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BRIEF REPORT

Use of Drug-level Testing and Single-genome Sequencing to Unravel a Case of Human Immunodeficiency Virus Seroconversion on Pre-exposure Prophylaxis

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Cases of seroconversion on pre-exposure prophylaxis (PrEP) should be carefully investigated, given their public health implications and rarity. We report a case of transmitted drug resistance causing seroconversion on PrEP in spite of high adherence, confirmed with dried blood spot and segmental hair drug-level testing and single-genome sequencing.

Keywords. PrEP; HIV seroconversion; adherence; single-genome-sequencing; PrEP failure.

For men who have sex with men, pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) is approximately 99% effective when taken daily and 96% effective when taken at least 4 times weekly, based on previous pharmacokinetic-pharmacodynamic modeling studies [1]. PrEP, in addition to facilitating increased diagnoses of human immunodeficiency virus (HIV) infections, providing HIV treatment and treatment as prevention, and aiding responses to HIV outbreaks, is a key component of the US End the HIV Epidemic Initiative.

PrEP failure can result from either low adherence or the acquisition of a resistant virus [2–7]. Objectively measured adherence in PrEP users around the time of suspected HIV seroconversion can help unravel whether PrEP failure resulted from inadequate adherence [2–7]. Segmental hair analysis is a novel technique that can be performed after a thatch of hair is cut into 1 cm segments, starting at the root, with each segment corresponding to 1 month of drug ingestion; this permits the assessment of PrEP adherence patterns over longer periods of time, depending on the hair length [2, 3]. To assess whether seroconversion occurred due to transmitted resistance, single-genome sequencing (SGS) can look for minority resistance mutations and estimate the duration of HIV infection after seroconversion [8]. Here, we describe a case of seroconversion on PrEP with a resistant virus in the setting of 100% self-reported adherence, with both adherence metrics (drug levels in hair and dried blood spots [DBS]) and SGS verifying the likely etiology of failure.

CASE REPORT

A 44-year-old man started daily TDF/FTC–based PrEP in December 2017 after a negative antigen/antibody (Ag/Ab) HIV test (Bioplex 2200) the day prior. He reported 100% adherence to PrEP, and HIV Ag/Ab tests were performed quarterly throughout 2018 and 2019, remaining negative throughout 2018, as well as in January and April 2019. He engaged in primarily insertive anal intercourse, with rare receptive anal intercourse, and did not use condoms.

In the beginning of June 2019, the participant complained of headache, sore throat, and chills. The physical examination demonstrated only cobblestoning of the oropharynx without exudate. A white blood cell count returned at 2800/microliter; the platelet count was 99 000/microliter. A fourth-generation HIV Ag/Ab test was reactive, but an HIV-1/HIV-2 antibody differentiation assay (Geenius) returned negative that day. He was told by phone that he had likely received a false-positive HIV test, given his high self-reported PrEP adherence. However, an HIV RNA level, also sent from that visit, returned 2 weeks later as 3 100 687 copies/milliliter, with a CD4+ count of 195 cells/mm^3 (21%). After the result returned, the TDF/FTC was discontinued and bictegravir/tenofovir alafenamide (TAF)/FTC was initiated; the viral load at this 2-week visit was 146 000 copies/milliliter. An HIV genotype (Quest) performed at the HIV treatment initiation visit returned with M184V, K70N, V179R, and P225H mutations in the reverse transcriptase (RT) gene.

METHODS

The patient provided written informed consent to enroll in the SeroPrEP study: a study of PrEP seroconversion

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approved by the Institutional Review Board of the University of California, San Francisco (NCT#02656511). A 4 cm–long hair sample was collected and analyzed at the University of California, San Francisco, Hair Analytical Laboratory in 1 cm segments; a DBS sample collected that day was analyzed at the Colorado Antiviral Pharmacology Laboratory [3]. Both laboratories use validated liquid chromatography tandem mass spectrometry methods to analyze concentrations of PrEP drugs/metabolites, as approved by the Clinical Pharmacology and Quality Assurance program. A stored serum sample collected 2 weeks prior to antiretroviral therapy initiation was sent for SGS at the National Cancer Institute's HIV Dynamics and Replication program, to look for minority resistance mutations [8].

RESULTS

The tenofovir-diphosphate concentration in the DBS sample collected on the day of HIV treatment initiation was 1683 femtomole/punch, consistent with estimated daily (7 days a week) adherence to TDF/FTC over the preceding 6 weeks. The tenofovir (TFV) level in the proximal 1 cm of hair, corresponding to the 4-week period prior to antiretroviral therapy initiation, was 0.035 nanograms (ng)/milligram (mg), consistent with dosing 7 days a week. The TFV hair concentration was 0.028 ng/mg in the 1 cm segment corresponding to 4–8 weeks prior to sample collection, consistent with adherence 5–6 times weekly. SGS identified a highly homogenous viral population, with all sequences containing the K70N, M184V, V179E, and P225H mutations in the RT gene, with an average pairwise distance of 0.05%. Minority resistance mutations were not observed. A partner genotype was not available in this case (Figure 1).

DISCUSSION

HIV seroconversion on PrEP can result from either poor adherence to PrEP or the acquisition of a resistant virus. Due to the public health concerns raised by PrEP failures resulting from the circulation of drug-resistant virus, it is important to examine the etiology of failure in such cases. Reports of PrEP failure from transmitted drug resistance should be interpreted only after objective adherence assessments over varying time periods and either an analysis of SGS from around the time of acquisition or comparison with a partner genotype. Inadequate adherence is the most common etiology of PrEP failure, and a comprehensive adherence assessment using objective metrics is required, given the limitations of self-reports [2].

Based on DBS and hair data from this case, average adherence to TDF/FTC around the time of the likely HIV infection was estimated to be at least 6 times a week, and likely closer to 7 times a week; these adherence levels are expected to confer very high efficacy based on previous pharmacokinetic-pharmacodynamic modeling studies [1]. SGS of serum obtained while the patient was still on PrEP, but after acquiring HIV, identified a highly homogeneous population of viral sequences. V179E and P225H are nonnucleoside reverse transcriptase inhibitor mutations (NNRTI), which would not be selected by TDF/FTC-2 drug therapy and were therefore transmitted. M184V is a resistance mutation in the RT gene, usually acquired in the setting of FTC/lamivudine exposure, eliminating FTC susceptibility. K70N is a rare, nonpolymorphic mutation that causes low-level resistance to TDF, selected among patients receiving tenofovir-based

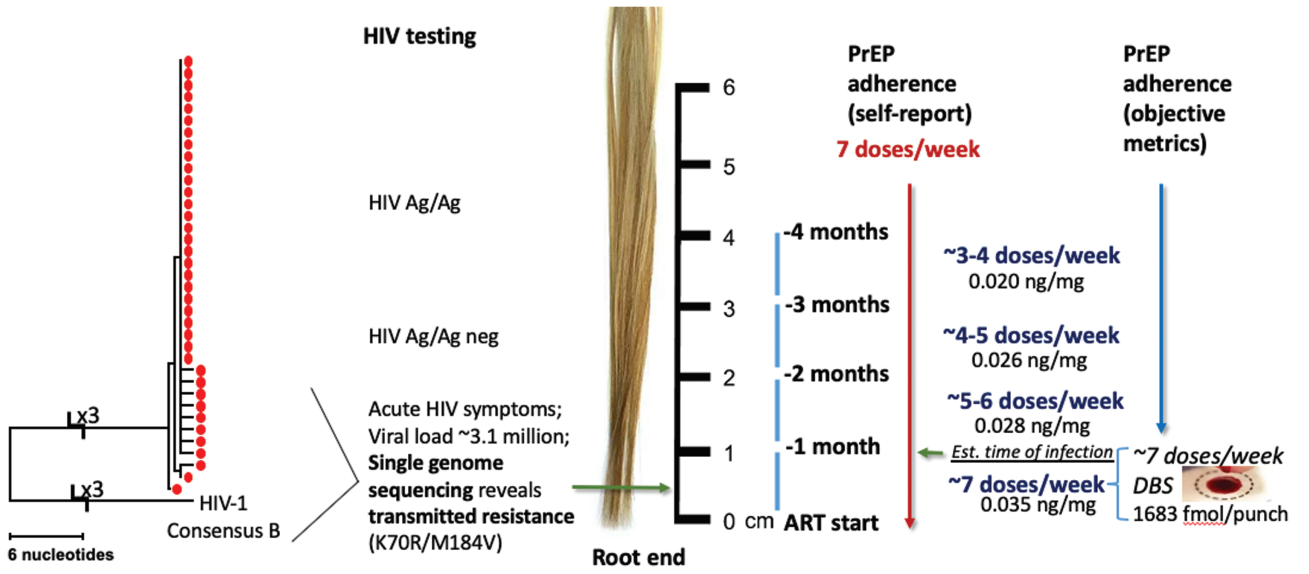

Figure 1. Illustration of adherence patterns and single-genome sequencing in this case, indicating that the patient likely acquired a transmitted, drug-resistant HIV strain while on PrEP with high adherence. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; PrEP, pre-exposure prophylaxis; DBS, dried blood spot.

compounds. Although a partner genotype was not available in this case, the etiology of PrEP failure was most likely transmission of a drug-resistant virus, as evidenced by a homogeneous viral population harboring both NRTI and NNRTI mutations, indicating an FTC-resistant and partially TDF-resistant virus, combined with PrEP drug levels in hair and DBS showing very high adherence around the time of HIV acquisition.

PrEP failure with resistance is most likely to occur in the setting of inadequate PrEP adherence and subsequent selective pressure on the virus with ongoing 2-drug PrEP after HIV seroconversion, resulting in the development of drug resistance [9]. The majority of resistance mutations observed in the setting of HIV seroconversion in PrEP clinical trials occurred when PrEP was inadvertently initiated during acute HIV infection, with the M184V mutation in particular developing very rapidly (within days) under selective pressure from only 2 NRTIs [10]. However, rarely, PrEP failure can occur under conditions of high adherence due to transmission of a resistant HIV virus. Despite high adherence, 6 cases of PrEP acquisition, which were verified via objective metrics, have been previously reported [2–7]. Of the 6 cases, 5 occurred with likely transmitted drug resistance to TDF and/or FTC (with all 5 having an M184V mutation). Notably, only 1 of these prior cases definitively confirmed transmission of resistance via SGS analysis and a paired partner genotype (Supplementary Table) [2].

Fewer than 1–3% of people living with HIV with unsuppressed viral loads harbor resistance mutations to either FTC or TDF/TAF in the United States, with resistance to both drugs being even rarer [2]. M184V is the most common resistance mutation impacting the NRTI class [2]. In a study with macaques, PrEP maintained high efficacy against viruses harboring M184V alone, likely because of retained tenofovir susceptibility and because M184V leads to compromised HIV viral fitness [11]. However, 6 of the 7 reported cases of PrEP failure among humans with high adherence that were due to transmitted drug resistance demonstrated a M184V-containing virus, and 4 of these cases had FTC resistance alone (Supplementary Table). The epidemiologic rarity of viruses resistant to FTC and/or TDF/TAF, the negative impact of M184V on viral fitness, and the high efficacy of PrEP contribute to the rarity of seroconversion on PrEP due to transmitted drug resistance.

Although this report supports transmitted acquisition of HIV, rather than the development of HIV drug resistance while on 2-drug PrEP, it is important to note that these cases remain exceedingly rare. For instance, not a single case of HIV seroconversion was reported over 3 years from a large cohort of patients on PrEP within an integrated health system [12]. Redoubling efforts to improve population-level viral suppression rates, such as those being intensified through the US End the HIV Epidemic Initiative, will make these events even rarer. Targeted outreach to people living with HIV with unsuppressed viral loads who are harboring resistance mutations to PrEP's components could be considered to prevent the transmission of potentially PrEP-resistant viruses.

In summary, we report a rare case of likely PrEP failure due to transmitted drug resistance, with both high adherence via objective adherence metrics and SGS analysis supporting this conclusion. The SGS analysis renders the transmission of a drug-resistant virus likely, as opposed to the evolution of resistance when PrEP was continued after HIV acquisition. Such cases, due to their public health implications, should be carefully adjudicated, and remain rare in spite of increasing PrEP use worldwide.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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References

1. Anderson PL, Glidden DV, Liu A, et al. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. Sci Transl Med **2012**; 4:151ra25.
2. Cohen SE, Sachdev D, Lee SA, et al. Acquisition of tenofovir-susceptible, emtricitabine-resistant HIV despite high adherence to daily pre-exposure prophylaxis: a case report. Lancet HIV **2018**; 6:E34–50.
3. Thaden JT, Gandhi M, Okochi H, Hurt CB, McKellar MS. Seroconversion on pre-exposure prophylaxis: a case report with segmental hair analysis for timed adherence determination. AIDS **2018**; 32:F1–4.
4. Colby DJ, Kroon E, Sacdalan C, et al. Acquisition of multidrug-resistant human immunodeficiency virus type 1 infection in a patient taking preexposure prophylaxis. Clin Infect Dis **2018**; 67:962–4.
5. Knox DC, Anderson PL, Harrigan PR, Tan DH. Multidrug-resistant HIV-1 infection despite preexposure prophylaxis. N Engl J Med **2017**; 376:501–2.
6. Hoornenborg E, Prins M, Achterbergh RCA, et al; Amsterdam Pre-Exposure Prophylaxis (PrEP) Project team in the Human Immunodeficiency Virus (HIV) Transmission Elimination Amsterdam Consortium (H-TEAM). Acquisition of wild-type HIV-1 infection in a patient on pre-exposure prophylaxis with high intracellular concentrations of tenofovir diphosphate: a case report. Lancet HIV **2017**; 4:e522–8.
7. Markowitz M, Grossman H, Anderson PL, et al. Newly acquired infection with multidrug-resistant HIV-1 in a patient adherent to preexposure prophylaxis. J Acquir Immune Defic Syndr **2017**; 76:e104–6.
8. Palmer S, Kearney M, Maldarelli F, et al. Multiple, linked human immunodeficiency virus type 1 drug resistance mutations in treatment-experienced patients are missed by standard genotype analysis. J Clin Microbiol **2005**; 43:406–13.
9. Mishra K, Huang J, Daskalakis D, Udeagu C. The impact of PrEP on drug resistance and acute HIV infection, New York City 2015–2017 [abstract# 107]. In: Program and abstracts of the Conference on Retroviruses and Opportunistic Infections (Boston, MA). Topics in Antiviral Medicine. International Antiretroviral Society-USA. San Francisco, California, **2019**.

10. Liegler T, Abdel-Mohsen M, Bentley LG, et al; *Iniciativa Profilaxis Pre-Exposición; or Pre-Exposure Prophylaxis Initiative in English* Study Team. HIV-1 drug resistance in the iPrEx preexposure prophylaxis trial. J Infect Dis **2014**; 210:1217–27.
11. Cong ME, Youngpairoj AS, Zheng Q, et al. Protection against rectal transmission of an emtricitabine-resistant simian/human immunodeficiency virus SHIV162p3M184V mutant by intermittent prophylaxis with Truvada. J Virol **2011**; 85:7933–6.
12. Marcus JL, Hurley LB, Hare CB, et al. Preexposure prophylaxis for HIV prevention in a large integrated health care system: adherence, renal safety, and discontinuation. J Acquir Immune Defic Syndr **2016**; 73:540–6.