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Comparative analysis of pre-existing HIV drug resistance mutations in proviral DNA via Next-Generation Sequencing and routine HIV RNA genotyping

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Abstract

Background: We investigated whether deep sequencing of archived HIV DNA of antiretroviral-naïve persons with acute/early HIV infection could identify transmitted drug resistance mutations (DRM), per the IAS drug resistance algorithm, that are not detected by routine bulk (consensus) sequencing.

Methods: Deep sequencing of HIV DNA from peripheral blood mononuclear cells and consensus sequencing from concurrent blood plasma (BP) was performed from antiretroviral (ART)-naïve adults with recent infection. We compared the prevalence of low-frequency (2–20%) and high-frequency (>20%) non-nucleoside reverse transcriptase inhibitor (NNRTI), nucleoside reverse transcriptase inhibitor (NRTI), and protease inhibitor (PI) DRM.

Results: Overall, 190 individuals were included, 72 (37.9%) with acute, 20 (10.5%) very early, and 98 (51.6%) with recent HIV infection. While all DRM detected in plasma appeared in archived proviral DNA, 9 high-frequency mutations were only detected in HIV DNA. These included 3 NRTI mutations, 4 NNRTI mutations, 1 PI mutation, and 1 H221Y (associated rilpivirine resistance) mutation. When considering DRM <20%, 11 NNRTI and 7 NRTI, 6 PI, and 3 F227L (associated doravirine resistance) mutations were found exclusively in HIV DNA. Interestingly, while 2 high-frequency M184V appeared in both DNA and RNA, low-frequency M184I were exclusive to HIV DNA (n=6). No participants experienced virologic failure after initiating ART during the median 25.39 ± 3.13 months of follow-up on treatment.

Conclusion: Although most high-frequency DRMs were consistently detected in HIV RNA and HIV DNA, the presence of low-frequency DRM in proviral DNA may be relevant for clinicians as these mutations could become dominant under drug selection pressure.

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Keywords

HIV drug resistance; Next-Generation Sequencing; Proviral DNA; Antiretroviral therapy

INTRODUCTION

Drug Resistance Mutations and HIV Genotyping

Routine drug resistance testing via bulk HIV genotyping using Sanger Sequencing before the initiation of antiretroviral therapy (ART) is the current standard of care.[1] Approximately 10–19% of persons newly diagnosed with HIV in resource-rich settings harbor variants with at least one transmitted drug-resistant mutation (DRM).[2,3] Thus, evaluating the impact of pre-existing DRM is critical for first line ART decisions. Bulk genotyping can detect DRMs that are present at a frequency of at least 20%, however it will not detect most low-frequency DRM.[4] The more sensitive, next generation sequencing (NGS) of archived proviral DNA might provide additional insight into less prevalent DRM that may emerge under the selective pressure of antiretroviral therapy.[5]

Collectively, low-frequency resistance mutations among HIV quasispecies have been associated with a higher risk of virological failure, poor immune recovery, accumulation of DRMs, higher frequency of treatment changes, and death.[6] However, related studies have also demonstrated opposing views regarding detection of low-frequency DRMs and the potential for associated virological escape.[7]As part of this study, we performed deep sequencing of HIV DNA from peripheral blood before initiation of ART from 190 adults enrolled in the San Diego Primary Infection Resource Consortium (PIRC). We compared the prevalence of low-frequency (2–20%) and high frequency (>20%) non-nucleoside reverse transcriptase inhibitor (NNRTI), nucleoside reverse transcriptase inhibitor (NRTI), and protease inhibitor (PI) mutations in archived HIV DNA to standard genotyping of plasma HIV RNA to determine if DRM archived in HIV DNA was associated with subsequent virological failure while receiving ART.[8]

M184V and M184I are the most frequently selected NRTI mutations in persons with relapsed viremia while receiving lamivudine (3TC) or emtricitabine (FTC)-containing regimens.[9] However, the detection of transmitted M184V/I is relatively low in ART naïve persons with HIV (PWH) (7%, n=14/204).[10] likely due to rapid reversion because of reduced replication capacity.[11] As the rapid replacement of M184V/I is associated with known fitness costs,[12] less is known regarding whether earlier sampling time and sequencing methods are relevant to successfully detecting these mutations. To better define this potential relationship, we analyzed participants with various DRM profiles and investigated any characteristic differences between those with and without transmitted M184V/I mutations.

MATERIALS AND METHODS

Study Cohort

This study included adults, age ≥18 years, with acute/early HIV infection enrolled in the San Diego PIRC. Individuals who met serologic and virologic criteria for acute and early

HIV infection was tested using a single-well limiting-antigen avidity enzyme immunoassay (Lag-Avidity EIA) and enrolled based on their estimated date of infection (EDI) calculated as previously reported.[13, 14] Briefly, EDI status is defined as follows: Acute (antibody [HIV Ab-]/antigen [Ag+]), Very Early (Ab+/Recency Assay consistent with infection <133 days), and Recent (recency assay consistent with infection <180 days or Ab+ with documented Ab- in last 365 days).[13, 14] Study participants were screened for NNRTI, NRTI, and PI mutations in blood plasma at baseline (bulk genotyping) before ART initiation (see Table S1; detailed list of transmitted DRM and frequencies). Participants were encouraged to initiate ART as soon as possible, often at the baseline visit (Day 0).

Plasma HIV RNA levels were measured at baseline (Day 0), weeks 2, 4, 8, 12, 24, 36, 48, and every 24 weeks thereafter (Cobas Amplicor HIV-1 test, Roche Molecular Systems, Pleasanton, California). Viral rebound was defined as a single viral load > 50 copies/mL after an undetectable level as previously described.[15,16] Samples were collected from January 2005 to February 2020 (Table S2). All individuals provided an informed, written consent approved by local institutional review before participation in this study.

Sequence Analysis

Blood Plasma—Bulk genotyping of HIV reverse transcriptase (RT) was performed to identify transmitted drug resistance (GeneSeq HIV; Monogram Biosciences, Inc., South San Francisco, CA or Viroseq v.2.0; Celera Diagnostics, Alameda, CA) in blood plasma before ART initiation.[17,18] Cumulative HIV RNA genotypes based on the IAS drug resistance algorithm[19] for each participant were created by adding up all mutations from genotypic tests for drug classes as previously described.[20]

Archived Proviral DNA—From the same blood samples used to assess blood plasma, NGS of HIV partial pol was performed to identify DRMs present in archived proviral DNA (peripheral blood mononuclear cells (PBMC)). Genotypic resistance testing on PR, RT, and IN from proviral HIV-1 DNA was performed using a modified version of the “deepTypeHIV” assay (SeqIT GmbH & Co. KG, Kaiserlautern, Germany). Briefly, genomic DNA was isolated from patient PBMCs (10^6 cells) using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Polymerase chain reaction (PCR) and nested PCR of PR and partial RT were carried out as previously described.[21] Amplicons were purified and quantified as previously described.[21] Only DRMs present at ≥2% of cleaned mapped reads were considered. APOBEC hypermutation filtering was applied to reduce the frequency of potentially spurious mutations.[22] NGS DRMs were categorized as high frequency and low frequency DRMs defined as >20% and 2–20% frequencies, respectively.

Statistical Analysis

Wilcoxon rank-sum tests were used to compare (1) time from EDI to first ART and (2) time from EDI to sampling date between those with and without M184V/I DRMs.

RESULTS

Cohort Characteristics

Of the 190 participants, EDI classifications were 37.9% acute (n=72/190), 10.5% very early (n=20/190), and 51.6% recent (n=98/190). Participants began ART a median of 82 days [IQR:34;139] after the EDI (Table S2).

DRM Prevalence (HIV RNA versus Archived Proviral DNA)

Overall, we detected NRTI, NNRTI and PI DRMs in 49 out of 190 participants (26%) through either standard sequencing or NGS methods. Standard genotyping of plasma HIV RNA detected DRMs in 22 out of 188 participants (12%). Specifically, we detected 4 high frequency NRTI (2 M184V, 2 M41L) mutations, 19 high frequency NNRTI (13 K103N/S, 6 E138A/G) mutations, and 3 high frequency PI (1 L90M, 1 M46I/L, 1 V82A/T) mutations. All the 26 NRTI, NNRTI, and PI DRMs were also detected in proviral DNA. An additional 9 high-frequency DRMs were detected exclusively in proviral DNA, including 3 NRTI (1 K70E, 1 L210W, 1 K219Q/E) mutations, 4 NNRTI (3 K103N/S, 1 Y188L) mutations, 1 PI (D30N) mutation and 1 H221Y (associated rilpivirine resistance) mutation (Other DRMs) (Fig. 1). Overall, 30 participants (16%) had detectable high frequency NRTI, NNRTI or PI DRM in either HIV RNA or HIV DNA.

We also detected 27 low-frequency NRTI, NNRTI, and PI DRMs in archived HIV DNA, including 8 NRTI (6 M184V/I, 1 L74V/I, 1 M41L) mutations, 11 NNRTI (7 M230L/I, 4 E138A/G) mutations, 5 PI (1 I50V, 3 M46I/L, 1 V82A/T) mutations and 3 F227L (associated doravirine resistance) mutations (Other DRMs). The low-frequency DRMs were all considerably lower than the 20% threshold aside from one E138A mutation with a frequency of 14%. Overall, 23 participants (12%) had detectable low frequency DRM in HIV DNA.

3TC/FTC ART regimen and M184V/I

A total of 137 (72%) of the 190 participants started ART that included either lamivudine (3TC) or emtricitabine (FTC). Eight of these 137 participants had M184V/I DRM detected prior to the start of ART. While 2 high frequency M184V mutations were detected in both archived DNA and HIV RNA samples, 6 low-frequency M184I mutations were found exclusively in HIV DNA (Fig 1). The 2 high frequency M184V mutations were found in participants who subsequently initiated FTC regimens, while 4 out of the 6 low-frequency M184I mutations were found in participants who subsequently initiated started 3TC/FTC regimens (Fig. 2). These individuals did not differ by demographics or HIV history compared to the entire cohort. The median pre-ART viral load and time from EDI to first ART were not statistically different ($P = 0.6672$). Additionally, the time from EDI to sampling date was not statistically different ($P = 0.5755$). Of note, the 8 participants began ART a median of 79 days [IQR:69;171] after the EDI.

HIV RNA level and Viral Rebound

Longitudinal viral loads for treated participants were monitored with an average follow-up of 25.39 ± 3.13 months following the start of ART. Despite identifying both high frequency

and low frequency DRMs in either archived proviral DNA or blood plasma, there was no evidence of viral rebound from any participants during the entire follow-up period, including the individuals identified with M184V/I and associated 3TC/FTC treatments (Fig.2) (see Table S3; detailed list of baseline ARV treatments).

DISCUSSION

Studies have shown that standard genotyping of HIV DNA detects significantly fewer resistance mutations than RNA genotyping.[23] However, deep sequencing of HIV DNA at lower sensitivity thresholds detected more mutations than standard HIV RNA genotyping. Our cohort similarly reaffirmed that NGS of archived DNA detected more DRMs than bulk genotyping of blood plasma. Specifically, we detected 5% more high-frequency DRM and 14% low frequency DRM in proviral DNA when compared to standard HIV RNA genotyping. It is unclear whether the DRM (low frequency or high frequency) identified in archived proviral DNA has any clinical significance and contributing to viral rebound or failure. Regarding M184V/I, there was no association between the detection and earlier DRM sampling dates following EDI. However, the detection of the 6 low frequency M184I only in archived DNA still suggests NGS as the more appropriate method of detection for these mutations. As previously reported,[24] we found no association between the presence of archived M184V/I and subsequent virological failure even in 4 participants who started 3TC based ART regimens.

Our observations are limited by the small cohort sample size, the short duration of follow-up for most of the participants, and other factors, such as possible differences in medication adherence. Additionally, although we used stored PBMC samples, our comparative analysis was limited because we did not have available plasma for NGS. Considering this limitation, we recognize previous studies which have validated NGS of RNA as a more sensitive method that detects additional minority mutations.[25] Potential determinants for the lack of viral rebound include starting treatment early and varying participants follow-up periods. “Considering the lack of follow-up DRM data, we acknowledge that M184V/I may have been present and further disappeared in plasma but archived in proviral DNA samples. As previously mentioned, FTC containing regimens are known to select for the presence of M184V/I if present.[9] In our cohort, 6/8 (2/2 high frequency and 4/6 low frequency) M184V/I mutations were identified in participants with FTC containing regimens. Moreover, we did not investigate integrase inhibitors (INSTI) due to the lack of sequencing data. A recent systematic review has shown that INSTI-associated transmitted drug resistance (TDR) is infrequent, revealing an overall median surveillance DRM prevalence of 0.5% over 75 related studies that had available sequences.[26] Additionally, INSTI regimens have been previously associated with minimizing M184V/I impact on virological response.[27] In our study, we found no evidence of virological failure in participants with preexisting M184V/I. This is in line with a recent study evaluating the impact of M184V/I on virologic response after switching to bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF). Here, the authors found that switching to B/F/TAF demonstrated durable efficacy in maintaining viral suppression including in those with preexisting M184V/I.[27] These limitations could lead us to underestimate the negative impact of detected archived DRM during primary infection on first-line ART.

Conversely, it is known that the mutation K65R is of concern as it confers cross-resistance to almost all other NRTIs.[28] Of note, none of the participants in our cohort had any evidence of K65R via bulk genotyping or NGS. Although ART regimens may be susceptible to DRMs present at baseline, beginning treatment early ensures that DRMs remain latent in the reservoir rather than continuously expanding.[29] Similar to preexisting studies, early ART initiation of our cohort after EDI led to sustained viral suppression despite existing DRMs. Overall, comparing deep sequencing of archived DNA and routine genotyping of HIV RNA has revealed that HIV DNA detects additional high frequency and low frequency DRMs. Given that we did not identify any effect on long term virologic outcomes, additional studies are necessary to determine if HIV proviral NGS has clinical applications prior to recommending performing pre-treatment genotypes on DNA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1. HIV Drug Resistance Mutation (DRM) prevalence

27 Low frequency DRMs were detected in archived DNA (14.2%). For high frequency DRMs, there were 3 nucleoside reverse transcriptase inhibitor (NRTI) mutations (1.6%, top and bottom right), 4 non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations (2.1%, top left), 1 protease inhibitor (PI) mutation (0.5%, bottom left) and 1 H221Y mutation (0.5%, middle left) (associated rilpivirine resistance)[19] found in only archived DNA. 2 high frequency M184V mutations appeared in both PBMC and blood plasma, 6 low-frequency M184I were found only in PMBC. Mutations detected in HIV RNA are represented in red. Mutations detected in HIV DNA were represented in blue for less than 20% and orange for greater than 20%.

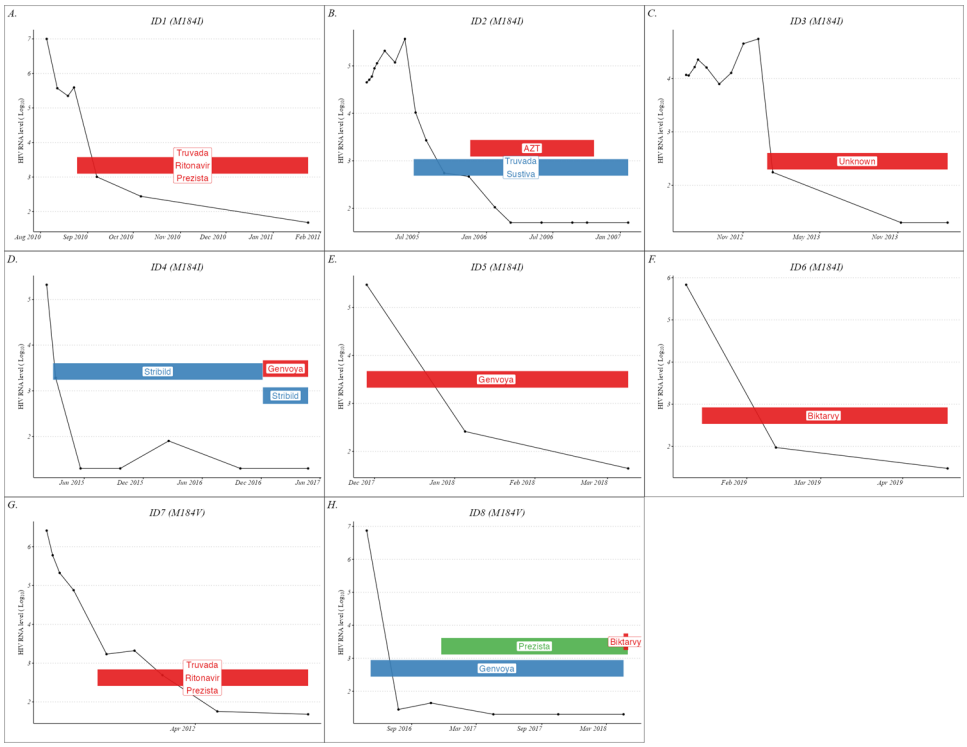

FIGURE 2. Viral Load Trajectory and ART Regimen in 8 participants harboring M184V/I

We detected 6 low-frequency M184I and 2 high frequency M184V mutations. Among them, 75% (2/2 M184V; 4/6 M184I) had 3TC/FTC regimens. Each horizontal bar represents different drug regimens for each participant.