Transmitted Drug Resistance and Phylogenetic Relationships Among Acute and Early HIV-1–Infected Individuals in New York City

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Background: Transmitted drug resistance (TDR) is critical to managing HIV-1–infected individuals and being a public health concern. We report on TDR prevalence and include analyses of phylogenetic clustering of HIV-1 in a predominantly men who have sex with men cohort diagnosed during acute/recent HIV-1 infection in New York City.

Methods: Genotypic resistance testing was conducted on plasma samples of 600 individuals with acute/recent HIV-1 infection (1995–2010). Sequences were used for resistance and phylogenetic analyses. Demographic and clinical data were abstracted from medical records. TDR was defined according to International AIDS Society–USA and Stanford HIV database guidelines. Phylogenetic and other analyses were conducted using PAUP*4.0 and SAS, respectively.

Results: The mean duration since HIV-1 infection was 66.5 days. TDR prevalence was 14.3% and stably ranged between 10.8% and 21.6% (P\text{trend} = 0.42). Nucleoside reverse transcriptase inhibitors resistance declined from 15.5% to 2.7% over the study period (P\text{trend} = 0.005). M41L (3.7%), T215Y (4.0%), and K103N/S (4.7%) were the most common mutations. K103N/S prevalence increased from 1.9% to 8.0% between 1995 and 2010 (P\text{trend} = 0.04). Using a rigorous definition of clustering, 19.3% (112 of 581) of subtype B viral sequences cosegregated into transmission clusters and clusters increased over time. There were fewer and smaller transmission clusters than had been reported in a similar cohort in Montreal but similar to reports from elsewhere.

Conclusions: TDR is stable in this cohort and remains a significant concern to both individual patient management and the public health.

Key Words: transmitted drug resistance, TDR, HIV-1, acute infection, phylogenetic analysis, MSM, NYC

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INTRODUCTION

Transmission of antiretroviral resistant HIV-1 variants [transmitted drug resistance (TDR)] is a dynamic process that varies temporally and geographically.\textsuperscript{1–3} Monitoring TDR trends informs current understanding of local drug resistance and treatment practices particularly in situations where initiating therapy is urgent. Characterizing HIV-1 during the acute or recent infection stage (AHI) may prove beneficial at the individual level\textsuperscript{4} and at the population level.\textsuperscript{5,6} Genotypic information obtained from resistance testing for patient management was used to describe transmission dynamics of HIV-1 infection in many settings including Canada,\textsuperscript{7} the United Kingdom,\textsuperscript{8,9} Switzerland, and the United States.\textsuperscript{10,11} Some studies suggest that there is more onward transmission of HIV-1 during AHI, likely due to high levels of viremia that characterize this infection phase.\textsuperscript{12} In some risk groups such as men who have sex with men (MSM), transmission during AHI may potentially have significant population impact due to behavioral–biological synergy: highly infectious individuals engaging in high-risk behaviors.\textsuperscript{13,14} Although some of these studies can be generalized to the local HIV-1 epidemic, sequence-linked demographic, behavioral, or clinical information are limited and our understanding of the drivers of these phylogenetic patterns remains incomplete.

In this study, we update patterns of TDR in HIV-1–infected individuals in New York City (NYC) and report sociodemographic and clinical correlates. Furthermore, we use phylogenetic analyses to examine the level of relatedness...
in viral sequences in this sample and correlate those measures to patient characteristics. Findings from these analyses will inform our understanding of TDR trends and AHI in NYC.

MATERIALS AND METHODS

Study Sample

All AHI participants were screened at the Aaron Diamond AIDS Research Center, Rockefeller University Hospital (RUH), between January 1995 and December 2010. Patients enrolled between 1995 and 2004 were reported previously1,2 but were included here for analyses of TDR trends and phylogenetic clustering. Participants were defined as AHI based on documentation of plasma HIV-1 RNA levels above 5000 copies per milliliter, with contemporaneously negative/indeterminate HIV enzyme immunoassay (EIA) or Western blot. Recent infections were defined by the following criteria: a positive HIV-1 EIA or Western blot and a documented negative HIV-1 EIA within the previous 6 months or a less sensitive (detuned) EIA optical density (OD) ≤0.5 for infection within 90 days and between 0.5 and 1 OD for infection within 180 days. Case definition was changed in 2002 to include patients who produced a documented negative HIV-1 EIA within the previous 12 months. The duration of infection was estimated as follows: (1) in patients who were symptomatic, estimated date of infection was 14 days before the onset of symptoms; (2) in participants who were asymptomatic, we used an algorithm developed by the Acute Infection and Early Disease Research Program15,16 in which the infection date was estimated as 24 days before the date of the preseroconversion EIA date; and (3) among patients with a nonreactive detuned, the duration was estimated as 72 days when the OD ≤0.4, and among those with an OD >0.4, the duration was estimated as 45+ (150×OD).15 Finally, in patients with a reactive detuned enzyme-linked immunosorbent assay and a documented negative and a first positive serology for HIV-1, the duration of infection is the midpoint between the 2 test dates minus 24 days. Written informed consent was obtained from all participants. The RUH Institutional Review Board approved the study protocol.

Sample Data Collection

Sociodemographic characteristics were obtained for study participants during registration at the RUH outpatient clinic. Medical, sexual, and drug-using histories were abstracted from the medical records. CD4+ T-cell counts were measured using fluorescent-activated cell sorter. HIV-1 RNA levels were measured in plasma samples using the Roche Amplicor HIV-1 Monitor v1.5 polymerase chain reaction assay (Roche Diagnostics, Branchburg, NJ).

Genotype Determination

HIV-1 RNA was extracted from pretreatment plasma samples using QIAmp viral extraction kit (Qiagen, Valencia, CA). This RNA then underwent single-tube reverse transcriptase–polymerase chain reaction and was used for nucleotide sequence analysis of the protease gene (codons 4–99) and reverse transcriptase (codons 1–247) using the TRUGENE HIV-1 G9 genotyping kit (Siemens Diagnostics, Berkeley, CA) and OpenGene DNA sequencing system as per protocol. Before 1999, genotypic analysis was performed as previously described.13

TDR Definition

Drug resistance mutations were classified using the International AIDS Society–USA consensus guidelines17 and the Stanford University HIV-1 Drug Resistance Database (http://hivdb.stanford.edu). Antiretroviral (ARV) resistance was defined by mutations at the following positions: M41L, A62V, K65R, D67N, T69ins, K70R, L74VI, Y115F, F116Y, Q151M, M184VI, T210W, T215YF, and K219QE for nucleoside reverse transcriptase inhibitors (NRTIs); L100I, K101EP, K103NS, V106AM, Y181CSV, Y188CLH, G190ASC, and M230L for nonnucleoside reverse transcriptase inhibitors (NNRTIs); and D30N, V32I, M46IL, I47VA, G48VM, IS0LV, IS4VTALM, L76V, V82AFTS, IS4V, N88S, and L90M for protease inhibitors (Pis). “Any resistance” was defined by the presence of any of the mutations cited above. Multidrug resistance was defined as dual-class resistance if mutations were present in only 2 ARV classes and as triple-class resistance if mutations were in 3 ARV classes: NRTIs, NNRTIs, and PIs. The prevalence of surveillance drug resistance mutations was also determined using the World Health Organization’s drug resistance definition.18

Statistical Analysis

Descriptive statistics were computed overall and by study period. Temporal trends were tested using the Cochran–Armitage or the χ² test of linear trend for categorical binary and analyses of variance for continuous variables, treating study period as a semicontinuous variable. The χ² test of independence and Wilcoxon-signed rank test were used to determine difference between any study period for categorical and continuous variables, respectively. Crude and adjusted prevalence ratios and 95% confidence intervals for the associations with selected correlates of TDR were estimated using logistic regression models.20 Analyses were performed using the SAS statistical software version 9.2 (SAS Institute, Cary, NC).

Geospatial Analysis

The geographic distribution of participants residing in NYC at the time of diagnosis was plotted using shape maps of United Hospital Fund (UHF) neighborhoods obtained from New York City Department of Health and Mental Hygiene (NYCDOHMH). TDR as a fraction of all study participants from that UHF within the sample was plotted. Because the sample was mostly male, the average prevalence of new male HIV diagnoses per 100,000 men was tabulated using data from NYCDOHMH and the US Census Bureau and plotted as the underlay, with cumulative AHI from our sample as the overlay. NYC prevalence was defined as the number of new HIV-1 diagnoses in men for each year between 2001 and 2009 at the UHF level relative to the population size for that
UHF, projected from the 2000 US Census Bureau data. Socioeconomic status of UHF was abstracted from the NYCODMH community health profile database. Each dot represented at least 5 male cases in our sample from that UHF during the 10-year period. Maps were generated using the ArcView software, ArcGIS v9.2 (ESRI, Redlands, CA).

Phylogenetic Analysis
Available viral sequences (n = 600) from TRUGENE spanning codons 3–99 of protease and 38–223 of reverse transcriptase and from in-house sequencing for fragments preceding 1999 were cut and concatenated to the same fragment length of 845 nucleotides. Sequences were multiple aligned using Clustal W in Bioedit v7.0.8 (Ibis Biosciences), gap squeezed at a tolerance level of 100% using the gap strip/gap squeeze tool on the LANL site (http://www.hiv.lanl.gov/content/sequence/GAPSTREEZE/squeeze_ready.html), and manually edited to complete alignment. HIV-1 subtype was determined using the REGA Subtyping Tool v2.0 (http://www.bioafrica.net/rega-genotype/html/subtypinghv.html).22 Sequences with bootstrap values <95% or no subtype assignment in REGA were assessed separately using the recombinant identification program (RIP 3.0) (http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html) and the jumping profile Hidden Markov Model.23 To minimize the bias due to convergent evolution from ARV selective pressures, codons for relevant positions in the protease region (30, 46, 50, 82, 84, and 90) and in the reverse transcriptase region (41, 67, 70, 101, 103, 106, 181, 184, 188, and 215) were deleted from aligned sequences. Separate data sets were created for the 2 larger subtype groups, B and CRF02_AG, for transmission cluster analysis, and sequences of the same subtype sampled at the same time were used to determine whether the non-B subtypes in our cohort that coclustered were true clusters.

Defining Transmission Clusters
The modaltest program was used to determine model parameters (PAUP*4.0) for the entire sequence set and for the subtype B sequences only. Monophyletic clusters were derived from multiple alignments of all concatenated subtype B sequences. The HXB2 (Genbank accession number: K03455) reference strain and NL4.3 were included to detect possible contamination. Within-subtype sequences were used to construct neighbor-joining phylogenies. Final phylogenetic relationships were determined using PAUP* version 4.0 software with the general time reversible model with gamma distribution and invariable site distribution.24 Branch supports were estimated using bootstrap analysis with 1000 replicates. Phylogenetic trees were visualized in Figtree v1.3.1. Monophyletic clusters were defined using a combination of the following cutoff values for genetic distances (branch lengths) and bootstrap values. Branch length cutoffs were ±0.015, and bootstrap cutoffs were 99% and 95%. We also determined the number of clusters with cutoffs of 95%, irrespective of branch length. A within-cluster maximum window period between estimated transmission events was determined as the difference between the earliest and latest infection dates.

RESULTS

Patient Characteristics
Between 1995 and 2010, 600 patients were confirmed with AHI (Table 1). Similar numbers of participants were enrolled during each of the 5 study periods, 1995–1999 and biennially from 2000 to 2010. Overall, the mean age of participants was 35 years (range, 15–69 years), and the mean CD4+ count was 466 cells per cubic millimeter (range, 88–1281 cells/mm3). Participants were predominantly male (97%) and non-Hispanic white (68%). The largest frequency of AHI cases resided in neighborhoods where HIV-1 prevalence was high as per NYCDOMH data (see Figure, Supplemental Digital Content 1, http://links.lww.com/QAI/A327). The overall mean duration of HIV-1 infection was 66.6 days and increased significantly from 58.2 days during the first study observation period to 66.4 days in the last and peaking at 83.4 days in the 2007–2008 period (P < 0.001). The mean plasma HIV-1 RNA at presentation was 5.1 log10 copies per milliliter (range 1.7–8.3 log10 copies/mL) but was significantly higher for the 2000–2002 period (5.6 log10 copies/mL, P = 0.005). During the period 2007–2010, for which data were available, a majority of the sample reported unprotected anal intercourse (52.8%) and 28.6% reported illicit drug use, with methamphetamine being the most commonly documented drug (14.3%). Additionally, 13.2% reported meeting a recent sexual partner online. Other sample characteristics remained stable over time, except race/ethnicity and NYC residence (Table 1).

HIV-1 Drug Resistance
The overall prevalence of TDR to any ARV class was 14.3% and did not vary significantly over the 15-year period, ranging between 10.8% and 21.6% (Table 2, PTrend = 0.42). Overall, TDR by ARV class was 8.3% (NRTIs), 6.8% (NNRTIs), and 4.0% (PIs). NRTI resistance declined significantly from 15.5% to 2.7% over the study period (PTrend = 0.005). Dual- and triple-class resistance was 3.2% and 0.8%, respectively. TDR prevalence was similar when the World Health Organization’s surveillance definition was used, although the overall prevalence of TDR was slightly lower (13.8%, data not shown). Despite small numbers, the prevalence of TDR by class and multidrug resistance was highest in neighborhoods with high HIV-1 prevalence but lower socioeconomic status (see Figure, Supplemental Digital Content 1, http://links.lww.com/QAI/A327).

Temporal trends in specific mutations were shown in Table 3. Mutations M41L (3.7%), T215Y/F/S/D/C/E (4.0%), K103N/S (4.7%), and L90M were most prevalent. All other TDR mutations were less than 2% prevalent. Between 1995 and 2010, prevalence of K103N/S increased from 1.9% to 8.0% (PTrend = 0.04). D67N (P = 0.03), K70R (P = 0.06), M184V (P = 0.11), L210W (P = 0.11), and K219Q (P = 0.02) decreased during the study period. The presence of M184V was associated with a lower HIV-1 log10 viral load.
Phylogenetic Analysis

A majority (96.8%) of the sequences were determined to be subtype B (n = 581). In declining prevalence, 1.7% of sequences were circulating recombinant forms CRF_02_AG (n = 10), 0.7% were subtype A1 (n = 4), 2 were subtype BD, and there were 2 CRF_01AE, and 1 C subtype. Using only subtype B sequences to construct the phylogenetic tree, the average branch length was 1.716, the mean base difference per site was 0.013, and the nucleotide difference per sequence was 45.7. When using the transmission cluster criteria of ≥0.015 branch lengths and ≥99% bootstrap values, 19.3% (112 of 581) of sequences clustered: There were 42 pairs, 4 clusters of 3 sequences, 2 clusters of 4, and 1 cluster of 8 sequences (Fig. 1). When the bootstrap cutoff value was relaxed to ≥95%, and using the same ≥0.015 branch lengths, 21.7% (126 of 581) of the sequences clustered; we identified another pair: 1 cluster of 3 and 1 cluster of 9. With the most relaxed criteria, 27.7% (161 of 581) of the sequences clustered. The mean branch length within the subtype B clusters was 0.028 (SD = 0.020; range, 0.009–0.106). For the non–B sequences, 1 cluster of 6 CRF02_AG variants was identified, dating from 2005 to 2007. The CRF02_AG cluster grew to 8 using the least rigorous criteria.

Transmission Cluster Characteristics

Under the strictest criteria for transmission clusters, coclustering sequences came from younger participants and had a longer estimated duration of infection (59.8 vs. 71.3 days; P = 0.05; Table 4). Transmission clusters have trended upward until 2003–2004; 6.7%, 16.8%, 23.5%, 21.6%, 24.4%, and 21.1% of sequences clustered in 1995–1999, 2000–2002, 2003–2004, 2005–2006, 2007–2008, and 2009–2010, respectively (P trend = 0.004). Patients with clustered sequences were more likely to report recent drug use (P = 0.05); although not statistically significant, they were more likely to find partners online. One
clustered pair had M46L, another had T215D, and a third had K103N, D67N, and K219Q. Also, a cluster of 3 had transmitted M41L. Clustering viruses were not significantly different from nonclustering viruses in other characteristics. In an analysis of those large clusters (>4) identified using the second criteria, there were no demographic characteristics predictive of large clustering and there was no clustering of TDR (data not shown). By the strictest cluster criteria, the mean transmission window period within clusters was 54.7 months (range, 1–200 months).

### TABLE 2. Transmitted Antiretroviral Resistance Trends Throughout the Study Observation Period*

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* Determined by χ² test for trend. N = 600; A62V, K65R, Y115F, F116Y, Q151M, G190A/S/C, P225H, and M230L were not identified in the RT region of the pol gene. L23I, L24I, V321I, G48V/M, G73S/T/C/A, L176V, and N83D, N88D/S were not identified in the PR region of the pol gene. Mutations in bold are considered major resistance mutations and are included in our calculations for Table 2.

** Transmitted Drug Resistance in NYC 2012 Lippincott Williams & Wilkins
Furthermore, it might not be measured in population-

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| TABLE 4. Demographic and Clinical Characteristics by Transmission Cluster Definition |
|-----------------|-----------------|-----------------|-----------------|
| Characteristic  | Nonclustered AHI (n = 472) | Clustered AHI (n = 109)* | P† |
| Age, mean ± SD (yrs) | 35.8 ± 8.3 | 32.7 ± 7.3 | 0.005 |
| HIV-1 viral load, mean ± SD (log_{10} copies/mL) | 5.08 ± 1.2 | 5.11 ± 1.2 | 0.85 |
| CD4 count, mean ± SD (cells/μL) | 471 ± 204 | 483 ± 212 | 0.62 |
| Recent drug use, % (n) | 3.7 (14/416) | 8.5 (8/94) | 0.05 |
| Use of Internet for coupling | 1.7 (7/416) | 4.3 (4/94) | 0.28‡ |
| Duration of infection, mean ± SD | 59.8 ± 50.6 | 71.3 ± 66.3 | 0.05 |

Does not include non-B subtype cluster.

‡ AHI, acute and early HIV infection. Includes pairs and larger clusters.

† Determined by t test.

‡ Determined by χ² test.

DISCUSSION

We have been tracking TDR in this cohort of newly infected individuals in NYC for 15 years and have reported our past observations.3–5

Here, we report that the prevalence of TDR remains stable, at 14.3% overall, and 10.8% in the most recent period. Although there is no statistically significant trend in TDR, all categories of resistance peaked in 2003–2004. The distribution of mutations is very similar to that reported in the European surveillance study Strategy to Control the Spread of HIV-1,29 with a predominance of the thymidine analog mutations, M41L and T215R/F, and L90M in the protease coding region. Specific mutations and polymorphisms, such as K103N and L33F, have increased. K103N is likely due to shifts in treatment regimens, and its continued transmission could impact the use of first-generation NNRTI agents as an initial treatment option in these individuals. Given current treatment recommendations, this pattern supports the use of ritonavir-enhanced PI-based regimens when treating HIV-1 infection urgently and the routine use of resistance testing to inform ongoing regimen decisions.26 The use of integrase inhibitor–based therapy could also be advocated. The transmission of M184V has steadily decreased since the 1990s. The declining trend likely may be due to more successful regimens but possibly due to the impaired viral fitness seen as lower pretreatment viral loads in ours and other studies.27 Furthermore, it might not be measured in population-level resistance assays as we have used, where strains comprising less than 10% viral quasi-species are not likely to be detected. Deep sequencing analyses of these transmitted viruses are ongoing to determine whether M184V transmissions continue but are missed by this assay or whether reversion to wild type after transmission is occurring.

The prevalence of TDR in this cohort of mostly MSM is similar to that reported in other developed countries. A 10-city survey of new diagnoses in the United States revealed a 14.6% prevalence of TDR.28 Surveillance programs in Europe and Israel have reported a stable prevalence of 8.4%.25 Population studies from the UK HIV drug resistance surveillance group reported 10%, but these were mostly chronic HIV-1 infections, and the data were collected through 2004 or 2006.29,30 In a more recent report of men with AHI in San Francisco, the prevalence of TDR had decreased to 15% in 2008–2009 from 24% in 2007.31 As treatment success improves with simpler regimens and more durable virologic suppression, we expect that TDR within the population would diminish as it had in other settings.32,33 However, the stable prevalence of TDR that remained in this sample suggests that we need to better understand the drivers of TDR in the population. Differences in sampling approaches and sample characteristics (eg, transmission risk, gender, racial/ethnic composition) and population parameters (eg, circulating HIV drug resistance among treatment-experienced individuals) could explain some of these variables. TDR defining mutations also have changed over time, and comparing trends must be interpreted with this understanding. Population-based surveys such as the UK HIV Drug Resistance surveillance can be generalized to the source population of HIV-1–infected individuals, but acute and recent HIV-infected individuals comprise such a small fraction of this sample that it is difficult to extrapolate and understand current resistance transmission trends. Data from our study were drawn from a highly selected sample and cannot be generalized to the population of HIV-1–infected individuals in NYC. Comparison of population-based and sample-based data provides insights into disparities in early HIV detection, a public health and clinical imperative, and allows some inference about the representativeness of samples.

Determining AHI from single time point measurements with assays like the detuned antibody assay used in our algorithm has been discouraged because of the possibility of misclassifying individuals with low viral loads and low CD4+ T-cell counts or an unknown history of antiretroviral therapy as AHI.34,35 However, such algorithms are done in the absence of clinical history and additional laboratory data. We believe that our stringent criteria of acute and early HIV-1 infection that include detailed clinical and laboratory data, high entry viral loads, exclusion of those who could enter the cohort with CD4 counts less than 200 cells per cubic millimeter unless additional laboratory and clinical data supported the diagnosis, and conservative cutoff values of the detuned assay reduced the likelihood of misclassification. Furthermore, in our sample, we further reduced misclassification by using multiple EIA methods (ie, documented negative EIA and Western blots) in substantial numbers of individuals. The 97% prevalence of subtype B variants is consistent with other studies in the United States.26 Interestingly, we only identified 1 non–B subtype variant before 2005, and 14 of the 19 subjects who had non–B subtypes were US born. We did not identify any geographic area associated with clustering but did find a higher prevalence of TDR in those regions of NYC with lower socioeconomic status. The outer boroughs of NYC are a particular public health focus due to the higher incidence (compared with Manhattan) and lower HIV testing and access to medical services. However, the numbers are too small to infer much from these data. Thus, identification of AHI individuals from these neighborhoods is required to better understand this pattern.

Using the strictest of 3 criteria for defining transmission clusters, we report that 19.3% of sequences tested
during AHI clustered. Our results are similar to those from a study of multiple centers across Europe (11%). However, the clustering observed in this study is considerably lower than that of an earlier report by Brenner et al, where 49.4% of the sequences coclustered, and other samples in Europe and Australia, where clustering was reported to be between 34% and 53%. Several factors may explain observed differences between transmission clusters in our study and other reports. Review of reports of other transmission networks shows that there is considerable variability in both analysis and definition of transmission networks. In this study, relaxing the criteria for defining transmission clusters increased the proportion of coclustering sequences from 19.3% to 27.7%. Furthermore, the sample in this study represented a relatively small fraction of HIV infections in NYC. This low sampling fraction, and the predominance of non-Hispanic white MSM in the sample, renders it less representative of HIV-1 infections in NYC overall. It is possible that more complete sampling would show more transmission clusters in the sample set and/or larger clusters than those observed here. Conversely, population-level sampling of NYC may show less transmission clusters than was observed in this more homogeneous cohort. It is possible that the NYC epidemic is more complex than other settings; mixing patterns may be more heterogeneous and dynamic, and in and out migration varies considerably from smaller North American cities like Montreal. These nuances in study context can produce differences and should be interpreted with caution.

CONCLUSIONS

NYC has among the highest documented HIV-1 infection prevalence in the United States. The diverse transient population in NYC makes routine monitoring of AHI, TDR, and transmission networks an imperative. The major strength (and limitation) of this cohort lies in the fact that the cohort was demographically stable over 15 years. The stability of this sentinel group increases the internal validity of this study to report trends in TDR and transmission networks over time. Variations in geographical residence of TDR, although not statistically significant, highlight the need for broader surveillance of TDR and a more comprehensive understanding of HIV-1 transmission networks in the New York Metro area. Complementing these biological data, other sociodemographic, behavioral, and virological information obtained in a systematic fashion would further inform targeted HIV-1 prevention efforts in NYC.

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