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Surveillance of HIV-1 Drug Resistance Among Naive Patients from Venezuela

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Abstract

We have studied 65 HIV-1 infected untreated patients recruited in Caracas, Venezuela with TCD4 counts ≥350/uL. The reverse transcriptase and protease sequences of the virus were sequenced, aligned with reference HIV-1 group M strains and analyzed for drug resistance mutations. Most of the viruses were subtype B genotype in both protease and RT genomic regions. Five of the 62 virus isolates successfully amplified showed evidence of recombination between protease and RT, with their protease region being non-B while their RT region was derived from subtype B. Four strains were found bearing resistance mutations either to NRTIs, NNRTIs or PIs. The prevalence of HIV-1 isolates bearing resistance mutations was therefore above the 5% threshold of WHO.

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Introduction

Highly active antiretroviral therapy (HAART) has resulted in a dramatic decrease in AIDS-related morbidity and mortality in developed countries. However, the emergence of HIV-1 drug resistance is a major concern for HAART efficacy [1,2]. Moreover, HIV-1 drug resistant variants can be readily transmitted, which participates to the expansion of drug resistance. Previous reports of the molecular epidemiology of HIV-1 in Venezuela have shown a predominant prevalence of clade B and isolated reports of subtype C, B/C recombinant and two CRF12_BF. [3-7]

HAART regimens have been adopted by the AIDS National Program in Venezuela since 1999. By the end of 2005, an approximate number of 15 000 patients were receiving HAART in Venezuela, which represented 84% of adults in need of therapy according to the program guidelines.[8] The available HIV drug resistance reports from the country show that a 3-fold increase of resistant viruses to RTIs, representing now 10% of the clinical samples, has been observed between 2001 and 2007.[3,7] Certainly this increase implies a significative rate of HIV drug resistance transmission . The present study aimed at monitoring the prevalence of genotypic drug resistance in a sample of asymptomatic untreated patients recruited at an outpatient network in Caracas according to the WHO survey threshold method recommendations. [9]

Materials and methods

All individuals enrolled in the study signed the informed consent and apply to the following criteria : adults with a positive HIV ELISA within the last 3 years verified through Western Blot, naive to antiretroviral treatment and with a TCD4 lymphocyte count ≥ 350 cells/uL. Blood was collected in ethylenediaminetetracetic acid (EDTA) tubes and plasma was separated and stored at -80°C. Plasmas were thawed, homogenized and applied to spots of Schleicher and Schuell 903 (now Whatman 903) filter paper cards with 5 spots of 50 ul of plasma. The samples were allowed to dry at room temperature for one hour in vertical position and placed in plastic bags with dessicants. They were stored at 4°C before being carried over at room temperature to Bordeaux, France during a 12-15 hour journey. The spots were cut with scissors and eluted in 220 ul /spot of a buffer containing PBS, Tween 20 and fetal calf serum as previously described.[10] The samples were then agitated at 4°C during one hour before being vortexed and 140 ul of the final elution were used for RNA extraction using QIAamp Viral RNA Mini Kit (Qiagen). The RNA was used in reverse transcription polymerase chain reaction (RT PCR) of reverse transcriptase (RT) and protease (Prot) respectively, using two sets of primers in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) thermal cycler. The outer and inner primers for RT and Prot are described in our previous publications.[10,11] The obtained fragments were sequenced

on both strands using the CEQ DTCS Quick Start kit on an automated sequencer Beckman CEQ 2000 DNA Analyser System in the Virology department of the University Hospital of Bordeaux as previously described[10,11]. The derived nucleotide sequences of the RT and Prot regions were aligned by the Clustal W 1.74 alignment program with reference sequences of HIV-1 M, N and O groups (plus SIV-CPZ for the Protaese tree) from the HIV Database Subtype Reference Alignment **(http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html).** Phylogenetic trees were inferred using the neighbor-joining method from matrix distances calculated after gapstipping of aligments, with a Kimura two-parameter algorithm. The mutations involved in antiretroviral resistance were recorded according to the algorithm of the French Agence Nationale de Recherche sur le SIDA (ANRS) (http://hivfrenchresistance.org). GenBank accession numbers for the sequences reported in this study are GQ202842 to GQ202974.

Results

Seventy HIV-1 infected naive outpatients were recruited in Caracas ; five patients were excluded from the study (2 with no results of TCD4 counts, 2 with TCD4 counts below 350/uL and one lost to follow up) ; the results were therefore valid for 65 patients. The studied population was as follows : 51 men (78.46%), 14 women (21.54%), 18-58 (median 32.57) years old, TCD4 range from 350 to 1320 (average 551, median 531) cells/uL, viral loads ranging from 763 to 500.000 (average 53 164, median 19 337) RNA copies/mL and an estimated first positive HIV ELISA ranging from 0 to 132 months (average 14.86, median six months).

Both Prot and RT were successfully amplified for 62 HIV-1 isolates of the 65 patients remaining in the study. For subtyping purposes only, nucleotide sequences of RT or Prot fragments from the 3 other viral isolates were also considered in the phylogenetic analysis. All analysed RT sequences clustered with subtype B (Figure 1). All but 5 Prot sequences were of subtype B ; the 5 non-B Prot sequences were assigned as B/F1 recombinant (two sequences), CRF06_cpx (2) and CRF01_AE-like recombinant (1) (Figure 2). As CRF06_cpx is rarely reported elsewhere than in Africa, we evaluated if the two identified strains could have a common ancestor and/or a particular recombination pattern. However, no unexpected breakpoints were seen when scanning both protease sequences and the highest blast scores corresponded to different CRF06_cpx reference sequences (data not shown). We then reanalysed both RT subtype B fragment in the context of previously known B strain sequences from Venezuela and found that they fall in distinct clusters of a maximum likelihood tree (data not shown). Thereby the two B/CRF06_cpx genomes were proven to be unlinked isolates which is also consistent with patient's records of unrelated infections. More extensive data would be necessary to classify these mosaic genomes. However, we can

conclude that the variability of HIV-1 in Venezuela is increasing together with recombination of different strains within a background of subtype B.

Analysis of the RT sequences exhibited polymorphic substitutions at positions which, according to the ANRS algorithm, are not associated with resistance (E122, D123, I135, K173, T200, E204, Q207, R211, V245) ; amino acid substitutions at positions of resistance were quite rare ; more interestingly, 2 resistance mutations to NNRTIs (K101E and K103N) were noted in separate isolates of subtype B while a third subtype B was found bearing two resistance mutations to NRTIs (D67N and K219Q) (Table 1).

Analysis of Prot sequences revealed frequent polymorphic amino acid substitutions at some positions of secondary resistance (M36, L63, V77) ; only one isolate (which did not bear resistance mutations to NRTIs and NNRTIs) exhibited major resistance mutations to PIs (L33F, M46I, V82T, I84V and L90M) (Table 1).

Discussion

Most of the HIV-1 isolates are of subtype B ; these results are consistent with previous data from the country showing that B viruses are largely predominant but novel recombinant forms to the local epidemic which need more extensive characterization, have been observed .

Some studies of resistance mutations have been done in Venezuela using proviral DNA from both treated and untreated patients but all of them included a low number of samples from untreated individuals and the results must be considered qualitative and not quantitative even if the authors gave percentages of prevalence of isolates with drug resistance mutations . Delgado et al[3] estimated to 3% RT mutations in untreated patients and reported a case of resistance to PIs . Dieudonne et al[6] highlighted the frequency of polymorphism at positions of seconday resistance in Prot of both treated and untreated patients ; they found resistance mutations to NRTIs and NNRTIs in one untreated individual of their study (7.7%). Bouchard et al[7] within our collaborative group confirmed the polymorphism of Prot particularly at position 77 and estimated to 10% the percentage of isolates with resistance mutations to NRTIs and NNRTIs.

The study here exhibits some differences : 1 It has been done using plasma samples when those cited above have been carried out on PBMCs and proviral DNA (it must be pointed out that either compartment seems suitable for the detection of mutations)[12,13] 2 It has been undertaken following the WHO criteria and the results should be considered more reliable. When we studied the RT and Prot sequences, we observed polymorphic substitutions particularly in the Prot region as already reported[6,7] . Four isolates (all of B subtype) exhibited drug resistance mutations : 2 to NNRTIs, one to NRTIs and one to PIs . Overall, these data show that the prevalence of HIV-1 isolates bearing resistance mutations to the drug classes

used in the country, is above the threshold of 5% and that a longitudinal survey of transmitted resistance will be necessary in a next future.

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Molecular characterization of the 62 isolates studied	RT : B subtype Prot : B subtype Total: 57	RT : B subtype Prot : B/F1 recombinant(1) URF_BF (1) CRF06_cpx(2) CRF01_AE(1) Total: 5
Drug resistance mutations	4 different isolates RT K101E (1) RT K103N (1) RT D67N+K219Q (1) Prot L33F+M46I+V82T+I84V+L90M (1)	

Table 1 . Molecular characterization of the HIV-1 isolates of the study with identified drug resistance mutations . Reverse transcriptase : RT ; protease : Prot

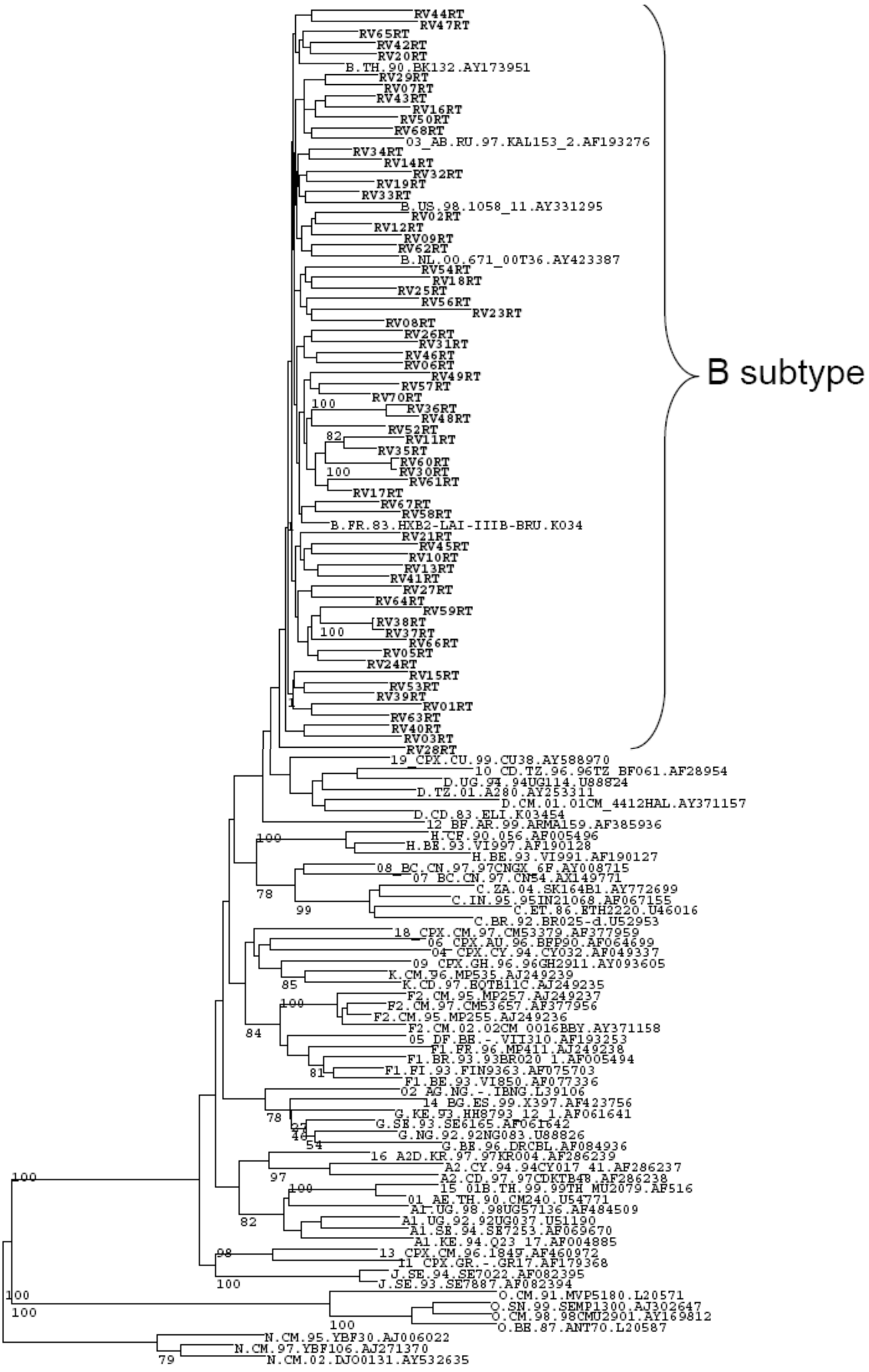

Figure 1: Neighbor joining tree of RT sequences. Subtype reference strains were included and N strain was used as root.

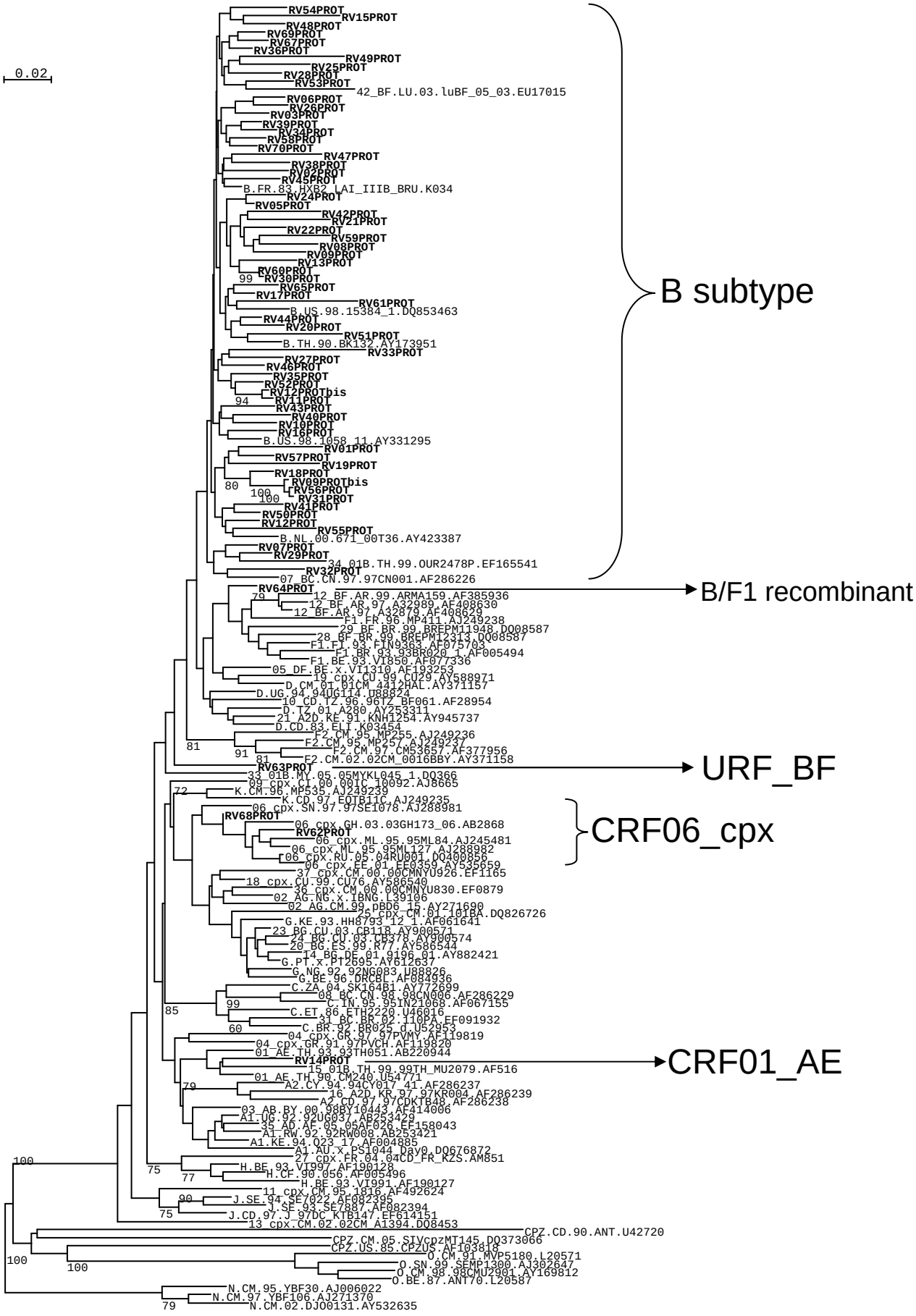

Figure 2: Neighbor joining tree of PROT sequences. Subtype reference strains were included and N strain was used as root.