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Genetic Diversity on the Integrase Region of the *pol* Gene among HIV Type 1-Infected Patients Naive for Integrase Inhibitors in São Paulo City, Brazil

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Abstract

The presence of mutations associated with integrase inhibitor (INI) resistance among INI-naive patients may play an important clinical role in the use of those drugs Samples from 76 HIV-1-infected subjects naive to INIs were submitted to direct sequencing. No differences were found between naive (25%) subjects and subjects on HAART (75%). No primary mutation associated with raltegravir or elvitegravir resistance was found. However, 78% of sequences showed at least one accessory mutation associated with resistance. The analysis of the 76 IN sequences showed a high polymorphic level on this region among Brazilian HIV-1-infected subjects, including a high prevalence of aa substitutions related to INI resistance. The impact of these findings remains unclear and further studies are necessary to address these questions.

The integration of viral cDNA into host chromosomes is promoted by the integrase (IN) viral enzyme and occurs in multisteps. The IN enzyme (32 kDa) is encoded by the *pol* gene and is composed of 288 amino acids (aa). The amino terminal domain (NTD) encompasses the aa 1 to 49 and contains the HHCC motif (Hys_{12}, Hys_{16}, Cys_{40}, Cys_{43}), a zinc finger that is important for catalytic function. The catalytic core domain (CCD) encompassing aa 50–212 carries the DDE motif (Asp_{64}, Asp_{116}, Glu_{152}), which coordinates the bivalent metallic cofactor (Mg_2), a requisite for IN catalytic activity. The carboxy-terminal domain (CTD) encompasses aa 213–288, binds nonspecifically to DNA, and is involved in the stability of the enzyme with DNA.[1,2]

The integrase inhibitors are a new class of antiretroviral drugs that act to prevent viral DNA integration into the host chromosome. Raltegravir (RAL) (*Isentress*, Merck, Germany), the first molecule approved for clinical use, in 2007, is a selective strand transfer inhibitor (STI) that acts by occupying the position of acceptor DNA. The elvitegravir (EGV) molecule, another STI, is currently in phase III clinical trials.[3,4] The STIs bind at the interface of the viral DNA-IN-divalent metal complex, preventing IN from binding target chromosomal DNA, and the strand transfer reaction is unable to proceed.[4]

More than 40 amino acid substitutions have been associated with resistance to RAL or EGV.[2] The RAL resistance develops by three distinct pathways: N115H/S, Q148K/R/H, or Y143R/C. The secondary mutations associated with the resistance pathways are L74M, E92Q, T97A, E138A/K, G140S, V151I, E157Q, G163KR, and D232N. The antiviral activity of EGV can be affected by the presence of mutations T66I/A/K, V72I, F12Y, T125K, G140C, Q148H, V151I, S153Y, M154I, and S230R.[5] Therefore, our study aimed to determine the prevalence of mutations or polymorphisms in the IN region that may impact the clinical use of INIs in a cohort in São Paulo City.

Seventy-six HIV-1-infected subjects, under clinical monitoring in our HIV out-patient clinic (ADEE3002/HCFMUSP) from a general University Hospital in São Paulo City, were randomly invited to participate in our study, from June to September 2008. The study was approved by the Ethical Research Board of the Hospital das Clínicas, Faculdade de

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Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples by Ficoll-Hypaque gradient density centrifugation. DNA was extracted from the PBMCs using the GFX Genomic Blood DNA Purification Kit (GE Healthcare, Little Chalfont, UK). Nested polymerase chain reactions (PCR) were amplified using the outer primers IN12 and IN13[6] or the alternative primers MW1 and MW2,[7] and the inner primers IN1[6] and INR[1] to the proximal region and to the distal region of the IN enzyme[6] were used (Table 1).

The second round PCR products were purified by the QIAquick Purification Kit (Qiagen, Hilden, Germany). The sequencing reaction was carried out according to the manufacturer's instructions using the ABI PRISM Big Dye Terminator v. 3.1 Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA), using the inner primers. The sequence reactions were run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequences proximal and distal were superimposed and analyzed using SeqScape v. 2.5.0 (Applied Biosystems, Foster City, CA). The HIV-1 subtypes were determined using the REGA HIV-1 and 2 Automated Sub-typing Tool v. 2.0[8] [http://www.bioafrica.net/rega-genotype/html/subtypinghiv.html]. The analysis of the sequences was performed using softwares BioEdit v. 7.0.8 (Ibis Biosciences, EUA). The mutations are described in the Stanford University HIV Drug Resistance Database[9] for RAL (E92Q, F121Y, E138A/K, G140A/S, Y143R/C/H, S147G, Q148H/R/K, N155H/S, E157Q; nonpolymorphic H183P, Y226D/F/H, S230R, D232N; polymorphic L74M, T97A, V151I, G153R, I203M, S230N), EGV (T66I, E92Q, F121Y, E138A/K, G140A/S, S147G, Q148H/R/K, S153Y, N155H/S, E157Q, R263K; nonpolymorphic H51Y, Q95K, Q146P), and additional mutations nonpolymorphic T125K, A128T, Q146K, N155S, and K160D and polymorphic V72I, A154I, V165I, and V201I (updated February 17, 2009). Statistical analysis was performed by Statistica v. 7.0 (Stat Soft. Inc., Tulsa, OK).

Our study involved 52 men and 24 women whose mean age was 40 years. All subjects were naive to integrase inhibitors; however, 57 (75%) of them were on highly active antiretroviral therapy (HAART) for an average of 7 years (1 month to 14 years) while 19 (25%) remained naive to HAART. The subjects on HAART had a mean T $CD4^+$ count of 517 cells/mm^3, and the naive patients had a mean T CD4 count of 518 cells/mm^3. The mean viral load for HAART patients was 20,751 copies/ml, while that for naive patients was 16581 copies/ml ($p = NS$). These similar laboratory data point to treatment failure among HAART patients. The very different number of patients between groups may also represent an analysis bias.

Analysis of the 76 IN aa sequences (B = 62, F1 = 7, C = 1, BF1 = 4, unknown = 2) revealed that 142 (49.3%) positions were polymorphic, with a mean of 15 (7–40) aa substitutions by sequence, when compared to the consensus B reference obtained in the Los Alamos Database. The NTD showed a degree of conservation of 35% and the CCD and the CTD showed 52% and 59%, respectively. These results agree with previous studies of the B subtype, ranging from 38% to 77% on the whole sequence, whereas NTD, CCD, and CTD were found to be less conserved in our samples than previously found.[1,3,10] There was no difference in conservation between naive and experienced groups (data not shown).

Regarding the resistance mutations, 78% of the sequences showed at least one mutation associated with reduced *in vitro* susceptibility to RAL or EGV; however, no primary mutation was found. The accessory mutation E157Q to EGV resistance was found in a single sequence. The RAL resistance V151I mutation was found in 23.7% of sequences, in addition to L74I (5.3%) and I203M (3.9%). Other aa substitutions associated with INI resistance were found, the most frequent being I72V (60.5), V201I (53.9), and S230N (10.5). Recently a study analyzing Brazilian samples from Rio de Janeiro (430 km from São Paulo) obtained similar results regarding primary and accessory mutations, but the frequency of the aa substitutions and the molecular signatures found suggest that the evolution of the mutation diverged in those two populations.[11]

We did not find primary mutations for INIs, either RAL or EVG, in our patients. This may reflect what happens in the population, since these drugs were not available for clinical use in Brazil until last year. In fact, a low prevalence of such mutations has been described for a larger database.[12] However, we did find high numbers of patients presenting accessory mutations. Actually, several investigators have reported similar results, but there is no consensus as to whether the presence of the secondary mutations, if not associated with primary mutations, can impair the use of INIs in the clinical setting, so the clinical relevance remains unclear.[1,3,10,12–14]

Curiously, three subjects (3.9%) on HAART showed E152K, an important and unexpected aa substitution for enzyme activity. Most previous studies have not described a similar finding[1,5,11]; however, Rhee *et al*. found this same aa substitution at low frequency within sequences of the GenBank database, and suggested that E152 may be particularly prone either to sequencing error or to sequence editing, as it has been demonstrated that mutations at any of the DDE residues abolish all catalytic activities of the protein.[10,15]

Table 1. Primers Used for the Amplification and Sequencing of the HIV-1 IN Region

Name	*Sequence 5′–3′*	*Position on HXB2*
IN12 (outer)	GCAGGATTCGGGATTAGAAG	4007–4026
IN13 (outer)	CTTTCTCCTGTATGCAGACC	5251–5270
MW1 (outer alternative)	CCACARGGATGGAAAGGATCACC	2998–3018
MW2 (outer alternative)	CTGGGGCTTGTTCCATCTRTCYTC	5557–5574
IN1 (inner proximal)	AAGGTCTATCTGGCATGGGTA	4137–4157
INR (inner proximal)	CCATTTGTACTGCTGTCTTAA	4743–4764
BH6 (inner distal)	CCTGGTAGCAGTTCATGTAG	4448–4467
BH4 (inner distal)	TCCCCTAGTGGGATGTGTACTTC	5200–5222

Finally, the study of 76 IN sequences from INI-naive subjects disclosed a high level of polymorphisms in this region among Brazilian HIV-1-infected subjects from São Paulo city. Natural polymorphisms have been found to be involved in INI resistance, but no phenotypic data are currently available regarding the impact on INI resistance level.[14] A high prevalence of accessory mutations and several polymorphisms were so common that we suppose they may not be linked to resistance. Natural polymorphisms associated with resistance to INIs represent an important clinical tool when the use of this antiretroviral class is contemplated, but the impact of the diversity of the integrase region on the clinical setting needs to be studied further. However, a high level of viral suppression using raltegravir, for example, has been found in clinical trials.[4]

Sequence Data

The sequence data obtained in this study are available in GenBank under Accession numbers 6Q864162–6Q864237.

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Disclosure Statement

No competing financial interests exist.

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