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HIV CRF08_BC AND X4 STRAIN ARE ASSOCIATED WITH POSING MAJOR NUCLEOSIDE AND NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS RESISTANCE MUTATIONS

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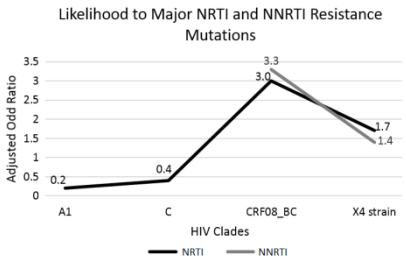
Graphical abstract



Abstract

In HIV treatment, surveillance of mutations inducing resistance to NRTIs (Nucleoside Reverse Transcriptase Inhibitors) and NNRTIs (Non-Nucleoside Reverse Transcriptase Inhibitors) is important. This study analyzed 2,071 HIV-1 genomic sequences directed to detection of major NRTIs and NNRTIs resistance mutations and viral tropism. CRF08_BC and X4 strain more likely had major NRTIs (adjusted odds ratio (aOR) 3.0, 95% CI 1.182-7.820, p=0.021 and aOR 1.7, 95% CI 1.233-2.368, p=0.001; respectively) and NNRTIs (aOR 3.3, 95% CI 1.281-8.365, p=0.013 and aOR 1.4, 95% CI 1.037-1.977, p=0.029, respectively) resistance mutations. Subtype A1 (aOR 0.2, 95% CI 0.069-0.702, p=0.011) and C (aOR 0.4, 95% CI 0.241-0.648, p<0.001) were associated with major NRTIs resistance mutations. The occurrence of major NRTIs and NNRTIs resistance mutations in HIV subtype A1, C, CRF08_BC, and X4 viruses should be a particular concern.

Keywords: HIV; NRTIs; NNRTIs; resistance; mutation

Abstrak

Dalam rawatan HIV, pengawasan terhadap mutasi yang menimbulkan rintangan terhadap NRTI (Nucleoside Reverse Transcriptase Inhibitors) dan NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitors) adalah penting. Kajian ini menganalisis 2,071 sekuens genom HIV-1 yang dilakukan deteksi mutasi mayor rintangan NRTI dan NNRTI serta tropisme virus. CRF08_BC dan strain X4 lebih sering untuk memiliki mutasi mayor rintangan NRTI (*adjusted odds ratio* (aOR) 3.0, 95% CI 1.182-7.820, p=0.021 untuk CRF08_BC dan aOR 1.7, 95% CI 1.233-2.368, p=0.001 untuk strain X4) dan NNRTI (aOR 3.3, 95% CI 1.281-8.365, p=0.013 untuk CRF08_BC dan aOR 1.4, 95% CI 1.037-1.977, p=0.029 untuk strain X4). Subtipe A1 (aOR 0.2, 95% CI 0.069-0.702, p=0.011) dan C (aOR 0.4, 95% CI 0.241-0.648, p<0.001) berkaitan dengan mutasi mayor rintangan NRTI. Terjadinya mutasi mayor rintangan NRTI dan NNRTI pada HIV subtipe A1, C, CRF08_BC, dan virus X4 harus menjadi perhatian.

Kata kunci: HIV; NRTI; NNRTI; resistensi; mutasi

1.0 INTRODUCTION

Human immunodeficiency virus-1 (HIV-1) is a highly mutable virus because of its error-prone replication [1]. To date, HIV-1 is divided into four groups (M, N, O, and P). Within the group M, nine subtypes are recognized (A to D, F to H, J, K), along with more than sixty circulating recombinant forms (CRFs) (www.hiv.lanl.gov). Due to this enormous diversity, developing effective drugs and vaccines are extremely difficult [2, 3].

Highly active antiretroviral therapy (HAART) is the most successful approach to improve the survival of HIV patients [4, 5]. There are four classes of antiretrovirals (ARVs) approved by the U.S. Food and Drug Administration, comprising Reverse Transcriptase inhibitors, Protease inhibitors, fusion/entry inhibitors, and Integrase inhibitors [3]. As Nucleoside and Non-Nucleoside Reverse Transcriptase Inhibitors (NRTIs and NNRTIs) are the first-line ARV regiments given to HIV patients [6], continuous surveillance in the rate of major mutations related to resistance to these drugs is important to monitor and estimate the level of resistance in circulating HIV strains. Here, we aimed to analyze the rate of major NRTIs and NNRTIs resistance mutations, as well as their associated factors.

2.0 EXPERIMENTAL

2.1 Sequence Data

In total, 2,071 aligned HIV-1 genomic sequences in the Los Alamos HIV database (http://www.hiv.lanl.gov/cgi-bin/NEWALIGN/align.cgi), consisting of numerous HIV subtypes and CRFs, were downloaded in fasta format. All sequences were manually edited in CLC Sequence Viewer 6.0 (www.clcbio.com) in order to separate the RT- and V3-encoding sequences.

2.2 Identification of Major NRTIs and NNRTIs Resistance Mutations and HIV-1 Tropism

The detection of major NRTIs and NNRTIs resistance mutations in HIV-1 sequences were conducted using MEGA6 software [7], in accordance with the most updated WHO list for surveillance of drug resistance mutations (http://hivdb.stanford.edu) [8]. The V3-encoding region in the *env* gene of all genomic sequences were submitted to Geno2pheno [coreceptor] 2.5 (http://coreceptor.geno2pheno.org/) for the identification of viral tropism. Significance levels were adjusted in accordance with recommendations from the European Consensus Group on clinical management of HIV-1 tropism testing [9].

2.3 Statistical Analysis

A two-sided Pearson chi-squared test or Fisher's exact test was performed to detect a difference in the rate of major NRTIs and NNRTIs resistance mutations with respect to HIV clades and tropism. A logistic regression was used to evaluate potential association. A p value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 21 software (IBM Corp, Armonk, NY, USA).

3.0 RESULTS AND DISCUSSION

The RT sequences of 2,071 HIV-1 isolates worldwide were analyzed. As many as 76.9% (1,592/2,071) and 23.1% (479/2,071) of them were predicted as R5 and X4 viruses, respectively.

Nine percent (186/2,071) and 9.8% (203/2,071) of the HIV-1 isolates had ≥1 major NRTIs (defined as the existence of M41L, K65R, D67N, T69Ins, K70E, K70R, L74VI, Y115F, Q151M, M184VI, L210W, T215FY, and/or K219QE) and NNRTIs (defined as the appearance of L100I, K101EP, K103NS, V106AM, E138AGKQ, Y181CIV, Y188LCH, G190ASE, and/or M230L) resistance mutations, respectively. M184VI, K65R, K70E, L74VI, and Y115F were identified in 58/2,071, 20/2,071, 1/2,071, 18/2,071, and 6/2,071 isolates, respectively. The Thymidine Analog Mutations (TAMs), M41L, D67N, K70R, L210W, T215FY, and K219QE were present in 50/2,071, 50/2,071, 49/2,071, 20/2,071, 84/2,071, and 58/2,071, respectively. T69Ins, a multidrug resistance (MDR) mutation, was absent. Another MDR mutation, Q151M, was observed in 5/2,071 isolates. Major NNRTIs resistance mutations, L100I, K101EP, K103NS, V106AM, E138AGKQ, Y181CIV, Y188LCH, G190ASE, and M230, appeared in 4/2,071, 8/2,071, 58/2,071, 6/2,071, 70/2,071, 57/2,071, 25/2,071, 27/2,071, and 6/2,071 isolates, respectively.

Major NRTIs resistance mutations were common in subtype A2, H, J, U, and CRFs, while major NNRTIs resistance mutations were frequent in subtype H and CRFs as well (Table 1). The high rates of major mutations in HIV-1 CRFs indicate the need of concern to this group. The proportion of major NRTIs resistance mutations was different with respect to subtype A1 ($p=0.007$), B ($p=0.001$), C ($p<0.001$), and CRF08_BC ($p=0.016$). While a significantly different proportion of NNRTIs resistance mutations was present in CRF08_BC ($p=0.025$).

As many as 13.8% (66/479) and 7.5% (120/1,592) of X4 and R5 viruses, respectively, contained major NRTIs resistance mutations. While major NNRTIs resistance mutations appeared in 12.3% (59/479) and 9.0% (144/1,592) of X4 and R5 isolates, respectively. X4 strain more likely had major NRTIs (OR 2.0, 95% CI 1.424-2.698, $p<0.001$) and NNRTIs (OR 1.4, 95% CI 1.024-1.949, $p=0.035$) resistance mutations.

Table 1 Distribution of major NRTIs and NNRTIs resistance mutations in HIV-1 subtypes and CRFs

Subtype	n (%)	Predicted tropism (n, %)		Major NRTIs resistance mutation		Major NNRTIs resistance mutation	
		R5	X4	n (%)	p	n (%)	p
A1	126 (6.1)	110 (87.3)	16 (12.7)	3 (2.4)	0.007[a]	9 (7.1)	0.300[a]
A2	3 (0.1)	2 (66.7)	1 (33.3)	1 (33.3)		0 (0.0)	
B	729 (35.2)	539 (73.9)	190 (26.1)	86 (11.8)	0.001[a]	80 (11.0)	0.186[a]
C	461 (22.3)	416 (90.2)	45 (9.8)	19 (4.1)	0.000[a]	53 (11.5)	0.165[a]
D	61 (2.9)	28 (45.9)	33 (54.1)	7 (11.5)	0.489[a]	3 (4.9)	0.193[a]
F1	24 (1.2)	19 (79.2)	5 (20.8)	3 (12.5)	0.470[b]	3 (12.5)	0.725[b]
G	36 (1.7)	32 (88.9)	4 (11.1)	2 (5.6)		1 (2.8)	
H	4 (0.2)	3 (75.0)	1 (25.0)	1 (25.0)		1 (25.0)	
J	4 (0.2)	3 (75.0)	1 (25.0)	1 (25.0)		0 (0.0)	
U	9 (0.4)	7 (77.8)	2 (22.2)	2 (22.2)		1 (11.1)	
CRF01_AE	259 (12.5)	145 (56.0)	114 (44.0)	22 (23.3)	0.769[a]	20 (7.7)	0.229[a]
CRF02_AG	74 (3.6)	70 (56.9)	4 (17.1)	3 (4.1)	0.131[a]	3 (4.1)	0.090[a]
CRF04_cpx	5 (0.2)	2 (40.0)	3 (60.0)	3 (60.0)		1 (20.0)	
CRF05_DF	3 (0.1)	3 (100.0)	0 (0.0)	2 (66.7)		0 (0.0)	
CRF07_BC	14 (0.7)	14 (100.0)	0 (0.0)	1 (7.1)		1 (7.1)	
CRF08_BC	24 (1.2)	21 (87.5)	3 (12.5)	6 (25.0)	0.016[b]	6 (25.0)	0.025[b]
CRF11_cpx	10 (0.5)	10 (100.0)	0 (0.0)	1 (10.0)		0 (0.0)	
CRF14_BG	5 (0.2)	1 (20.0)	4 (80.0)	1 (20.0)		1 (20.0)	
CRF16_A2D	2 (0.1)	0 (0.0)	2 (100.0)	1 (50.0)		0 (0.0)	
CRF17_BF	7 (0.3)	6 (85.7)	1 (14.3)	2 (28.6)		1 (14.3)	
CRF18_cpx	4 (0.2)	4 (100.0)	0 (0.0)	1 (25.0)		1 (25.0)	
CRF20_BG	2 (0.1)	2 (100.0)	0 (0.0)	1 (50.0)		0 (0.0)	
CRF23_BG	2 (0.1)	2 (100.0)	0 (0.0)	1 (50.0)		0 (0.0)	
CRF27_cpx	2 (0.1)	1 (50.0)	1 (50.0)	0 (0.0)		1 (50.0)	
CRF28_BF	5 (0.2)	3 (60.0)	2 (40.0)	1 (20.0)		0 (0.0)	
CRF29_BF	7 (0.3)	4 (57.1)	3 (42.9)	2 (28.6)		3 (42.9)	
CRF31_BC	3 (0.1)	3 (100.0)	0 (0.0)	0 (0.0)		1 (33.3)	
CRF35_AD	21 (1.0)	17 (81.0)	4 (19.0)	0 (0.0)		1 (4.8)	
CRF38_BF	5 (0.2)	4 (80.0)	1 (20.0)	1 (20.0)		1 (20.0)	
CRF39_BF	3 (0.1)	0 (0.0)	3 (100.0)	3 (100.0)		3 (100.0)	

CRF40_BF	4 (0.2)	2 (50.0)	2 (50.0)	3 (75.0)	2 (50.0)
CRF43_02G	4 (0.2)	4 (100.0)	0 (0.0)	1 (25.0)	0 (0.0)
CRF45_cpx	5 (0.2)	5 (100.0)	0 (0.0)	1 (20.0)	0 (0.0)
CRF46_BF	7 (0.3)	5 (71.4)	2 (28.6)	3 (42.9)	2 (28.6)
CRF48_01B	3 (0.1)	0 (0.0)	3 (100.0)	0 (0.0)	1 (33.3)
CRF54_01B	3 (0.1)	3 (100.0)	0 (0.0)	1 (33.3)	1 (33.3)
CRF57_BC	3 (0.1)	2 (66.7)	1 (33.3)	0 (0.0)	2 (66.7)

[a]Chi-square test.
[b]Fisher's exact test.

In the analysis considering viral subtype and tropism, subtype A1 (adjusted OR (aOR) 0.2, 95% CI 0.069-0.702, p=0.011) and C (aOR 0.4, 95% CI 0.241-0.648, p<0.001) were associated with a lower likelihood to contain the major NRTIs resistance mutations. In contrast, CRF08_BC and X4 strain were associated with a higher likelihood to have major NRTIs (aOR 3.0, 95% CI 1.182-7.820, p=0.021 and aOR 1.7, 95% CI 1.233-2.368, p=0.001, respectively) and NNRTIs (aOR 3.3, 95% CI 1.281-8.365, p=0.013 and aOR 1.4, 95% CI 1.037-1.977, p=0.029, respectively) resistance mutations. X4 strain is the HIV-1 which uses CXCR4 chemokin receptor to mediate viral entry and has always been associated with rapid progression to Acquired Immunodeficiency Syndrome (AIDS) [10]. The results of the present study suggest that the emergence of major NRTIs resistance mutations may be one of the mechanism by which X4 viruses are associated with accelerated disease progression.

4.0 CONCLUSION

Numerous major NRTIs and NNRTIs resistance mutations are present in HIV CRFs. Subtype A1 and C are associated with major NRTIs resistance mutations. CRF08_BC and X4 viruses are at a higher likelihood to pose major NRTIs and NNRTIs resistance mutations. The occurrence of major NRTIs and NNRTIs resistance in HIV subtype A1, C, and CRF08_BC should be a concern.

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