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Sequence Note

HIV Type 1 Drug Resistance among Naive Patients from Venezuela

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ABSTRACT

In this study, we characterize proviral DNA of 20 HIV-1 asymptomatic antiretroviral-naive patients from Venezuela in *env*, *gag*, and *pol* genes regions. Results from both *env/gag* HMA subtyping and phylogenetic analysis of *pol* partial sequences led to the description of clade B in all cases. Nevertheless, the high prevalence of polymorphisms was particularly evident among the protease sequences. A 10% prevalence of major resistance mutations to RTIs was found. Our data also suggested that the protease polymorphisms I62T and V77T could be considered as molecular markers of the subtype B local epidemic. In addition, we show how proviral DNA can be used as a reliable tool to follow trends of resistance mutation transmission.

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] Moreover, HIV-1 drug-resistant variants can be readily transmitted, which is a factor in the spread of drug resistance.

HAART regimens have been adopted by the AIDS National Program in Venezuela since 1999. At present, approximately 10,000 adults are receiving treatment in Venezuela,[2] which represents 80% of adults in need of therapy according to the program guidelines. Therefore the present study monitors the prevalence of genotypic drug resistance and HIV-1 variability among asymptomatic-naive patients recruited at an outpatient network in Caracas. Surveillance of HIV-1 antiretroviral resistant mutations has been consistently based on genotyping from plasma RNA since this virus source is supposed to represent the currently circulating virus more accurately than proviral DNA. Nevertheless, the high correspondence among these compartments within treated patients has been shown.[3] And more recently, peripheral blood mononuclear cells have been documented as a source of archival proviral DNA and a reliable predictor of drug resistance.[4] We have therefore chosen to analyze proviral DNA.

Individuals enrolled in the study signed the informed consent and meet one of the following criteria: positive ELISA within the past 3 years verified through Western blot or HIV-positive serologic test plus a $CD4^+$ lymphocyte count $\geq$450 cells/mm^3.

Proviral DNA was extracted from EDTA blood samples with the Qiagen DNA extraction kit (QIAGEN GmbH, Holden, Germany). A nested polymerase chain reaction (PCR) for *gag* (460 bp) and *env* (600 bp) heteroduplex mobility assay (HMA) subtyping was carried out as previously described.[5,6] Additionally, reverse transcriptase (RT) and protease (Pr) *pol* gene regions were amplified and sequenced with primers described elsewhere.[7] Pol amplified products were purified using the Concert Nucleic Acid Purification System kit (Life Technology, Gibco BRL, Germany), and then submitted to a sequencing facility (Cesaan/IVIC, Altos de Pipe, Venezuela), using a Big Dye Terminator kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 377 sequencer. The PCR products were sequenced in both senses with sequencing primers A(35)–NEI(35) for RT and 5′prot 2–3′ prot 2 or 3′ prB–5′ prB for the Pr region.[7]

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Sequence edition was performed manually with the BioEdit software (Sequence Alignment Editor, version 5.0.9). Additionally, for genotypic interpretation and subtyping of isolates, edited sequences were submitted to the HIV resistance mutation database of Stanford University.[8]

Sequences alignments were performed with Clustal W 1.7.[9] Phylogenetic trees were constructed with the neighbor-joining method and the reliability of the branching orders was determined by 1000 times bootstrap.

Twenty HIV-1-infected naive outpatients (women = 3; men = 17) coming from Caracas and the central area of the country were studied. The average $CD4^+$ count was 772 ± 388 cells/mm^3 and the time since first positive ELISA was 2 ± 2.5 years.

All isolates succeeded in the amplification the of *env*, *gag*, and RT regions. In the Pr region 3 of 20 isolates (15%) failed all attempts of amplification.

According to HMA results (not shown) and phylogenetic trees (Fig. 1), all viruses were shown to cluster with HIV-1 subtype B. However, high bootstrap values were observed for Ven-N24 and Ven-N6 with CRF07_BC reference sequences in the protease tree as well as for Ven-N10 with CRF03_AB reference sequences in the reverse transcriptase tree. Therefore this set of sequences was also submitted to the NIH-NCBI genotyping tool,[10] which confirmed subtype B in the three cases throughout their entire length. Indeed, subtype B has been consistently reported to be the principal HIV-1 strain circulating in Venezuela.[11–14] Both Ven-N10 and N24 sequences correspond to viral strains of unrelated patients. The higher divergence of sequences from patients Ven-N10 and Ven-N24 could be due to a longer time of infection (~9 years since the first positive serology) as compared to other patients diagnosed less than 3 years ago and with viral loads above 7000 copies/ml. In other

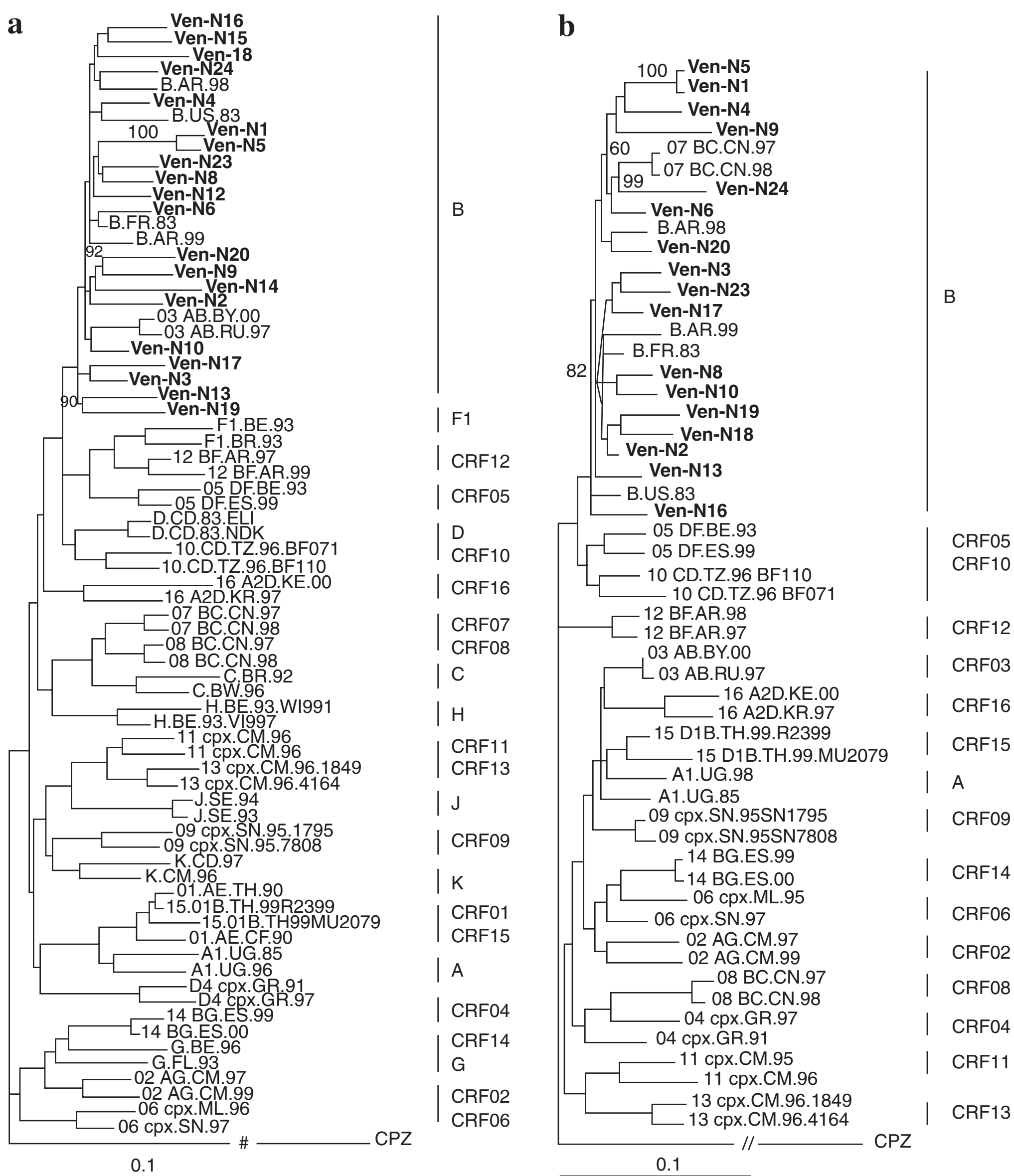

FIG. 1. (**a**) Reverse transcriptase tree (538 bp alignment relative to HXB2). (**b**) Protease tree (334 alignment relative to HXB2).

Table I. Frequency of Mutations per Codon Position in the Reverse Transcriptase and Protease Region of HIV-1 Isolates from the Study

Codon position and amino acid substitution	*Frequency*	*%*
Reverse transcriptase ($n = 20$)		
K101Q/R	3	15
V108I	1	5
Y188F	1	5
G190D	1	5
K219Q	1	5
P225S	1	5
F227I	1	5
Protease ($n = 17$)		
L10I	2	11.7
E35D	5	29.4
M36I	1	5.8
R41K	6	35.2
I62V/T	3	17.6
L63H/P/Q/T/S	15	88.2
V77I/T	3	17.6

words, we could be looking at viruses representing different chronological moments of the local epidemic.

High bootstrap values (100%) among sequences Ven-N1 and Ven-N5 were verified in both trees and therefore confirmed a related source of rural infection within this couple.

Drug resistance mutations were identified only in RT: one nucleoside RT inhibitor mutation (K219Q) belonging to the mutations selected by the thymidine analogs, and one nonnucleoside RT inhibitor mutation (V108I) in two different patients. The global prevalence of drug-resistant viruses was thus 10% (2 of 20 patients). Additionally, atypical RT mutation Y188F was present.

The 10% prevalence of resistant viruses represents more than 3-fold the proportion previously found by Delgado *et al*. in 2001 among Venezuelan naive patients.[11] Mutations Y188F and K219R were also found in RT sequences from a naïve patient from Venezuela harboring a recombinant virus *Cgag/Brt/Cenv* reported by our group.[14]

Together these findings suggest transmission of HIV-1 resistance to RT inhibitors in the local epidemic. Given the small number of patients included in our study, we must, however, be cautious in evaluating any dynamic trend concerning this transmission.

Overall, when comparing the frequency of mutations and polymorphisms per codon position at the RT vs. Pr region (Table 1), we find that the frequency of mutations is clearly higher in the protease region, as already described.[15]

A rare polymorphism, I62T (Table 1) was found as a single mutation in isolate 10. Therefore we sequenced the same time point RNA and found two other nucleotide substitutions (L19I and K45R) but not I62T. Polymorphism I62T was first reported in 2001 in a naive patient from Venezuela.[11]

Additionally, rare mutations V77T to PIs and F227I to RTIs were identified in two different patients (Table 1). Both mutations have been previously reported among patients receiving antiretroviral therapy (ART) and harboring either subtype B infections in the case of V77T[16,17] or CRF02-AG infection for F2271 carriers.[18,19]

The V77T mutation is an uncommon substitution, particularly among naive patients. When sequencing RNA quasispecies at the same time point, V77T was found again, emphasizing the stability of this mutation across the different viral compartments at a set point.

This study shows a high rate of RTIs in an HIV-infected sample from Venezuela. It also highlights the role of mutations I62T and V77T as putative molecular markers of the subtype B local epidemic. Finally, it shows how proviral DNA can be used as a reliable tool to follow trends in the transmission of resistant mutations.

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