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Reverse Transcriptase (RT) Substitution at Codons 208 and 228 Among Treatment-experienced HIV-1 Subtype-C Infected Indian Patients Is Strongly Associated with Thymidine Analogue Mutations (TAMs)

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Summary

In HIV-1 subtype C infected populations in south India, we searched for novel mutations associated with failing antiretroviral therapy that included nucleoside reverse transcriptase (RT) inhibitors. HIV-1 RT sequences were generated from treated and untreated groups and each nucleotide position was analysed with appropriate corrections for multiple testing. We found that nonsynonymous mutations at positions 208 and 228 were strongly associated with the presence of thymidine analogue mutations in the treated group, and were not present at all in the naïve group. The role of these substitutions on treatment outcomes and the evolution of drug resistance in HIV-1 subtype-C infected populations warrant further investigation.

Keywords

HIV-1 Drug Resistance Mutation; HIV-1 Subtype C; HIV in India; Thymidine Analogue Mutation (TAMs); HIV-1 RT mutation at codon 208; 228; HIV Drug Resistance in India

The emergence of HIV-1 drug resistance within a HIV-infected individual depends on the genetic background of the virus and the selection pressure of the therapy. A variety of mutations in HIV reverse transcriptase (RT) have been associated with nucleoside reverse transcriptase inhibitor (NRTI) exposure, which give rise to a range of drug susceptibilities and viral replication capacity. The genetic variability of HIV-1 is the result of several factors including the high rate of replication, recombination, the low fidelity of HIV-1 RT, and the lack of proofreading activities during the RNA-dependent DNA synthesis^{1–3}. In India, subtype C is the most prevalent genetic background of circulating HIV-1, and reverse

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transcriptase (RT) inhibitors [zidovudine (AZT)/stavudine (D4T) + lamivudine (3TC) + nevirapine (NVP)/efavirenz (EFV)] are the most commonly prescribed first-line drugs. Resistance to AZT and D4T results from the sequential accumulation of thymidine analog resistance mutations (TAMs) at reverse transcriptase (RT) codons 41, 67, 70, 210, 215, and 219. Data from several studies suggest that TAMs mostly occur in two distinct clusters. The TAM-1 cluster includes mutations that occur with T215Y (including M41L, L210W, and sometimes D67N), and the TAM-2 cluster includes mutations that occur with K70R (including D67N, T215F, and K219Q) ^{4,5}. Most of these observations have occurred in the setting of drug resistance that has developed in the viral genetic background of HIV-1 clade B, and it is unclear if other mutations can arise in the background of HIV-1 subtype C. To this end, we investigated HIV-1 RT sequences generated from antiretroviral naïve and experienced patients who were infected with HIV subtype C in southern India to determine mutational patterns associated with treatment status.

YRG Centre for AIDS Research and Education (CARE) is a non-profit medical and research institution in Chennai, India, that provides medical care to >15,000 HIV-infected individuals. The patients attending this centre are treated according to WHO treatment guidelines. Patients were seen every 3 months or as clinically indicated. CD4 cell count monitoring was performed every 3–6 months. HIV-1 plasma viral load monitoring was not standard of care due to cost factor. We sequenced the RT region (codons 1 – 240) of 100 treatment naïve and 350 patients who were failing their initial HAART regimen (treatment experienced). Genotyping was performed using validated home-brew assay (reverse-transcriptase nested polymerase chain reaction), as previously described ⁶. Sequences were aligned (ClustalX) to an Indian subtype C reference (C.IN.AF067155) and examined for HIV-1 subtype in REGA v2 ⁷. The sequence data were analyzed using the Stanford HIV Drug resistance database available at <http://hivdb.stanford.edu> in Jan 2011. Nucleotide and residue positions in sequences collected from antiretroviral naïve and experienced patients were compared using the Chi-squared test and with continuity correction. Logistic regression was then used to examine associations between mutations that were statistically different between the two groups and the presence of established RT resistance mutations, as determined by the Stanford HIV Resistance Database ⁸. Bonferroni correction was applied to all tests to correct for multiple comparisons, and a Bonferroni corrected p-value<0.05 was considered statistically significant. Mutations present as mixtures were excluded from the analysis.

In univariate analysis, mutations in the HIV-1 RT coding region at codons 36 (81% vs. 76%; p<0.01), 48 (84% vs 59%; p<0.01), 60 (94% vs. 79%; p=0.01) and 173 (66% vs. 33%; p<0.01) were higher among treatment naïve patients, while substitution at codons 179 (15% vs. 2%; p<0.01) and 207 (79% vs. 63%; p=0.02) were higher among treatment experienced patients. Substitutions at codons 208 (7% vs. 0%; p= 0.14) and 228 (9% vs. 0%; p=0.04) and were observed only among treatment experienced patients. A non-synonymous substitution at position L228H was associated with the presence of K70R (OR: 4.0; p<0.01) and K219 (OR: 5.02; p<0.01). Whereas, a non-synonymous substitution in codon H208Y was strongly associated with the presence of TAMs: M41L (OR: 6.46; p<0.01), T215Y (OR: 5.31; p<0.01) and D67N (OR: 7.24; p<0.01).

When antiretroviral therapy fails to fully suppress HIV RNA replication, new viral variants can emerge that have decreased susceptibility to the antiretroviral agents. These new variants may escape from the drugs by accumulating mutations. Concerning the RT coding region, many studies have identified new mutations that are associated with NRTI treatment ^{9–11}, but their exact role in the development of NRTI resistance remains unclear. The emergence of TAMs in treated patients occurs in an orderly manner and their accumulation is associated with increasing levels of resistance. The magnitude of thymidine

analogue resistance conferred by TAMs can also be modulated by other nucleoside analogue mutations⁴. Such is the case for the M184V mutation commonly seen in regimens containing lamivudine or emtricitabine which causes high-level resistance to lamivudine and emtricitabine, moderate resistance to didanosine and abacavir, and increases the susceptibility to zidovudine, stavudine and tenofovir¹². Another example is the fact that the prevalence of H208Y is more often found in the setting of multiple TAMs, which indicates its emergence with prolonged selective pressure from NRTI and its likelihood of being an accessory mutation⁵. Although limited by sample size in the treatment naïve group, RT non-synonymous substitutions at positions 208 and 228 were strongly associated with the presence of TAMs in the viral genetic background of HIV-1 subtype C. The role of these substitutions on treatment outcomes, as well as the evolution of drug resistance in HIV-1 subtype C infected populations warrants further investigation.

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Frequency of RT drug resistance mutations and its association with non-drug resistance mutations at codon position 208 & 228

Table 1

DRMs	Frequency (%)	No mutation at 208/228 (n=295)	L228 (n=30)	H208 (n=25)	p value
41L	124	97	8	19	0.0000494
210W	43	32	5	6	0.1174172
215Y/F	165	133	13	19	0.000144
215Y	88	65	8	15	0.0109392
215F	42	33	5	4	0.5536695
67N	139	105	14	20	0.0000542
70R	83	59	15	9	0.0003719
219Q/E	60	39	13	8	0.0000207
65R	19	15	3	1	0.4994518
184V	252	202	27	23	0.0030269
151M	23	20	0	3	0.1891056
103N	103	81	13	9	0.144823
106M/A	48	41	2	5	0.3495263
179D/E	8	5	1	2	0.1186349
181C	116	98	13	5	0.1867823
190M	95	84	4	7	0.2052696