Short Communication: Phylogenetic Evidence of HIV-1 Transmission Between Adult and Adolescent Men Who Have Sex with Men

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Abstract

HIV-1 incidence among youth, especially men who have sex with men (MSM), is increasing in the United States. We aimed to better understand the patterns of adolescent HIV-1 acquisition, to help guide future prevention interventions. We conducted a study combining epidemiologic and HIV-1 *pol* sequence data from a retrospective cohort of HIV-infected adults and adolescents in Seattle, WA between 2000 and 2013. Adolescents were defined as 13–24 years of age at the time of first HIV-1 care. Maximum-likelihood phylogenetic trees were reconstructed to identify putative viral transmission clusters of two or more individuals, followed by multivariable regression tests of associations between clustering and demographic and clinical parameters. The dataset included 3,102 sequences from 1,953 individuals; 72 putative transmission clusters were identified, representing 168 individuals (8.6%). MSM and MSM/intravenous drug use (IDU) were positively associated with clustering, with aOR 3.18 (95% CI: 1.34–7.55) and 2.59 (95% CI: 1.04–6.49), respectively. African American race was negatively associated with clustering (aOR 0.54 95% CI: 0.32–0.91). Twenty-five clusters contained one adolescent and five clusters contained two adolescents. Other individuals who clustered with adolescents were predominantly male (95%), white (85%), and either MSM (66%) or MSM/IDU (16%), with a greater mean age (34 years vs. 22 years; $p < .01$). In this Seattle cohort, HIV-1 transmission linkages were identified between white male adolescents and older MSM adults. Interventions aimed at age-discrepant pairs may reduce HIV-1 infections in adolescent males.

Keywords: adolescent, transmission, phylogenetics

Introduction

SINCE 2006, HIV-1 incidence has increased among young men who have sex with men (MSM).^{1–3} Adolescents are at increased risk of HIV-1 acquisition because of high rates of unprotected sex, sexually transmitted infections, transactional sex, multiple sex partners, and intravenous drug use (IDU), coupled with low risk awareness.^{4–6} HIV-1 *pol* genotypes, collected routinely upon diagnosis or before treatment initiation, can be used to identify clusters of viral genetic similarity that reflect putative HIV-1 transmission relationships.^{7–10} Epidemiological traits of the individuals can then be evaluated for associations with cluster membership. Traits overrepresented in clusters are inferred to be associated with higher rates of HIV-1 transmission.¹¹ Few

phylogenetic studies of HIV-1 have included individuals <18 years of age.^{3,4,26} To our knowledge, no studies have used phylogenetics to specifically understand the risk factors for HIV-1 transmission among adolescents.

Methods

Study population

We performed a retrospective analysis of HIV-1-infected individuals identified from the University of Washington (UW) HIV Cohort described previously¹² or Seattle Children's Hospital (SCH) with one or more HIV-1 *pol* genotypes collected between February 2000 and March 2013. Individuals with perinatal or transfusion acquisition were

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excluded. Adolescents were defined as 13–24 years of age at time of entry into HIV-related care. Demographic and clinical characteristics recorded at first HIV-1 genotyping were obtained from the UW HIV Information System (UWHIS) and SCH. Individual data assessed in this study included the following: age at first recorded HIV-1 care, age at HIV-1 *pol* genotyping, sex, race, CD4+ T cell count and plasma viral load at first HIV-1 care, housing status (homeless or not) at date of first HIV-1 genotyping, and HIV-1 risk group, including MSM, heterosexual (HET), IDU, MSM who also reported intravenous drug use (MSM/IDU). Sequences have been deposited in GenBank with accession numbers: KY034691 to KY037792.

Sequence analysis

Partial HIV-1 *pol* gene sequences (length = ~1,080 nucleotides (with 2 substantially shorter sequences, 600 and 800 bp, also included) from amino acid positions 6 to 99 of protease and 1–253 of reverse transcriptase, relative to HXB2 numbering) were obtained from the University of Washington Clinical Virology Laboratory. Sequences were aligned using MUSCLE¹³ within Geneious v8,¹⁴ followed by manual adjustment. Maximum-likelihood phylogenetic trees were reconstructed using FastTree 2.1.5^{15,16} rooted with HXB2, assuming the GTR model of nucleotide substitution with gamma-distributed substitution rate heterogeneity, also within Geneious v8. Node support was estimated using FastTree SH-like (likelihood based) approach.^{15,16} Phylogenies

were reconstructed using datasets including: (1) only the first genotype collected from each individual, to avoid erroneously excluding an individual from a cluster due to viral evolution away from an earlier variant and (2) separately, all available sequences from each individual, to increase the detection of transmission linkages from individuals who transmitted later in their infection. To the alignments, we added 500 randomly selected U.S. HIV-1 subtype B *pol* sequences (from www.hiv.lanl.gov) to confirm Seattle-based clusters. UW and SCH sequences were assigned to HIV-1 subtypes using Recombinant Identification Program¹⁷ and were corroborated by assessment of the phylogeny with added subtype reference sequences. The possibility for phylogenetic errors introduced by convergent evolution due to drug resistance mutations was assessed by repeating the phylogenetic reconstruction and clustering analyses after removal of regions known to encode amino acids associated with antiretroviral drug resistance.

Cluster identification

Phylogenetic clusters were defined as: (1) sequences with a shared ancestral node containing only sequences from our Seattle-based dataset; (2) node support values¹⁶ >0.95; and (3) maximum pairwise genetic distances ≤0.015 nucleotide substitutions/site, using ClusterPicker.¹⁸ These node support and distance thresholds are conservative for molecular epidemiological studies of *pol*.^{19,20} To allow for identification of more clusters, we identified clusters observed in either of the

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY POPULATION AND ODDS RATIOS FOR PHYLOGENETIC CLUSTERING OF HIV-1 *POL* SEQUENCES IN UNIVARIABLE AND MULTIVARIABLE LOGISTIC REGRESSION MODELS

Characteristic	Total, N = 1,953 n (%)	In cluster, N = 168 n (%)	Univariable OR (95% CI)	p ^a	Multivariable ^b OR (95% CI)	p ^a
Sex						
Female	276 (14)	18 (11)	Ref.	—	Ref.	—
Male	1,663 (85)	150 (89)	1.42 (0.86–2.36)	.17	0.59 (0.25–1.36)	.21
Transgender ^c	14 (1)	0 (0)	NA ^c	—	NA ^c	—
Age group						
Adult	1,579 (81)	133 (79)	Ref.	—	Ref.	—
Adolescent ^d	374 (19)	35 (21)	1.12 (0.76–1.66)	.56	1.15 (0.76–1.73)	.52
Race						
White	1,227 (69)	121 (80)	Ref.	—	Ref.	—
African American	373 (21)	18 (12)	0.46 (0.28–0.77)	.003	0.54 (0.32–0.91)	.02
Other ^e	179 (10)	12 (8)	0.66 (0.36–1.21)	.18	0.78 (0.42–1.46)	.44
Risk group						
Heterosexual	349 (18)	16 (10)	Ref.	—	Ref.	—
IDU	206 (11)	14 (8)	1.52 (.72–3.18)	.27	1.77 (.79–3.96)	.17
MSM	987 (51)	102 (61)	2.40 (1.40–4.12)	.002	3.18 (1.34–7.55)	.009
MSM/IDU	394 (20)	35 (21)	2.03 (1.10–3.73)	.02	2.59 (1.04–6.49)	.042
Homelessness						
Not homeless	977 (92)	105 (91)	Ref.	—		
Homeless	86 (8)	10 (9)	1.09 (.55–2.18)	.80		
HIV-1 subtype						
B	1,870 (96)	168 (100)				
non-B	83 (4)	0 (0)				

^aChi-squared test used for categorical variables and unpaired two-tailed *t*-test used for comparing means of continuous variables.

^bMultivariable model included sex, age group, race, and HIV-1 risk group as covariates.

^cAll transgender were male-to-female, this group included too few individuals to include in either regression model.

^dAdolescents were defined as ages 13–24 years at the time first engaged in HIV-1 care.

^e“Other” races include Asian, Black African, Hawaiian, Pacific Islander, Multiracial, and American Indian.

IDU, intravenous drug use; MSM, men who have sex with men.

Bold represents *p* value of less than or equal to 0.05.

two phylogenies (i.e., using “only first sequences obtained from each individual” or “all sequences obtained”). We were unable to identify the direction of transmission (i.e., the source and recipient individuals) due to unknown dates of seroconversion.

Statistical analysis

To identify predictors of cluster membership, univariable logistic regression analysis was performed using each covariate followed by multivariable analysis using characteristics with $p < .2$, regardless of effect size in the univariable analysis, except adolescent age, the primary covariable of interest.

To identify risk factors associated with HIV-1 infection of adolescents, we focused on clusters that contained at least one adolescent, termed “adolescent clusters.” The adolescent’s risk factors were compared to data from others within the

cluster. The “other” individual(s) (regardless of adolescent or adult status) were interpreted to be the “exposure” individual. The demographic and epidemiological traits of this exposure individual were assumed to be proxies for the trait frequency in the transmission network for which the clusters are representative.²¹ Chi-squared tests and unpaired two-tailed t -tests were used to compare frequencies of categorical and continuous variables, respectively. We also compared (1) individuals within adolescent clusters to individuals within nonadolescent clusters to assess differences between these two high transmission groups and (2) adolescents within clusters to adolescents not in clusters to assess differences between adolescents in high and low transmission groups.

Results

The dataset for our analyses included 3,102 *pol* sequences from 1,953 individuals; 374 (19%) were adolescents, with a

TABLE 2. DISTRIBUTION AND TRAITS OF HIV-1-INFECTED INDIVIDUALS LIVING IN SEATTLE, ≥ 13 YEARS OF AGE, WHO WERE OR WERE NOT IN PHYLOGENETIC CLUSTERS WITH AT LEAST ONE ADOLESCENT

	74 clusters with ≥ 1 adolescents ^a			Clusters without adolescents Adults, N=94	p ^{e,f}	Not in clusters		
Trait	Adolescents ^b , N=35	Others, N=57 ^c	p ^{d,e}			Adolescents, N=339	Adults, N=1,445	p ^{c,g}
Sex, <i>n</i> (%)								
Male	30 (86) ^h	54 (95)	.14	82 (87)	.33	266 (78)	1,247 (86)	.56
Female	5 (14)	3 (5)		12 (13)		70 (21)	188 (13)	
Transgender	0 (0)	0 (0)		0 (0)		3 (1)	11 (1)	
Age								
Median (IQR) in years	22 (21, 24)	32 (27, 40)	<.001	36 (29, 42)	<.001	22 (20, 23)	34 (29, 40)	.17
Race, <i>n</i> (%)								
White	28 (85)	47 (85)	.55	62 (77)	.47	223 (71)	883 (67)	.23
African American	4 (12)	4 (7)		11 (14)		62 (20)	293 (22)	
Other	1 (3)	4 (7)		8 (10)		28 (9)	139 (11)	
HIV risk group, <i>n</i> (%)								
Heterosexual	3 (9)	3 (5)	.87	10 (11)	.72	68 (20)	265 (18)	.16
IDU	3 (9)	7 (13)		8 (9)		27 (8)	165 (12)	
MSM	24 (69)	37 (66)		54 (57)		168 (50)	717 (50)	
MSM/IDU	5 (14)	9 (16)		22 (23)		73 (22)	286 (20)	
Homelessness								
No	19 (86)	36 (88)	.87	60 (95)	.10	172 (89)	700 (93)	.70
Yes	3 (14)	5 (12)		3 (5)		21 (11)	55 (7)	
Baseline CD4 (cells/ μ L)								
Median (IQR)	463 (343, 654)	436 (254, 672)	.47	430 (260, 571)	.45	313 (134, 503)	360 (186, 539)	.004
Baseline plasma HIV-1 RNA (log ₁₀)								
Median (IQR)	2.88 (2.61, 3.07)	2.69 (2.53, 2.96)	.27	2.87 (2.46, 3.05)	.98	2.85 (2.56, 3.04)	2.82 (2.41, 3.03)	.37

^aClusters defined by FastTree likelihood-based node support ≥ 0.95 and maximum pairwise genetic distance $< 1.5\%$.

^bAdolescents excluded patients with perinatally-acquired or transfusion-acquired HIV who were 13–24 years of age at time engaged in HIV-1 care.

^cCharacteristics for “Others” in adolescent clusters were counted once per adolescent; because five adolescent clusters had two adolescents, the demographic characteristics for 18 individuals (10 adolescents and 8 adults) in the “Others” column were counted twice.

^dComparison between adolescents in adolescent clusters and “Others” in adolescent clusters.

^eChi-squared test used for categorical variables and unpaired two-tailed t -test used for comparing means of continuous variables.

^fComparison between those in nonadolescent cluster and total (adolescents and adults) in adolescent cluster.

^gComparison between adolescents not in a cluster and adolescents in a cluster.

^hNumber of patients (%), unless otherwise specified.

Bold represents p value of less than or equal to 0.05.

median age of 22 years (IQR: 20–23; mean: 21.48) (Table 1, Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/aid). Median time span between earliest HIV care and sequencing was 5 years (IQR: 0, 11 years). Data on homelessness were available for only 1,063 of 1,953 individuals (54%). Using all sequences from each individual, we identified 54 phylogenetic clusters. Using just the first sequence from each individual, we identified 42 clusters. Combined, there were 72 separate clusters included in subsequent analyses (24 clusters were found in both phylogenies). A total of 35 adolescents (at the time of entry into HIV care) were in clusters (10%), including 25 clusters with one adolescent and five with two adolescents. There was only one cluster composed solely of adolescents, which included just two individuals. “Other” individuals within adolescent clusters were predominantly male (95%), white (85%), and MSM (66%), who were older (median age 32 years) (Table 2). Twelve percent of “other” individuals were homeless. Adolescents were similar to “others” within adolescent clusters with regard to sex, race, HIV-1 risk group, homelessness, baseline CD4, and viral load. In addition, adolescents in clusters were similar to adolescents not in clusters with respect to age, sex, race, HIV-1 risk group, homelessness, and first recorded HIV viral load. However, adolescents in clusters had significantly higher median baseline CD4 counts than adolescents not in clusters (463 cells/ μ L vs. 313 cells/ μ L). Inclusion or exclusion of sequence encoding amino acids known to be associated with antiretroviral drug resistance had no significant effects on the phylogenetic reconstruction or clustering analyses.

Discussion

Phylogenetic analyses revealed that Seattle adolescents in putative HIV-1 transmission clusters likely acquired HIV-1 through sexual pairing with older adults. The observed age differential of 12 years supports previous epidemiologic research reporting intergenerational sex to be a risk factor for HIV-1 acquisition in young MSM.^{22–25} This is also consistent with a national study that found that 36% of MSM 13–24 years had a potential transmission partner who was >5 years older.²⁶ Studies have shown that young MSM in age-discrepant pairs are more likely to engage in unprotected sex and receptive anal intercourse with older partners and report drug use and sexually transmitted infections, all of which increase their risk of acquiring and transmitting HIV-1.^{5,6,22,27,28} Qualitative research has suggested that the participation in these behaviors may stem from differences in financial or social status.^{22,29} The higher baseline CD4 counts in adolescents, who clustered in phylogenetic analysis compared with those adolescents who did not, suggest one of two possibilities: (1) that the clustered adolescents were the recipients rather than the source of HIV-1 infection or (2) that the clustered adolescents were earlier in their course of disease. We think the former explanation more probable since individuals at younger ages generally have less time since sexual debut.

Limitations to our study, similar to all studies with retrospective sampling of *pol* genotypes, relate to uncertainties regarding the proportion of the HIV-1-infected population studied, and our inability to identify the transmission networks for all infected adolescents (10% of adolescents in our study were in a phylogenetic cluster), and particularly for young women. The UW and SCH HIV clinics provide the

majority of free and/or government-sponsored HIV care in the Seattle metropolitan area. Thus, it is possible that many Seattle adolescents were infected by individuals residing in other geographic regions or by individuals who receive care funded by private insurance or who do not seek or receive HIV care. The majority of Seattle’s HIV-infected individuals who receive care outside of the UW and SCH networks obtain care in private practice settings financed by private insurance. These individuals are likely of older age, higher socioeconomic status, and maintain viral suppression. The unavailability of these individuals’ sequences could bias our results toward an underestimate of the age differentials within adolescent clusters. The lack of data related to homeless status may have limited our sensitivity to assess associations with cluster membership (either generally or among adolescents). Finally, HIV-1 transmission risk factors were reported by the individuals themselves, which may have led to a reporting bias against stigmatized behaviors such as IDU and MSM. Since we found that both MSM and MSM/IDU were significantly associated with clustering, omission of these behaviors would likely have biased our findings toward the null.

In conclusion, our phylogenetic study of HIV-1 *pol* sequences in Seattle finds that a significant number of adolescent HIV-1 transmission events are associated with age-discrepant sexual pairing, specifically among white MSM. This suggests that the MSM community may benefit from new prevention strategies aimed at age-discrepant pairs.

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