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Short communication

Primary HIV-1 drug resistance in the C-terminal domains of viral reverse transcriptase among drug-naïve patients from Southern Brazil

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ABSTRACT

Background: Major and accessory drug resistance mutations have been recently characterized in the C-terminal RT subdomains of HIV-1, connection and RNase H. However, their presence in treatment-naïve patients infected with HIV-1 non-B subtypes remains largely unknown.
Objectives: To characterize the patterns of primary resistance at the C-terminal RT subdomains of HIV-1 infecting subjects in the southern region of Brazil, where HIV-1 subtypes B and C co-circulate.
Study design: Plasma viral RNA was extracted from patients recently diagnosed for HIV infection (2005–2008). The protease and reverse transcriptase regions were PCR-amplified and sequenced. Infecting HIV subtypes were assigned by phylogenetic inference and drug resistance mutations were determined following the IAS consensus and recent reports on C-terminal RT mutations.
Results: The major mutation to NNRTI T369I/V was found in 1.8% of patients, while A376S was present in another 8.3%. In the RNase H domain, the compensatory mutation D488E was more frequently observed in subtype C than in subtype B ($p = 0.038$), while the inverse was observed for mutation Q547K ($p < 0.001$). The calculated codon genetic barrier showed that 22% of subtype B isolates, but no subtype C, carried T360, requiring two transitions to change into the resistance mutation 360V.
Conclusions: Major resistance-conferring mutations to NNRTI were detected in 10% of RT connection domain viral sequences from treatment-naïve subjects. We showed for the first time that the presence of specific polymorphisms can constrain the acquisition of definite resistance mutations in the connection and RNase H subdomains of HIV-1 RT.

1. Background

The human immunodeficiency virus type 1 (HIV-1) is classified into four groups, M-P, and HIV-1M can be further divided into nine subtypes (A–D, F–H, J and K), in addition to circulating recombinant forms (CRFs) and unique mosaics.[1–3] HIV-1M is responsible for the AIDS pandemic and its distribution is characterized by regional founder events.[4,5] The most prevalent HIV-1M subtype is C, accounting for nearly 50% of infections in 2007.[5]Despite the use of successful highly active antiretroviral therapy, HIV acquires drug resistance mutations (DRM) to all clinically approved drugs available.[6] Both classes of reverse transcriptase (RT) inhibitors, nucleoside (NRTI) and non-nucleotide (NNRTI), act in the viral RT N-terminal polymerase domain, where all DRM were initially characterized. A new mechanism of RT resistance was recently proposed, in which mutations in the RNase H (RNH) and connection (CN), the C-terminal RT subdomains, increase resistance to thymidine analogues by decreasing RNH enzymatic activity.[7,8] A dual role of CN mutations N348I and T369I/V for both NRTI and NNRTI has also been demonstrated.[9–12] Mutations A376S and Q509L were shown to confer major resistance to NVP,[12] while other mutations only potentiate the resistance conferred by TAMs.[12,14–17]

Currently, CN and RNH subdomains are not included in resistance genotyping assays, but their clinical impact is controversial and remain poorly characterized.[13,18,19] Limited studies have attempted to evaluate these mutations among drug-naïve

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subjects.[20] Moreover, virtually all studies so far conducted assessed RT C-terminal mutations in subtype B.

2. Objectives

Brazil is reference to universal and free access to HAART. Despite the predominance of HIV-1B in the country,[5] the southern region of Brazil is featured by a high prevalence of HIV-1C. This region permits the analysis of the primary resistance in HIV-1B and C from treatment-naïve patients. Our objective was to analyze the prevalence of DRM in the HIV-1 RT C-terminal subdomains of HIV-1B and C, and to compare their genetic barriers to DRM acquisition.

3. Study design

Plasma samples of 245 treatment-naïve HIV$^+$ subjects diagnosed between January 2005 and December 2008 and consecutively seeking care at Hospital Universitário de Rio Grande, Southern Brazil were collected. All patients signed a written consent to participate in the study.

Viral RNA was extracted with QIAamp Viral RNA kit (QIAGEN) from 140 μl of plasma and was submitted to RT-PCR using M-MLV-RT and Taq platinum polymerase (Invitrogen). The protease and RT regions were amplified in two fragments; the first nested PCR encompassed the entire protease and RT polymerase (~1228 bp), as previously described[21]; the second one harbored the RT CN and RNH domains (962 bp), using primers 5′tggatgggttatgaact3′ and 5′cagtctacttgtccatgcatggcttc3′ in the first round, and 5′atacagaagttagtgggaaaa3′ and 5′cattgctctccaattactgtgatatttctcatg3′ in the second. When the amplification was not successful, the *pol* region subdomains were amplified separately according to established protocols.[20–22] PCR products were sequenced using the Big Dye v.3.1 kit (Applied Biosystems). Sequences were generated in an automated ABI3130XL apparatus and edited with SeqMan v7.0 (DNASTAR). Sequences were aligned in BioEdit v7.0[23] with HIV-1 references from the Los Alamos Database (http://hiv-web.lanl.gov/). Subtypes were determined through phylogenetic inference using neighbor-joining and Kimura's two-parameter, with 1000 bootstrap replicates, using MEGA 4.1.[24]

DRM genotyping was done by aligning viral sequences with HXB2 in BioEdit. DRM in the CN and RNH domains considered here were G335D, N348I, A360V, T369I/V, A371V, A376S, A400T, D488E, Q509L and Q547K, for their recognized phenotypic role in drug resistance.[12,14–17] Protease and RT polymerase sequences were genotyped using the Stanford HIV Drug Resistance algorithm.[25]

Viral sequences were grouped by subtype and the frequency of primary DRM for each genomic region was determined. Comparisons of mutation frequencies were performed with two-tailed Fisher exact tests and *p*-values below 0.05 were considered significant.

Sequences were grouped by subtype (B or C) and the composition of each codon associated with DRM was determined. The number and nature of nucleotide of changes needed to turn a wild-type codon into a resistant codon was determined. Comparison of polymorphism frequencies for each subtype was performed with two-tailed Fisher's exact tests.

HIV sequences reported in this study were submitted to the GenBank nucleotide database and were assigned the accession numbers JN010440–JN010780.

4. Results

We successfully PCR amplified and sequenced RT CN and/or RNH fragments of 83.7% (205/245) viruses. Table 1 describes demographic, behavioral, clinical and laboratorial characteristics of the casuistic analyzed. No differential characteristics were seen for the 40 subjects for whom no viral sequence data was obtained (data not shown), with exception of the mean HIV viral load, which was significantly lower the unsuccessful group ($p = 0.06$). This fact may explain, at least in part, our inability to generate viral sequences from that group of patients. HIV-1C was responsible for 64% (132/205) of infections, while HIV-1B accounted for 22% (45/205), followed by 14% (28/205) of other forms.

Table 1
Demographic, behavioral, clinical and laboratorial characteristics of the studied population, HU-FURG, Rio Grande, Brazil, 2005–2008 ($n = 205$).

Characteristic	*n* (%)
Age (average ± SD)	35.4 ± 11.7
Gender	
Male	116 (56.6)
Transmission route ($n = 175$)[a]	
Heterosexual	135 (77.1)
MSM	18 (10.3)
Intravenous (IDU/transfusion)	22 (12.6)
HIV$^+$ partner	41 (20)
Partner on ART ($n = 182$)[a]	9 (4.9)
CDC clinical classification	
A	109 (53.2)
B	25 (12.2)
C	71 (34.6)
CDC immune classification	
1	56 (27.3)
2	69 (33.7)
3	80 (39)
Median CD4+ T-cell counts (cells/mm^3) (IQR_{50})	301 (105–532)
Median HIV-1 plasma VL (cp/ml) (IQR_{50})	35,078 (9,851–133,944)
Sequenced HIV fragments ($n = 205$)	
Protease	141 (68.8)
Polymerase RT	87 (42.4)
Connection RT	164 (80.0)
RNase H RT	168 (81.9)

[a] Number of individuals for which the variable was available.

The major mutations N348I and Q509L were not observed in our dataset. However, T369I/V was seen in 1.8% (03/168) of patients, while A376S was presented in another 8.3% (14/168). Overall, 10.1% (17/168) of the isolates presented any major CN DRM. G335D and A400T were the most frequent compensatory mutations, with proportions of 54% and 33% HIV-1B and C, respectively. G335D was classified as a polymorphism in subtype C ($p < 0.001$ in comparison with B), while A400T was found in half of subtype B viruses ($p < 0.001$ in comparison with C). D488E was more frequently observed in HIV-1C than B ($p = 0.038$); the inverse was observed for Q547K ($p < 0.001$). The primary resistance in the protease region was 2% (03/141), and 6% (05/87) in the RT polymerase region (Table 2). We did not observe any correlation between CN or RNH DRMs and mutations in RT polymerase, as no virus carried concomitant primary mutations in N- and C-terminal RT regions (data not shown). When analyzing viral isolates with polymerase

Table 2
Patterns of primary drug resistance at protease and polymerase RT genomic regions, Rio Grande, Brazil, 2005–2008.

Patient ID	HIV-1 subtype classification	Protease region ($n = 141$)	Polymerase RT region ($n = 87$)
J63	B	I54V, N88S	K103N, M184V
J99	CRF31_BC	0	K103N
J150	B	–	D67N, T215V, K219Q/N
J156	B	T74P	–
J162	C	0	K219N
J265	URF_BC	0	K103N
J348	URF_F1B	M46I, I54V, V82A	–
Total (%)		2.1	5.7

Table 3
Codon genetic barriers to drug resistance mutation acquisition at the C-terminal RT domains of HIV-1 of subtypes B and C.

RT pos	WT	Codon	Sub B (43/35)[a] (%)	Sub C (103/118) (%)	*p*	DRM	Codon	Change
335	G335	GGC/GGT	90	18	<0.001	335D	GAC/GAT	1 ts
		GGA/GGG	3	2	NS	335D	GAC/GAT	1 tv, 1 ts
348	N348	AAT/AAC	100	100	NS	348I	ATT/ATC	1 tv
360	A360	GCT/GCC/GCA/GCG	79	100	<0.001	360V	GTT/GTC/GTA/GTG	1 ts
	T360	ACT/ACC/ACA/ACG	21	–	<0.001	360V	GTT/GTC/GTA/GTG	2 ts
369	T369	ACT/ACC/ACA/ACG	85	94	NS	369V369I	GTT/GTC/GTA/GTG	2 ts
							ATT/ATC/ATA/ATG	1 ts
	A369	GCA	15	4	NS	369V369I	GTA	1 ts
							ATA	2 ts
371	A371	GCT/GCA	100	100	NS	371V	GTT/GTA	1 ts
376	A376	GCT/GCC/GCA/GCG	59	87	<0.001	376S	TCT/TCC/TCA/TCG	1 tv
	T376	ACT/ACC/ACA/ACG	28	5	<0.001	376S	TCT/TCC/TCA/TCG	1 tv
	V376	GTT/GTC/GTA/GTG	3	–	NS	376S	TCT/TCC/TCA/TCG	1 ts, 1 tv
400	A400	GCT/GCC/GCA/GCG	34	81	<0.001	400T	ACT/ACC/ACA/ACG	1 ts
	S400	TCT/TCC/TCA/TCG	–	2	NS	400T	ACT/ACC/ACA/ACG	1 tv
488	D488	GAT/GAC	100	100	NS	488E	GAA/GAG	1 tv
506	I506	ATT/ATC/ATA/ATG	100	100	NS	506L	CTT/CTC/CTA/CTG	1 tv
509	Q509	CAA/CAG	100	96	NS	509L	CTA/CTG	1 tv
	K509	AAA	–	4	NS	509L	CTA	2 tv
547	Q547	CAA/CAG	97	100	NS	547K	AAA/AAG	1 tv

NS, not significant; ts, transition; tv, transversion.
[a] Numbers in parentheses correspond to the number of connection/RNase H sequences for each subtype used in the calculations.

and CN regions sequenced ($n = 53$), the overall frequency of primary resistance was 15%.

The genetic barrier analysis is depicted in Table 3. Viruses carrying T360 need two transitions to change to 360V, while those with A360 need one transition. Twenty-one percent of HIV-1B isolates but no HIV-1C carried T360, suggesting that the former require more changes to acquire 360V. The polymorphism T369 needs one transition to change to 369V, and two to change into 369I. We also detected a higher genetic barrier for changing V376 (one transition and one transversion) into 376S compared to A376 and T376 (one transition). Viruses carrying S400 require one transversion to acquire T400, compared to those carrying A400 (one transition).

5. Discussion

Primary drug resistance has been estimated between 8% and 14% in developed settings,[26–31] whereas in Brazil recent studies showed lower rates of transmitted resistance (7–8%).[32,33] However, the C-terminal RT domains have not been previously surveyed. Mutations N348I, A360V and Q509L have been detected in low frequency in treatment-naïve subjects (<3%),[13,18,19,22] in agreement with our study. Although the observation of A376S at 8.3% is noteworthy, this should be interpreted with care. A376S has been associated with reduced susceptibility to NVP,[12] but the fold-change values were below the biological cut-off for that drug.[34] T369I/V was found in only 1.8% of our patients.

G335D was recently characterized as potentiating TAM resistance.[17] Here, it was profusely detected in HIV-1C. The effect of G335D remains unknown in HIV-1C, and further phenotypic studies are warranted. A400T is also polymorphic in HIV-1 subtypes, and its role was equivalent in HIV-1B, C and CRF01_AE.[14,35] The phenotypic role of the remaining RT polymorphisms seen here remains undetermined for non-B subtypes.

Major DRM to NNRTI was detected in 10% of RT CN domain of viruses from treatment-naïve subjects. This highlights their importance and potential inclusion in resistance genotyping. We also showed polymorphisms displayed by different HIV-1 subtypes that can potentially affect DRM acquisition. Further studies are necessary to elucidate the impact of these mutations on antiretroviral treatment of individuals infected with distinct HIV-1 subtypes.

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Competing interests

None declared.

Ethical approval

Committee of Ethics in Research, Universidade Federal do Rio Grande, Rio Grande, Brazil (protocol no. 63/2008).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2011.09.005.

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