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Short Communication: Analysis of Selection Pressure and Mutational Pattern of HIV Type 1 Reverse Transcriptase Region Among Treated and Nontreated Patients

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Abstract

Variation of the HIV-1 subtype C reverse transcriptase region (RT) resulting in response to the selective pressures of drug therapy remains poorly characterized. Here, we compared the genetic variation resulting in the presence and absence of antiretroviral drug selective pressures on HIV-1 subtype C RT among nontreated and treated patients. The nucleotide variability, nonsynonymous and synonymous ratio, and the positively selected mutations were determined by comparing the RT sequences isolated at two time points among nontreated (baseline and follow-up) and treated patients (baseline and treatment failure). Compared to the nontreated patients, the intrapatient nucleotide variability, the number of nonsynonymous and synonymous substitutions was significantly higher among the treated patients. Among the mutations positively selected, the frequency of D121Y, I135R, and Q207E increased and the frequency of mutation S48T decreased significantly during treatment failure. Further studies are essential to discover the role of these mutations during treatment in HIV-1 subtype C.

The reverse transcriptase (RT) enzyme of the human immunodeficiency virus (HIV-1) converts viral genomic single-stranded RNA into double-stranded DNA. It is the essential step in the HIV-1 life cycle and therefore the RT region has been a target of antiretroviral therapy (ART). As an intrinsic property, HIV-1 RT lacks a proofreading function and this error-prone nature of RT together with the high rate of virus production sustained by HIV-1 infection *in vivo* contributes to the continuous generation of new viral variants.[1,2] The variability is further increased by antiretroviral drugs, resulting in mutations that have a selective advantage during drug pressure.[3–6] Studying the effect of drug treatment on HIV-1 variation is important in understanding the emergence of drug resistance and disease pathogenesis. Here we compared the genetic variation resulting from the presence and absence of ARV drug selective pressures on HIV-1 subtype C RT among nontreated and treated patients.

HIV-1-infected nontreated patients ($n = 18$) and patients treated ($n = 16$) for >6 months and failing the first line regimen were included. The demographic, clinical, and laboratory characteristics of the study groups are presented in Table 1. Peripheral blood samples from nontreated patients were collected during enrollment (baseline) and after a period of 8–12 months (follow-up). From the treated patients peripheral blood samples were collected at treatment failure and respective pretherapy (baseline) plasma samples that were collected before 12–16 months of therapy were retrospectively obtained from the archives (–70°C freezers). The study protocol was approved by the Institutional Review Board of the Y.R. Gaitonde Centre for AIDS Research and Education and the written informed consent was obtained from all the participants included in the study.

HIV-1 RNA was isolated using the QIAamp viral RNA kit (QIAGEN, Inc., USA). HIV-1 RT (region 20–240) was amplified from cDNA using nested polymerase chain reaction (PCR) as described earlier[7] with appropriate controls. Bidirectional population sequencing of purified products was done using an ABI 3100-*Avant* genetic analyzer (Applied Biosystems, USA). All the sequences were edited using the Seqscape

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Table 1. Demographic, Clinical, and Laboratory Characteristics of HIV-1-Infected ART-Treated and Nontreated Patients[a]

Characteristics	*Treated* (n = *16*)	*Nontreated* (n = *18*)
Gender		
Male, *n* (%)	10 (62.5)	11 (61)
Female, *n* (%)	6 (37.5)	7 (39)
Age (years)/mean (±SD)		
Male	37 (3.9)	33 (4)
Female	33 (3)	30 (2)
$CD4^+$ T cell count (cells/μl)/median (IQR)		
Baseline	161(53–182)	503 (366–672)
Follow-up	318 (55–302)	548 (315–885)
ART regimen		
AZT + 3TC + NVP or EFV, *n* (%)	6 (37.5)	None
d4T + 3TC + NVP or EFV, *n* (%)	8 (50)	
ddI + 3TC + NVP or EFV, *n* (%)	2 (12.5)	

[a]AZT, zidovudine; ddI, didanosine; d4T, stavudine; EFV, efavirenz; NVP, nevirapine; 3TC, lamivudine; ART, antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; SD, standard deviation.

software (Applied Biosystems, USA, version 2.5). The nucleotide variability and the Jukes–Cantor correction for multiple hits of the proportion of observed nonsynonymous (*dN*) and synonymous substitutions (*dS*) and its ratio (*dN/dS*) were determined by comparing the RT sequences isolated at two time points among nontreated (baseline and follow-up) and treated (baseline and treatment failure) patients using SynSCAN.[8] Similarly the codon sites evolving under the influence of positive Darwinian selection were identified by comparing the RT sequences isolated at two time points among nontreated and treated patients using HyPhy with the codon substitution model MG94.[9] Drug resistance mutations were identified using the Stanford HIV-1 drug resistance database (http://hivdb.stanford.edu/). The Mann–Whitney *U* test was used to compare the variables between the groups. The statistical analysis was performed using SPSS 13.0 statistical software (Chicago, IL). The GenBank accession numbers of the HIV-1 RT sequences described here are EU429988 through EU430023, EU545198 through EU545213, and EU545214 through EU545229.

The RT sequences from all the study subjects were subtype C and no intersubtype recombinants were observed. The intrapatient nucleotide variability [median (IQR)] of treated patients [5.3% (3.1–7.6)] was significantly higher ($p < 0.03$) compared to nontreated patients [1.9% (0.9–2.45)]. Similarly, the *dN*, *dS* significantly ($p < 0.001$) increased among treated [median (IQR): *dN* 0 (0–0); *dS* 0.02 (0–0.04)] compared to nontreated patients [median (IQR): *dN* 0.04 (0.02–0.04); *dS* 0.12 (0.05–0.15)]. Although previous studies have shown a reduction in the genetic variability of HIV during drug therapy,[10–12] higher intrapatient gene variability observed among the treated patients in the present analysis could be attributed to viral escape from drug pressure, thereby enhanced replication efficiency, which in turn led to greater genetic variation.[13] Several studies have found an inverse relationship between the rate of viral diversification and host disease progression,[14–18] whereas others have not.[19–21] The limitation of the present investigation is that the history of HIV seroconversion for the study population is not known and hence the consequence of drug treatment on the intrahost nucleotide variation could not be delineated from the duration of HIV infection. It should also be noted that earlier studies have shown that a higher selection pressure will be imposed by drug therapy[22–24] and the strains resistant to nucleoside reverse transcriptase inhibitors (NRTIs) can increase HIV-1 mutation frequencies.[25,26] However, in the present analysis, the ratio of *dN/dS* was <1 among the treated [median (IQR): 0.2 (0.08–0.25)] and nontreated patients [median (IQR): 0 (0–0)], which was similar to earlier reports.[27–31] This implies that the RT region is highly conserved because of structural/functional constraints and consequently any mutations in this region will be deleterious to the virus.[32,33]

Among nontreated patients, two (11%) had transient drug resistance mutations associated with NRTIs and nonnucleoside reverse transcriptase inhibitors (NNRTIs) such as V108IV (6%) and Y181CY (6%), respectively, at baseline, which was observed to be wild type at follow-up. Among the NRTI mutations observed among the treated patients, M184V (62.5%) was predominant, followed by T215F/Y (19%). Mu-

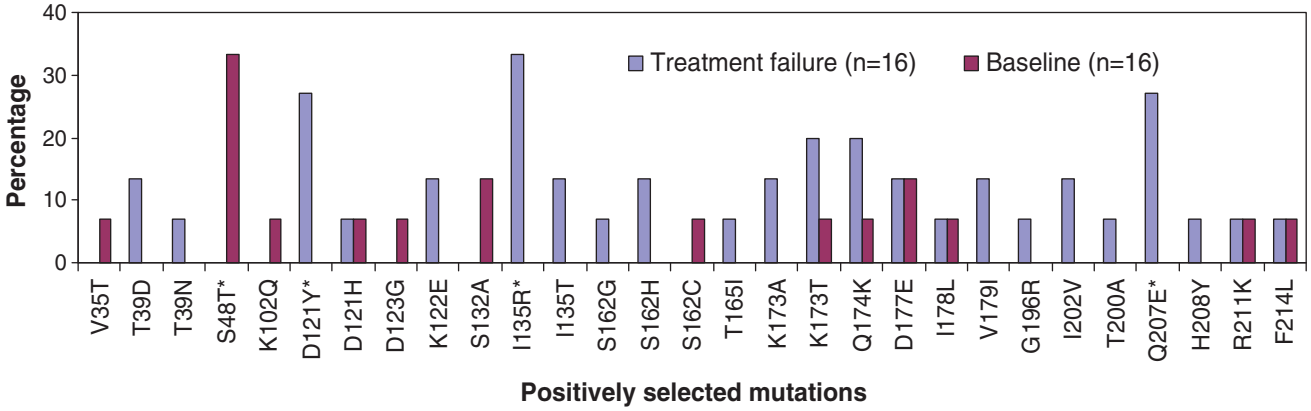

FIG. 1. Frequency of positively selected mutations in the RT sequences at baseline and treatment failure among the ART-treated patients. *$p < 0.05$ (Mann–Whitney *U* test). (Color image can be found at www.liebertonline.com/aid).

tations K219Q, M41L, D67N, and T69D was observed in 13% and K65R, K70R, V75I, V118F, and Q151M occurred in 6%. Among NNRTI mutations, Y181C (31%) was predominant followed by K103N/S, V106M, and G190A, which occurred in 25%. A98G/S and K101E were observed in 19% and 13%, respectively.

Positive selection of mutations D121Y, K122E, D123G, I135T, Q174R, I195L, I202V, Q207N, and R211K each at a frequency of 5.5% (1/18) was observed in the RT sequences amplified from nontreated patients. However, treated patients demonstrated positive selection ($dN/dS > 1$) of 29 mutations, among which the frequency of D121Y, I135R, and Q207E was observed to be significantly increased ($p < 0.001$) and the frequency of S48T significantly decreased ($p < 0.001$) during treatment failure compared to the baseline (Fig. 1). A study of B/C recombinants in China by Liao *et al*.[34] has shown that both D121Y and I135R are the common subtype C and subtype B polymorphisms, respectively. Moreover, in the same study, position 207 was also found to be a polymorphic site. However, mutations at position 135 and 207 have been reported to be associated with reduced susceptibility to nevirapine and zidovudine, respectively, in subtype B and D viruses.[35–39] Kantor *et al*.[40] reported that treatment has a greater effect at position 121 in subtype C viruses. Previous investigations have revealed that the positive selection of beneficial mutations is an important mechanism in HIV evolution, both for drug resistance[41,42] and immune escape.[43–47] Even though mutations at position 135 and 207 in RT are known to be associated with a reduction in susceptibility to nevirapine and zidovudine in HIV-1 subtype B and D viruses,[35–39] it is not clear if D121Y is related to drug resistance. Mutation S48T, observed to be negatively associated with treatment failure, is a common polymorphism that occurred at >50% of drug-naive patients in the present analysis similar to other studies of subtype C viruses.[7,48–50] This finding shows that mutation S48T might be deleterious in terms of viral replication in the presence of resistance mutations, thus increasing the level of the genetic barrier to drug resistance.

In conclusion, the present analysis reveals the higher selection pressure and genetic variability of HIV-1 subtype C RT during ART. Among the treated patients, a few positively selected mutations in RT not yet included as a candidate for drug resistance increased in frequency during treatment failure. A limitation of this study is the smaller sample size and population sequencing, which warrant further investigations that may provide new perspectives concerning the existence of polymorphisms that could influence the development of immune escape or drug resistance in HIV-1 subtype C viruses.

Author Disclosure Statement

No competing financial interests exist.

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