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.
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 \pm 388 cells/mm\(^3\) and the time since first positive ELISA was 2 \pm 2.5 years.

All isolates succeeded in the amplification 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 (\approx 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

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**FIG. 1.** (a) Reverse transcriptase tree (538 bp alignment relative to HXB2). (b) Protease tree (334 alignment relative to HXB2).
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. Mutations Y188F and K219R were also found in RT sequences from a naïve patient reported by our group.14

A rare polymorphism, I62T (Table 1) was found as a single point RNA and found two other nucleotide substitutions (L19I and K45R) but not I62T. Polymorphism I62T was first reported in 2001 in a naïve patient 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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REFERENCES


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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