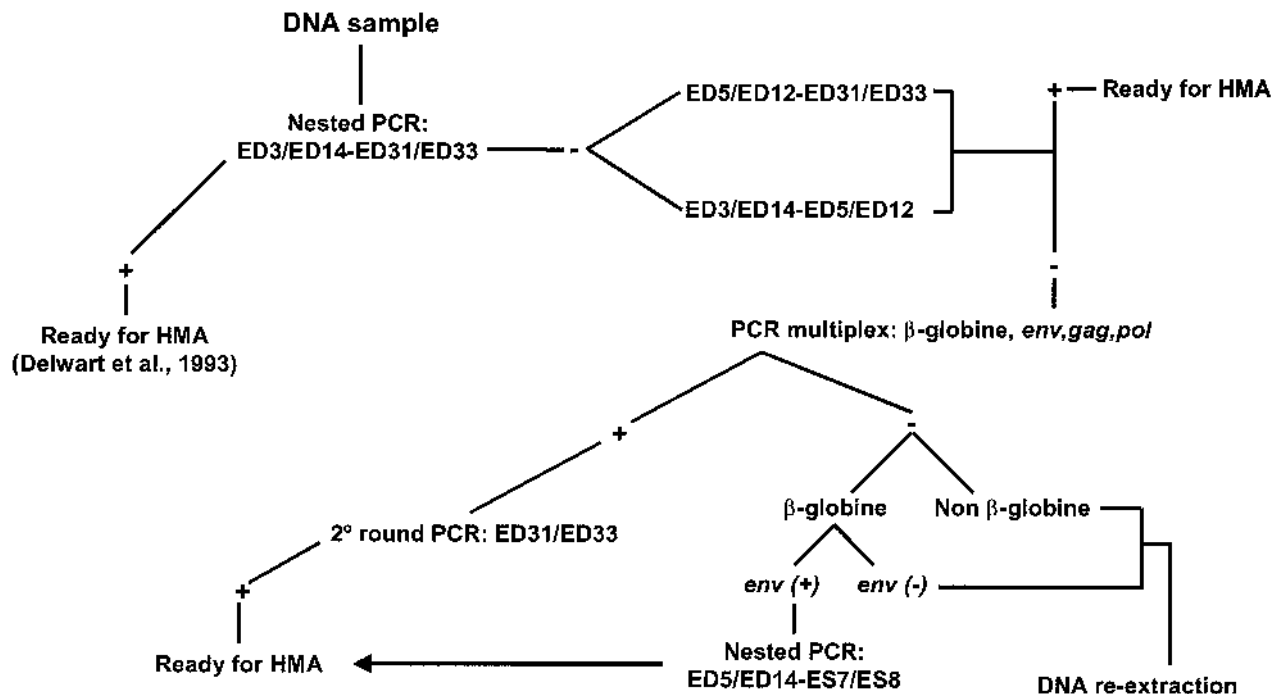


FIG. 1. PCR algorithm used on HIV-1 proviral DNA samples from Venezuela, 1998 to 1999.

HIV-1 subtyping was determined by *env* heteroduplex mobility assay (HMA) (28,29,31) using PCR amplified reference plasmids of HIV-1 subtypes B-F, or A-H in doubtful cases. The NIH AIDS Research and Reference Reagent Program provided the HMA HIV-1 *env* subtyping kit. In order to identify B'' GWGR subtype B samples, 5  $\mu$ L of ED31/ED33 PCR products were further digested with 6 U of *Fok I* restriction enzyme (11).

### Sequencing of PCR Products

A sample of 5  $\mu$ L of the C2-V3 ED31/ED33 PCR reaction was submitted to enzymatic treatment with 1  $\mu$ L of shrimp alkaline phosphatase (10 U/ $\mu$ L) and 1  $\mu$ L of exonuclease I (2 U/ $\mu$ L) hydrolytic enzymes according to the manufacturer's protocol and directly sequenced using the dideoxy chain termination method kit (DNA sequencer model 377, Applied Biosystems, Foster City, CA, U.S.A.), using the Big Dye Terminator kit (Applied Biosystems, Foster City, CA, U.S.A.). The PCR products were sequenced in both senses with ED31, ED33, and ES7 oligonucleotides as sequencing primers. Alignment of multiple nucleotide and predicted amino acid sequences was performed with the University of Wisconsin Genetic Computer Group (GCG) package. The subtype F *env* sample identified in the current study was further sequenced in the protease and reverse transcriptase (RT) regions using, respectively, DP10/DP11 (outer)/DP16/DP17 (inner) protease primer sets (12) and RT9/RT12 (outer)/RT1/RT4 (inner) RT primer sets (32) to complement its analyses.

Phylogenetic analysis was done using the CLUSTAL W software (33). Phylogenetic tree constructions were performed using the neighbor-joining method, and the reliability of the branching orders was determined by the 100 times bootstrap method. The final trees were visualized using the Tree View program (34). The intrasubtype distances were calculated using the Kimura two-parameter with pairwise gap deletion based on the MEGA program (35). GenBank accession numbers for HIV-1 *env* sequences from Margarita Island and Caracas, Venezuela are assigned from AF467716 to AF467725 (Accession numbers for 99VenC13 protease and RT sequences are, respectively, AF467727 and AF 467726).

### Statistical Analysis

The  $\chi^2$  or Fisher exact test was employed to evaluate possible associations, and a *p* level of < .05 was defined as statistically significant.

## RESULTS

Caracas and Margarita Island samples were substantially different in sociodemographic information. The Caracas sample (*n* = 50) was composed basically of men (46 men, 4 women), while the Margarita Island sample (*n* = 22) had exactly the same number of men and women. Patients from Margarita Island were younger (half were aged 18–30 years), contrasting with a concentration (64%) of patients from Caracas aged 31 to 44 years. Roughly 73% of the Margarita Island patients were supposed to be infected by heterosexual transmission, while the vast majority (70%) of patients from Caracas were gay men. CD4 counts were roughly com-

parable in patients from these two cities (data not shown).

### DNA Amplification and HIV Subtyping

With the primer sets ED3/14-ED31/33 we could amplify 60% DNA products from the Margarita Island samples and 82% of the Caracas samples, respectively. The remaining DNA products were analyzed with other primer sets according to the algorithm presented in Figure 1. There was no correlation between primer sets ED3/14-ED31/33 amplification capacity with either exposure category or CD4<sup>+</sup> counts in the two samples. A significantly (*p* = .038) higher discrimination capacity of primer sets ED3/14-ED31/33 was found for the Caracas samples, if compared with the Margarita Island samples, pointing to a higher intrasubtype B variability in the samples of Margarita Island vis-à-vis the samples from the continent, possibly due to the close link of the Island with the Caribbean subepidemic.

Three cases (99VenM1, 99VenC17, and 99VenC30) failed all PCR attempts of amplification. We repeated the blood screening with new samples obtained from 2 of these patients (99VenM1 and 99VenC17), and again we failed to amplify proviral DNA. Viral load (Nuclisens NASBA Diagnostics HIV-1 RNA QT) in these patients were 700 copies/mL and 14,000 copies/mL for 99VenM1 and 99VenC17, respectively, and their CD4<sup>+</sup> counts were >350 cells/mL. Both patients remained asymptomatic during the period they were followed (1998–1999) and did not receive any kind of antiretroviral drugs in this period. As PCR sensitivity in our study could successfully amplify from >20 copies of proviral DNA, we tried alternative sources of genomic material, obtaining cDNA from the plasmatic RNA retrotranscription. In these 2 cases, amplification was carried out with a multiplex PCR directed to viral *env*, *gag*, and *pol* genes, and a positive band for *gag* gene was observed only for the sample of patient 99VenC17 (data not shown).

We identified the subtype B HMA profile of HIV-1 in 20 (90.9%) of the Margarita Island patients and in 47 (94.0%) of the Caracas study group. One patient in the Caracas sample (99VenC13) showed the subtype F migration pattern. He was a 34-year-old gay man, with advanced immunosuppression (CD4<sup>+</sup> counts of 122 cells/mm<sup>3</sup>). He was presumably infected between 1994 and 1996, when traveling abroad. HMA result was indeterminate for one sample (99VenM11) from the Margarita sample, which was further selected for DNA sequencing.

According to the *FOKI* RFLP, none of the subtype B virus described in this study exhibited the typical GWGR subtype B profile in the V3 loop.

A subset of 11 samples, including the indeterminate HIV-1 subtype from the Margarita Island group (99VenM11), the subtype F *env* sample (99VenC13) from the Caracas group, and 9 subtype B samples, was selected for the C2-V3 *env* sequencing (Fig. 2A). The phylogenetic tree confirms that sample 99VenC13 does cluster within the F clade in the C2-V3 *env* region with a high bootstrap value. CRF-12 B/F reference samples (36) from Argentina (ARMA 185 and ARMA 159) were included in this analysis and also clustered within the subtype F branches. However, sample V62, a B/F recombinant virus previously described from Venezuela (26), clustered with the subtype B references in the *env* C2-V3 region. The remaining samples from Caracas and Margarita Island clustered into the B subtype with a bootstrap value of 70%, reflecting a higher interpatient diversity in this group. Indeed, no specific clustering could be detected for the HIV-1 samples from Venezuela, which were interspersed in branches of subtype B reference samples from other South American countries and from the United States. Moreover, pairwise distance among the subtype B specimens was, on average, 18.7% at the nucleotide level.

Additional phylogenetic analysis of *env* subtype F sample 99VenC13 in the protease and RT regions (Fig. 2B, C) revealed a mosaic genome clustering with subtype F references in the protease region, whereas for the RT fragment, this sample clustered in a separated branch with the two CRF-12 B/F ARMA 185 and ARMA 159 references with high bootstrap value (95%), indicating a recombinant profile in this fragment.

The predicted amino acid sequences of the *env* gp120 C2-V3 region obtained from Venezuelan HIV-1 strains are presented in Figure 3. Nine subtype B and one subtype F amino acid sequences were aligned with B and F reference sequences previously reported from other South American countries [Argentina (14), Bolivia (18), Brazil (13), and Uruguay (17)] and with one subtype F sequence from Romania (37). A broad spectrum of tetrameric amino acid sequences was observed at the crown of the V3 loop, including sequences containing

the GPGRTW (99VenM11 and 99VenC28) and GPG-GAF (99VenC47) motives for subtype B. The GPGR motif was also verified in the subtype F sequence from Venezuela, as described for other South American subtype F samples (37), distinguishing them from the Romanian subtype F viruses.

## DISCUSSION

Based on *env* and *pol* (PR and RT) HMA and nucleotide sequence analyses, respectively, this study identifies the presence of a new HIV-1 B/F recombinant virus in Venezuela (isolate 99VenC13). This sample was obtained from a gay man with advanced immunosuppression who was presumably infected between 1994 and 1996 when traveling abroad. Another B/F recombinant virus was previously described in Venezuela (26), but in that case, the *env* and RT regions were subtyped as B, while the PR was F, thus corresponding to an *Fpro/Brt/Benv* mosaic genome. In the current study, however, sample 99VenC13 clustered with subtype F reference samples in both *env* and PR regions and with the CRF\_12 B/F references (ARMA 159 and ARMA 185) in the RT, corresponding to an *Fpro/B/Frt/Fenv* recombinant genome. This particular observation could be linked to the increasing description of recombinant viruses and CRFs worldwide (3), including the recent description of CRF\_12 B/F and other B/F recombinant viruses in some South American countries (5,15,36,38). The relevant differences between continental and islander's samples suggest HIV-1 variability in Venezuela should be further explored. Recent studies carried out in Argentina (39) show a wide circulation of B/F recombinant strains among different exposure categories in Buenos Aires. The signature on the top of the V3 loop of 99VenC13 (GPGRVV), associated with its clustering with sub-subtype F1 reference samples in the phylogenetic analysis, allows us to consider this subtype F strain in the F1 subgroup (40), typically observed in South American subtype F strains (4,9).

The presence of mosaic genomes highlights the need to improve subtyping protocols with the molecular analysis of at least two distinct viral regions to identify the circulation of recombinant forms and dual infection

**FIG. 2.** Phylogenetic analysis of Venezuelan HIV-1 subtypes. **(A)** C2-V3 *env* sequences were compared with reference sequences of the HIV-1 group M subtypes available in the Los Alamos database, HIV-1 B and F samples from South America countries (13,14,17,18), and CRF\_12 B/F representative samples (36). **(B)** Protease and **(C)** reverse transcriptase sequences of sample 99VenC13 were respectively compared with reference sequences of the HIV-1 group M subtypes available in the Los Alamos database and CRF\_12 B/F representative samples (36). Sequence CPZgab was used as outgroup. Aligned fragments of 420bp (C2-V3), 300bp (protease), and 500bp (reverse transcriptase) were analyzed as described in the text, and bootstrap values for 100 replicates are listed at the major subtype branches. Venezuelan samples sequenced in this study are italicized in bold. GenBank accession numbers are specified in the Material and Methods section.



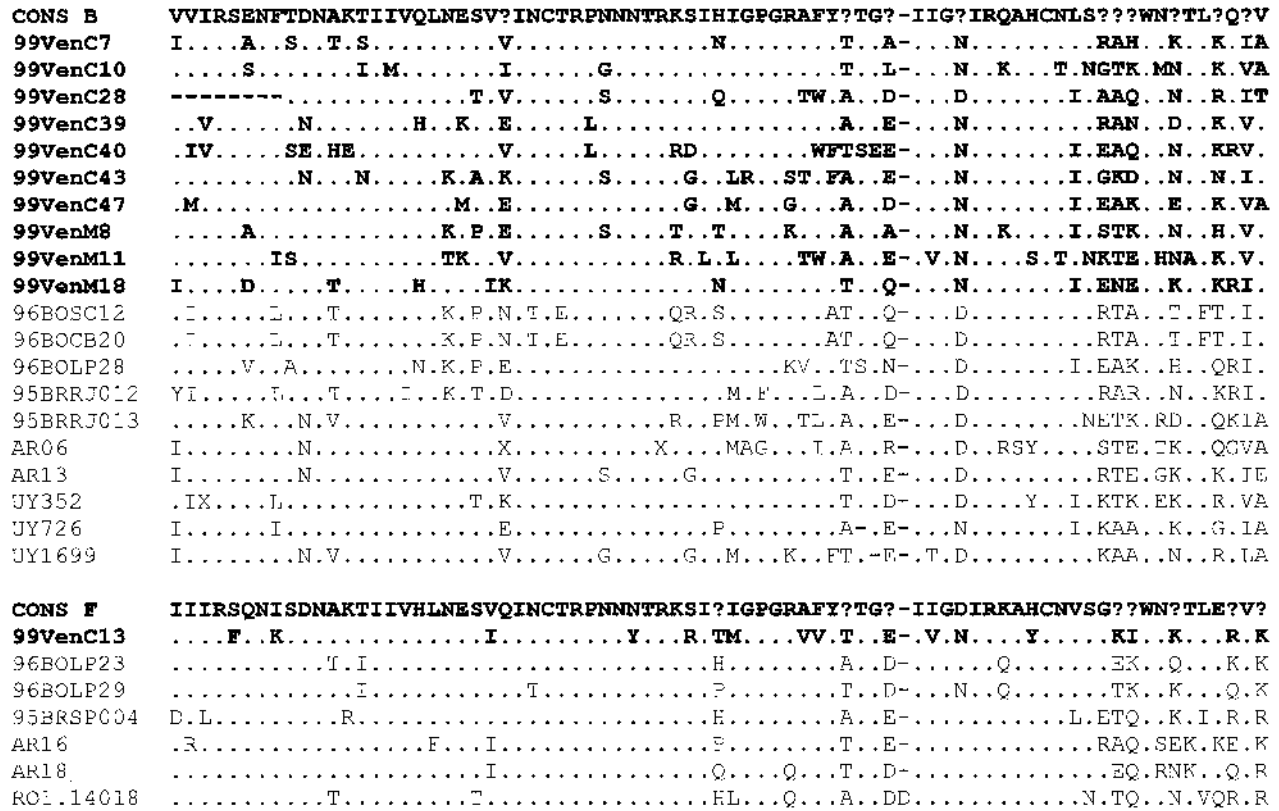


FIG. 3. Alignment of deduced C2-V3 env region amino acid sequences obtained from Venezuelan HIV-1 strains compared with the subtype B and F consensus sequences and individual reference samples from Argentina (14), Bolivia (18), Brazil (13), Uruguay (17), and Romania (37). Dots indicate identity with the consensus sequence, and dashes indicate gaps introduced to optimize the sequence alignment.

with different subtypes (41). For this purpose, an HMA env/gag protocol has been recently developed (42). In addition, today’s effort on HIV pol genotyping as a tool to document HIV-1 strains resistant to antiretroviral drugs has lead to a valuable source of data for phylogenetic analysis and subtyping worldwide (26,43).

In the B subtype samples, we found motifs GPGGAF and GPGRTW, which were never described before among Venezuelan HIV-1 B subtypes (24,25), probably reflecting the evolving molecular epidemiology of HIV-1 in Venezuela. Nevertheless, motif GPGGAF (99VenM11 and 99VenC47) in the top of the V3 loop was previously described in subtype B strains circulating in Brazil (4). Deserving special attention is the finding of the signature GPGRTW (99VenC28), which, to the best of our knowledge, was never described before for subtype B strains circulating in Latin American countries. The Venezuelan B subtypes cluster within different geographical HIV-1 subtype B reference samples, pointing to an important diversity, especially considering the small number of B strains analyzed in this study. Alignment of the C2-V3 region with Venezuelan reference subtype B samples previously described (24,25) was not

possible because of the difference of the fragment region evaluated in such samples, which provided a very small alignment extension when tested.

The study highlights the pressing need to comprehensively monitor HIV-1 molecular epidemiology in a geographic region in which the HIV/AIDS subepidemic is still rapidly spreading and becoming more complex and interactive, combining South America continental characteristics with patterns closely related to the Caribbean subepidemics.

**Acknowledgments:** The authors thank Natalia Sandoval from the Anticancer Society Laboratory at Margarita Island for her important support. The authors also thank Hector Bárcenas (“AIDS for AIDS”) for his invaluable help with the volunteers from Caracas.

REFERENCES

1. Korber B, Kuiken C, Foley B, et al. *Human retroviruses and AIDS. Theoretical biology and biophysics.* Los Alamos, NM: Los Alamos National Laboratory, 1998.
2. Robertson DL, Anderson JP, Bradac JA, et al. HIV-1 nomenclature proposal. *Science* 2000;288:55-6.
3. HIV Sequence Database. <http://hiv-web.lanl.gov/CRFs/CRFs.html>.
4. Morgado MG, Sabino ES, Shpaer EG, et al. V3 region polymorphism in HIV-1 from Brazil: prevalence of subtype B strains di-

- vergent from North American/European prototype and detection of subtype F. *AIDS Res Hum Retroviruses* 1994;10:569–76.
5. Sabino E, Shpaer EG, Morgado MG, et al. Identification of human immunodeficiency virus type 1 envelope genes recombinant between subtypes B and F in two epidemiologically linked individuals from Brazil. *J Virol* 1994;68:6340–6.
  6. Da Costa S, Schechter M, Shindo N, et al. Sequence and phylogenetic analysis of glycoprotein 120 of an HIV type 1 variant (GWGR) prevalent in Brazil. *AIDS Res Hum Retroviruses* 1995;11:1243–5.
  7. Pinto M, Tanuri A, Schechter M. Frequency of subtypes of HIV-1 in participants of a cohort study in Rio de Janeiro, Brazil, as suggested by endonuclease digestion patterns of the protease gene [abstract 1:133]. Presented at the XI International Conference on AIDS, Vancouver, 1996.
  8. Sabino E, Diaz R, Brigido R, et al. Distribution of HIV-1 subtypes seen in an AIDS clinic in São Paulo City, Brazil. *AIDS* 1996;10:1579–84.
  9. Galvão-Castro B, Couto-Fernández JC, Mello MA, et al. The Brazilian network for the HIV-1 isolation and characterization. A nationwide effort to systematically monitor HIV-1 diversity in Brazil: preliminary results. *Mem Inst Oswaldo Cruz* 1996;91:335–8.
  10. Morgado MG, Guimarães ML, Gripp CBG, et al. and The Hospital Evandro Chagas AIDS Clinical Research Group. Molecular epidemiology of HIV-1 in Brazil: high prevalence of HIV-1 subtype B and identification of an HIV-1 subtype D infection in the city of Rio de Janeiro, Brazil. *J Acquir Immune Defic Syndr* 1998;18:488–94.
  11. Morgado MG, Guimarães ML, Neves I Jr, et al. and The Hospital Evandro Chagas AIDS Clinical Research Group. Molecular epidemiology of HIV in Brazil: polymorphism of the antigenically distinct HIV-1 B subtype strains. *Mem Inst Oswaldo Cruz* 1998;93:383–6.
  12. Tanuri A, Vicente AC, Ostuki K, et al. Genetic variation and susceptibilities to protease inhibitors among subtype B and F isolates in Brazil. *Antimicrob Agents Chemother* 1999;43:253–8.
  13. Brazilian Network for the HIV-1 Isolation and Characterization. HIV-1 diversity in Brazil: genetic, biological and immunological characterization of HIV-1 strains in three potential HIV vaccine evaluation sites. *J Acquir Immune Defic Syndr* 2000;23:184–93.
  14. Campodonico M, Janssens W, Heyndrickx L, et al. HIV type 1 subtypes in Argentina and genetic heterogeneity of the V3 region. *AIDS Res Hum Retroviruses* 1996;12:79–81.
  15. Marquina S, Leitner TH, Rabinovich RD, et al. Coexistence of subtypes B, F, and B/F *env* recombinant of HIV type 1 in Buenos Aires, Argentina. *AIDS Res Hum Retroviruses* 1996;12:1651–4.
  16. Arsteinen AW, Coppola J, Brown A, et al. Multiple introductions of HIV-1 subtype E into the western hemisphere. *Lancet* 1995;346:1197–8.
  17. Fernández RD, Jansson M, Halapi E, et al. Genetic analysis of V3 domain sequences obtained from Uruguayan HIV type 1-infected individuals. *AIDS Res Hum Retroviruses* 1996;12:1491–3.
  18. Velarde-Dunois KG, Guimarães ML, La Fuente C, et al. Molecular characterization of human immunodeficiency virus type-1 infected individuals from Bolivia reveals the presence of two distinct genetic subtypes B and F. *AIDS Res Hum Retroviruses* 2000;16:1921–6.
  19. Navas MC, Letourneur F, Gomas E, et al. Analysis of the V3 loop sequences from HIV type-1 infected patients from Colombia, South America. *AIDS Res Hum Retroviruses* 1999;15:1141–4.
  20. Cabello A, Cabral M, Vera ME, et al. Analysis of V3 loop sequences from 10 HIV type 1-infected AIDS patients from Paraguay. *AIDS Res Hum Retroviruses* 1995;11:1135–7.
  21. Lara C, Sallberg M, Johansson B, et al. The Honduran human immunodeficiency virus type 1 (HIV-1) epidemic is dominated by HIV-1 subtype B as determined by V3 domain sero- and genotyping. *J Clin Microbiol* 1997;35:783–4.
  22. Rivera L, Rodríguez C, Novitsky V, et al. HIV-1 subtype B is a predominant virus of AIDS epidemic in Mexico [abstract 19]. Presented at the International Symposium on HIV, Leukemia and Opportunistic Cancers, Marrakech, May 23–28, 1999.
  23. Desgranges C, Fillon S, Letourneur F, et al. HIV-1 subtypes in Santiago, Chile. *AIDS* 1998;12:1563–5.
  24. Quiñones-Mateu ME, Dopazo J, Esté JA, et al. Molecular characterization of human immunodeficiency virus type 1 isolates from Venezuela. *AIDS Res Hum Retroviruses* 1995;11:605–16.
  25. Quiñones-Mateu ME, Esteban D. Nucleotide diversity in three different genomic regions of Venezuelan HIV type 1 isolates: a subtyping update. *AIDS Res Hum Retroviruses* 1999;15:73–9.
  26. Delgado E, León-Ponte M, Villahermosa ML, et al. Analysis of HIV type 1 protease and reverse transcriptase sequences from Venezuela for drug resistance-associated mutations and subtype classification: a UNAIDS study. *AIDS Res Hum Retroviruses* 2001;17:735–58.
  27. Centro de investigaciones Económicas y Sociales, ONUSIDA, PNUD & UNICEF. *El VIH-SIDA en Venezuela: análisis de situación y recomendaciones*. Caracas: 1998:1–149.
  28. Delwart E, Shpaer EG, McCutchan FE, et al. Genetic relationships determined by a DNA heteroduplex mobility assay. *Science* 1993;262:1257–61.
  29. Bachmann MH, Deltwart E, Shpaer EG, et al. and WHO Network for HIV Isolation and Characterization. Rapid genetic characterization of HIV type 1 strains from four World Health Organization-sponsored vaccine evaluation sites using a heteroduplex mobility assay. *AIDS Res Hum Retroviruses* 1994;10:1345–53.
  30. Zazzi M, Romano L, Brasini A, et al. Simultaneous amplification of multiple HIV-1 *DNA Seqs* from clinical specimens by using nested-primer polymerase chain reaction. *AIDS Res Hum Retroviruses* 1993;9:315–20.
  31. Delwart EL, Herring B, Learn GH Jr, et al. *Heteroduplex mobility analysis HIV-1 env subtyping kit, protocol version 3*. Rockville, MD: NIH AIDS Research and Reference Program, 1995.
  32. Brindeiro R, Vanderborcht B, Caride E, et al. Sequence diversity of the reverse transcriptase of human immunodeficiency virus type 1 from untreated Brazilian individuals. *Antimicrob Agents Chemother* 1999;43:1674–80.
  33. Thompson JD, Higgins D, Gibson T. CLUSTAL W—improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22:4673–80.
  34. Page RD. TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 1996;12:357–8.
  35. Kumar S, Tamura K, Jakobsen IB, et al. Molecular evolutionary genetics analysis, The Pennsylvania State University, PA: 2001. MEGA version 2.0.
  36. Carr JK, Avila M, Carrillo MG, et al. Diverse BF recombinants have spread widely since the introduction of HIV-1 into South America. *AIDS* 2001;15:F41–7.
  37. Banda CL, Ramos A, Pieniazek D, et al. Epidemiological and evolutionary relationship between Romanian and Brazilian HIV-1 subtypes F strains. *Emerg Infect Dis* 1995;1:91–3.
  38. Vicente ACP, Otsuki K, Silva NB, et al. The HIV epidemic in the Amazon basin is driven by prototypic and recombinant HIV-1 subtypes B and F. *J Acquir Immune Defic Syndr* 2000;23:327–31.
  39. Thomson MM, Villahermosa ML, Vazquez E, et al. A B/F intersubtype-recombinant HIV-1 strain with recombination in Pol is widely circulating in Buenos Aires, Argentina [abstract 176]. Presented at the 7th Retrovirus Conference, San Francisco, January 2000.
  40. Triques K, Bourgeois A, Saragosti S, et al. High diversity of HIV-1 subtype F strains in Central Africa. *Virology* 1999;259:99–109.
  41. Janini LM, Pieniazek NJ, Peralta JM, et al. Identification of single and dual infections with distinct subtypes of human immunodeficiency virus type 1 by using restriction fragment length polymorphism analysis. *Virus Genes* 1996;13:69–81.
  42. Heyndrickx L, Janssens W, Zekeng L, et al. and Study Group on Heterogeneity of HIV Epidemics in African Cities. Simplified strategy for detection of recombinant human immunodeficiency virus type 1 group M isolates by *gag/env* heteroduplex mobility assay. *J Virol* 2000;74:363–70.
  43. Descamps D, Calvez V, Izopet J, et al. and ANRS Antiretroviral Resistance Study Group. Prevalence of resistance mutations in antiretroviral-naïve chronically HIV-infected patients in 1998: a French nationwide study. *AIDS* 2001;15:1777–82.