Published in final edited form as:

J Acquir Immune Defic Syndr. 2011 November 1; 58(3): e94–e95. doi:10.1097/QAI.0b013e318232a13c.

HIV-1 Reverse Transcriptase Nucleotide Substitutions in Subtype C Infected Drug Naïve and Treatment Experienced Patients in South India

Shanmugam Saravanan, PhD^{1,*}, Vidya Madhavan, PhD¹, Pachamuthu Balakrishnan, PhD¹, Sunil S. Solomon, PhD¹, Nagalingeswaran Kumarasamy, PhD¹, Kenneth H. Mayer, MD², Greer Waldrop, BA¹, Suniti Solomon, MD¹, and Davey M. Smith, MD^{3,4}

¹YRG CARE, Chennai, India

²Brown University, USA

³University of California San Diego, LaJolla, CA, USA

⁴Veterans Affairs Healthcare System, San Diego, CA, USA

Summary

In this large cohort of patients infected with HIV-1 subtype C, virologic failure during antiretroviral therapy was associated with high rates of resistance; however, many nucleotide changes were not congruent with previously documented resistance associated mutations. These data may have implications in interpreting genotypic resistance of HIV-1 subtype C during therapy failure.

Keywords

G to A hypermutation in Indian HIV-1 subtype C; HIV-1 drug resistance mutation; HIV-1 RT nucleotide substitution; HIV-1 Primary Drug Resistance; HIV-1 Drug Resistance in Naïve

HIV-1 can evolve to escape from antiretroviral therapy (ART) and host immune responses^{1–3}. The pattern of nucleotide changes in these evolutionary scenarios is dependent on the genetic background of the virus. Given the large genetic diversity of HIV-1 worldwide, the development of ART resistance is most likely different between subtypes. We studied the mutational patterns occurring during virologic failure of ART among patients infected with HIV-1 subtype C in India.

Two groups of individuals infected with HIV-1 subtype C in southern India were investigated. One group (n=58) was ART naïve with viral loads > 3000 HIV RNA copies/mL (COBAS Amplicor HIV-1 Monitor, Version 1.5, Roche Molecular Systems Inc., Pleasanton, CA, USA) and the other group (n=524) was receiving first-line ART that

* Author for correspondence: Dr. S. Saravanan, PhD, Assistant Professor, Y.R. Gaitonde Centre for AIDS Research and Education (YRG CARE), Voluntary Health Services (VHS) Hospital, Taramani, Chennai - 600 113, INDIA, saravanan@yrgcare.org, Ph: 044-22542929, Fax: 044-22542939, www.yrgcare.org.

Conflicts of interest: DMS has received research support from Pfizer but not as it pertains to this study.

consists of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-NRTI (NNRTI), including combinations of zidovudine (AZT)/stavudine (d4T) plus lamivudine (3TC) plus nevirapine (NVP)/efavirenz (EFV) and whose viral load was > 3000 HIV RNA copies/mL. HIV RNA was extracted from blood plasma using Qiagen RNA MiniAmp kit (Qiagen, Valencia, CA, USA) and bulk sequences of HIV-1 reverse transcriptase (630 base pairs) were generated, as previously described validated in-house genotyping assay ⁴. Sequences were aligned (ClustalX) to an Indian subtype C reference (C.IN.AFo67155) and examined for HIV-1 subtype (REGA v2) ⁵, nucleotide diversity, mismatches, transition and transversion mutations (Highlighter HIV LANL) ⁶, average pairwise distance (TN69 model in HyPHY) ⁷, positive selection (REL in HyPHY) ⁷, drug resistance associated mutations (IAS-USA and Stanford HIV Resistance Database) ^{8,9} and APOBEC signatures G > A hypermutation (Hypermur HIV LANL) ¹⁰. Comparisons were made using one-way ANOVA and Tukey's multiple post-tests (Prism v5.03).

Hypermutation was not identified in either group and overall nucleotide diversity was higher in the treated group (average pairwise distance 0.043 vs. 0.078). Compared to the ART naïve group, the treated group had higher mean number of: G to A (14.6 vs. 15.9), A to G (13.91 vs. 16.02) and G to C (0.59 vs. 1.48) single nucleotide transitions and the dinucleotide GT to AT substitution (4.09 vs. 4.76) (all $p < 0.001$, Table 1). Overall, the A to G transition was the predominant pattern observed in resistance associated mutations in the treated group. Specifically, the M184V (ATG to GTG) mutation was present in 78.7% of the treated group and was characterized by an A to G transition. Other observed resistance associated mutations included 37% K103N (AAA to AAC), 33% Y181C (TAT to TGT), 33% D67N (GAC to AAC), 32% G190A (GGC to GCC) 30% with M41L (ATG to TTG), 27% T215Y (ACC to TAC), 23% K70R (AAG to AGG), 18% K101E (AAA to GAA) and 13% V106M (GTG to ATG) (Figure 1). These data are consistent with previous reports that found the most prevalent thymidine analogue mutations in HIV-1 subtype C from Africa included D67N and T215Y. Also, consistent with previous reports, ^{3,11,12} we did not observe high rates of the M184I substitution, which is caused by a G to A hypermutation, and commonly occurs prior to the selection and fixation of the M184V mutation. As expected, there was a trend for these resistance sites to demonstrate positive selection in the ART treated versus naïve groups ($p = 0.06$), and the only drug resistance associated mutation in the ART naïve group was the K219Q and it was found in only one individual.

The diversity of HIV-1 subtypes is a challenge for prevention and treatment programs worldwide. The differences among HIV-1 subtypes may have a profound impact on clinical management and surveillance of drug resistance, particularly as ART is expanded to patients infected with non-subtype B HIV-1. As expected, in this large cohort of patients infected with HIV-1 subtype C, virologic failure during ART was associated with high rates of resistance. Although this study does not allow for a direct comparison between subtype C versus B rate of drug resistance development, this is an important issue that should be investigated in well-designed clinical trials that allow for uniform monitoring of ART failure. Interestingly, virologic failure during ART was also associated with greater numbers of nucleotide changes, but many patterns of these changes were not specific to the observed resistance associated mutations. These patterns likely represent the mutational predilection

of the replicating viral population while under ART pressure. The clinical ramifications of these predilections remain unclear and should be investigated in the future.

Acknowledgments

We are grateful to the clinical and laboratory staff at YRG Centre for AIDS Research and Education, VHS, Chennai, India, for their facilitation of the study. DMS has received research support by grants from the National Institutes of Health: AI69432, AI043638, MH62512, MH083552, AI077304, AI36214, AI047745, AI74621, AI080353, and the James B. Pendleton Charitable Trust.

References

1. Preston BD, Dougherty JP. Mechanisms of retroviral mutation. *Trends Microbiol.* 1996; 4:16–21. [PubMed: 8824790]
2. Quinones-Mateu M, Weber J, Rangel H, Charkabarty B. HIV-1 fitness and antiretroviral drug resistance. *AIDS Rev.* 2001; 3:223–242.
3. Rezende LF, Drosopoulos WC, Prasad VR. The influence of 3TC resistance mutation M184I on the fidelity and error specificity of human immunodeficiency virus type 1 reverse transcriptase. *Nucleic Acids Res.* 1998; 26:3066–3072. [PubMed: 9611256]
4. Saravanan S, Vidya M, Balakrishnan P, et al. Evaluation of two human immunodeficiency virus-1 genotyping systems: ViroSeq 2. 0 and an in-house method. *J Virol Methods.* 2009; 159:211–216. [PubMed: 19490976]
5. de Oliveira T, Deforche K, Cassol S, Salminen M, Paraskevis D, Seebregts C, Snoeck J, van Rensburg EJ, Wensing AMJ, van de Vijver DA, Boucher CA, Camacho R, Vandamme AM. An Automated Genotyping System for Analysis of HIV-1 and other Microbial Sequences. *Bioinformatics.* 2005; 21(19):3797–3800.
6. Los Alamos HIV databases & HIV Database Tools. <http://www.hiv.lanl.gov/>
7. Pond SK, Muse SV. Site-to-site variation of synonymous substitution rates. *Mol Biol Evol.* 2005; 22(12):2375–85. [PubMed: 16107593]
8. Johnson VA, Brun-Vézinet F, Clotet B, Günthard HF, Kuritzkes DR, Pillay D, Schapiro JM, Richman DD. Update of the drug resistance mutations in HIV-1: December 2010. *Top HIV Med.* 2010; 18(5):156–63. [PubMed: 21245516]
9. Liu TF, Shafer RW. Web Resources for HIV type 1 Genotypic-Resistance Test Interpretation. *Clin Infect Dis.* 2006; 42(11):1608–18. [PubMed: 16652319]
10. Rose PP, Korber BT. Detecting hypermutations in viral sequences with an emphasis on G → A hypermutation. *Bioinformatics.* 2000; 16(4):400–401. [PubMed: 10869039]
11. Keulen W, Boucher C, Berkhout B. Nucleotide substitution patterns can predict the requirements for drug-resistance of HIV-1 proteins. *Antiviral Res.* 1996; 31:45–57. [PubMed: 8793008]
12. Novitsky V, Wester CW, DeGruttola V, Bussmann H, Gaseitsiwe S, Thomas A, Moyo S, Musonda R, Van Widenfelt E, Marlink RG, Essex M. The reverse transcriptase 67N 70R 215Y genotype is the predominant TAM pathway associated with virologic failure among HIV type 1C-infected adults treated with ZDV/ddI-containing HAART in southern Africa. *AIDS Res Hum Retroviruses.* 2007; 23(7):868–78. [PubMed: 17678469]

Figure 1.
Frequency of drug resistance mutations in the reverse transcriptase region

Table 1

Nucleotide substitutions among ART naïve and HAART experienced groups.

Nucleotide substitution	Naïve (n=58) Mean (SD)	First-line Failing Patients (n=522) Mean (95% CL)	Naïve vs First-line Failing
A to G	13.91 (13.29 – 14.54)	16.02 (15.75 – 16.30)	<.001
G to A	14.6 (14.23 – 14.98)	15.95 (15.73 – 16.17)	NS
T to C	10.1 (9.746 – 10.46)	10.65 (10.48 – 10.82)	NS
C to T	9.483 (9.113–9.852)	9.686 (9.544 – 9.828)	NS
GA to AA	7.224 (7.046 – 7.402)	7.764 (7.428 – 8.101)	NS
GT to AT	4.086 (3.815 – 4.357)	4.755 (4.629 – 4.880)	< .001
C to A	3.155 (2.979 – 3.331)	3.921 (3.819 – 4.023)	NS
A to C	1.966 (1.696 – 2.235)	2.593 (2.483 – 2.702)	NS
A to T	1.229 (0.9953 – 1.463)	2.331 (2.214 – 2.447)	NS
GA to AG	1.45 (1.153 – 1.675)	2.002 (1.724 – 1.915)	NS
GC to AC	1.879 (1.695 – 2.064)	1.878 (1.816 – 1.940)	NS
T to G	1.5 (1.323 – 1.677)	1.611 (1.542 – 1.679)	NS
T to A	1.358 (1.187 – 1.530)	1.564 (1.485 – 1.642)	NS
G to C	0.5862 (0.4153 – 0.7571)	1.478 (1.411 – 1.546)	<.001
C to G	1.12 (1.027 – 2.13)	1.354 (1.302 – 1.406)	NS
G to T	0.569 (0.4123 – 0.7256)	1.299 (1.173 – 1.425)	NS

¹ All p values calculated with the one-way ANOVA test.