Substantial Multiclass Transmitted Drug Resistance and Drug-Relevant Polymorphisms Among Treatment-Naïve Behaviorally HIV-Infected Youth

Allison L. Agwu, M.D., ScM,¹ James Bethel, Ph.D.,² Lisa B. Hightow-Weidman, M.D., M.P.H.,³ John W. Sleasman, M.D.,⁴ Craig M. Wilson, M.D.,⁵ Bret Rudy, M.D.,⁶ and Bill G. Kapogiannis, M.D.,⁷ for the ATN 061 Team and the Adolescent Medicine Trials Network for HIV/AIDS Interventions

Dear Editor:

We estimated the prevalence of transmitted drug resistance mutations (TDRMs) and drug-relevant polymorphisms (DRPs) in primary HIV infection in a cohort of youth (ages 18–24) and determined whether demographic or clinical characteristics were associated with prevalence of TDRMs or DRPs. We report the results of a substudy of Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) 061, an ongoing study of highly active antiretroviral therapy (HAART) initiation in HIV-positive youth who have CD4⁺ T cells (CD4) of >350 cells/mm³ with subsequent deintensification. Subjects were screened from 15 ATN U.S. and Puerto Rico sites and from 7 International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) U.S. sites. The study was approved by each participating institution's Institutional Review Board. Subjects were eligible for ATN 061 if they were 18–24 years of age, had a CD4 T-cell count >350/mm³, HIV-1 RNA viral load (VL) ≥1000 copies/mL, and were HAART-naïve with the exception of ARVs for the prevention of mother to child transmission for a single pregnancy with documentation of virologic suppression (*n*=5). Enrollment began in 2006 and was completed in 2010. All subjects had a screening genotype and those without specific mutations that were exclusion criteria for ATN 061 (I50L; I84V; N88D/S, D30N; V32I; L33I/F/V; M46I/L; I47V/A; G48V; I50V/L; I54V/L/A/M/T/S; L76V; V82A/F/T/S/L; L90M, or major RT mutations Q151M and 69 insertion complex) were eligible.

The screening genotypes from all patients were evaluated for protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI), and nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) major transmitted drug resistance mutations (TDRMs) and drug relevant polymorphisms (DRPs) according to World Health Organization (WHO) and International AIDS Society (IAS) algorithms.^{1,2} Alone, DRPs may not confer resistance, however, they may lead to com-

pensatory changes that may confer a fitness advantage to the virus.

All of the 130 eligible subjects had screening genotypes. The median age was 21.4 years, 86.9% were male, 69.2% black, and 99.2% acquired HIV sexually (Table 1). Twelve of 130 subjects (9.2%) had a total of 24 TDRMs: 5 of 24 (20.8%) NRTI; 9 of 24 (37.5%) NNRTI; and 10 of 24 (41.7%) PI. Two of 12 subjects with TDRMs (16.7%) had single, 8 of 12 (66.7%) double, and 2 of 12 (16.7%) triple class resistance; 10/130 (7.7%) had 2 or more class resistance. No factors were significantly associated with identification of TDRMs.

DRPs were found in 105 of 130 (80.8%) subjects including the 12 subjects with TDRMs who all had additional DRPs (Tables 1 and 2). Geographic region was significantly associated with having a DRP (Table 2). Due to a significant interaction of VL with the MSM HIV acquisition risk category (approximately 0.3 log₁₀ higher VL in MSM risk group, *p*=0.0448), two multivariate models were created, one that includes HIV VL with geographic region and a separate model that includes HIV acquisition risk with geographic region. In both models, Midwestern and Western geographic regions were associated with a decreased likelihood of DRPs as compared with the South (adjusted odds ratio [AOR] 0.166 [95% confidence interval {CI} 0.047–0.591] and AOR 0.093 [95% CI 0.024–0.360]), respectively). For each log₁₀ increase in VL, the odds of having a DRP increased by 2.118 (AOR 2.118 [95% CI 1.033–4.344]). Additionally, MSM HIV acquisition risk category versus heterosexual acquisition risk was independently associated with an increased likelihood of having DRPs (AOR 4.016 [95% CI 1.251–12.890]). No other demographic or clinical associations were identified.

In this geographically diverse study of U.S. youth, 9.2% had TDRMs and 7.7% had multiclass resistance. These rates are an underestimation as youth known to have harbored particular resistance mutations were excluded by the parent study and

¹Department of Pediatric and Adult Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland.

²Westat, Rockville, Maryland.

³Department of Medicine, Division of Infectious Diseases, University of North Carolina, Chapel Hill, North Carolina.

⁴Department of Pediatrics, Division of Allergy, Immunology, and Rheumatology, University of South Florida, St. Petersburg, Florida.

⁵Department of Epidemiology, University of Alabama at Birmingham, Birmingham, Alabama.

⁶Department of Pediatrics, New York University School of Medicine, New York, New York.

⁷Pediatric, Adolescent, and Maternal AIDS Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland.

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS WITH TRANSMITTED DRUG RESISTANCE MUTATIONS AND DRUG RELEVANT POLYMORPHISMS

	<i>All subjects</i>	<i>No resistance</i>	<i>TDRM^a</i>	<i>DRP</i>	<i>p Value^b</i>
Age (years)					
Mean (SD)	21.4 (1.7)	21.0 (2.0)	21.4 (1.7)	21.5 (1.7)	0.4998
Median (range)	21.4 (18–25)	20.7 (18–25)	21.3 (19–25)	21.5 (18–25)	
Gender					
Male	113	20 (17.7)	11 (9.7)	82 (72.6)	0.5658
Female	17	5 (29.4)	1 (5.9)	11 (64.7)	
Race/ethnicity					
Hispanic	24	8 (33.3)	3 (12.5)	13 (54.2)	0.4681
Non-Hispanic white	9	1 (11.1)	0 (0.0)	8 (88.9)	
Non-Hispanic black	90	14 (15.6)	9 (10.0)	67 (74.4)	
Asian/Pacific Islander	3	1 (33.3)	0 (0.0)	2 (66.7)	
Other	4	1 (25.0)	0 (0.0)	3 (75.0)	
Region of country					
Midwest	24	8 (33.3)	2 (8.3)	14 (58.3)	0.0162
Northeast	24	4 (16.7)	1 (4.2)	19 (79.2)	
South	62	5 (8.1)	8 (12.9)	49 (79.0)	
West	20	8 (40.0)	1 (5.0)	11 (55.0)	
Receipt of PMTCT					
Yes	5	3 (60.0)	0 (0.0)	2 (40.0)	0.1884
No	12	2 (16.7)	1 (8.3)	9 (75.0)	
CD4 (cells/mm³)					
Mean (SD)	511 (159)	563 (194)	510 (173)	497 (145)	0.3093
Median (range)	480 (246–1107)	548 (299–1089)	444 (264–961)	469 (246–1107)	
CD4 category (cells/mm³)					
< 350	16	4 (25.0)	1 (6.3)	11 (68.8)	0.1356
350–500	54	5 (9.3)	6 (11.1)	43 (79.6)	
501–750	51	12 (23.5)	4 (7.8)	35 (68.6)	
> 750	9	4 (44.4)	1 (11.1)	4 (44.4)	
Duration of infection (years)					
Mean (SD)	1.3 (1.5)	1.1 (0.7)	1.0 (1.5)	1.4 (1.6)	0.4370
Median (range)	0.9 (0.0–9.8)	1.1 (0.1–2.4)	0.4 (0.1–5.6)	0.8 (0.0–9.8)	
HIV acquisition risk					
High-risk heterosexual contact	23	7 (30.4)	3 (13.0)	13 (56.5)	0.1762
Male-to-male sexual contact	105	17 (16.2)	9 (8.6)	79 (75.2)	
Other heterosexual contact	1	1 (100)	0 (0.0)	0 (0.0)	
Unknown	1	0 (0.0)	0 (0.0)	1 (100)	
Viral load					
Mean (SD)	4.12 (0.64)	3.94 (0.80)	4.19 (0.50)	4.16 (0.61)	0.4560
Median	4.17 (2.2–6.1)	4.07 (2.2–5.6)	4.24 (2.8–4.7)	4.17 (2.7–6.1)	
Viral load					
< 1000	7	4 (57.1)	1 (14.3)	2 (28.6)	0.0421
1000–10,000	44	8 (18.2)	1 (2.3)	35 (79.5)	
10,001–100,000	72	12 (16.7)	10 (13.9)	50 (69.4)	
> 100,000	7	1 (14.3)	0 (0.0)	6 (85.7)	

^aThese 12 subjects with TDRMs (transmitted drug resistance mutations) also had additional DRPs (drug relevant polymorphisms).

^b*p* Values for categorical variables are calculated using Fisher's exact test and provide a test of whether rates of resistance (no resistance versus TDRM versus DRP) vary by the categories shown on the left-hand column. The *p* values for continuous variables are based on the Wilcoxon test and provide a test of whether there is an association between rates of resistance and the continuous variable shown in the left-hand column.

SD, standard deviation; PMTCT, prevention of mother to child transmission.

not captured by the substudy. The multiclass resistance rate was higher than previously reported from similar sites.³

Reported rates of DRPs have ranged from 60% to 90%, in line with the 80.8% rate we found.^{4,5} The significance of these high DRP prevalence rates is unclear, however, it may be important to continue to monitor as there may be an impact on treatment response to both current and future PIs. Due to their potency and higher genetic resistance barrier, PI-based regimens are particularly important for youth who tend to have lower treatment adherence.⁶ Additionally, as youth are a

group where HIV vaccines may be particularly targeted both for treatment and primary prevention, high prevalence rates of polymorphisms may also be of significance.

We report that MSM HIV acquisition risk and higher screening VL were associated with an increased risk of having DRPs, while being in the Midwest and West was associated with a decreased risk of having DRPs compared to the South. Other studies of resistance in youth have not shown differences by geographic location.^{3,7} However, the Southern region encompasses locales (e.g., Florida) with high incidence

TABLE 2. FACTORS ASSOCIATED WITH THE PRESENCE OF DRUG RELEVANT POLYMORPHISMS AT BASELINE IN THE UNIVARIATE ANALYSIS

<i>Factor</i>	<i>Odds ratio</i>	<i>95% lower CI</i>	<i>95% upper CI</i>	<i>p Value</i>
Age	1.158	0.897	1.494	0.2602
Male gender	1.896	0.600	5.988	0.2757
Race/ethnicity				
Hispanic	0.462	0.101	2.100	0.3173
Non-Hispanic black	1.220	0.307	4.845	0.7777
Non-Hispanic white and other	Reference			
Region				
Midwest	0.175	0.050	0.611	0.0062
Northeast	0.417	0.101	1.713	0.2248
West	0.121	0.033	0.438	0.0013
South	Reference			
CD4 count	0.998	0.995	1.000	0.0781
Years since diagnosis	1.177	0.818	1.693	0.3806
Acquisition risk				
Male-to-male sexual contact	2.559	0.946	6.922	0.0643
Heterosexual contact	Reference			
Log₁₀ viral load	1.791	0.881	3.639	0.1074

and prevalence of HIV infection⁸; the finding of significant regional differences in the DRP rates may be of concern and in need of further evaluation. Increasing drug resistance among newly infected adult MSM has been reported; and 45% of known HIV-positive and previously treated adult men (age 26–64) in a community sample in a large, urban city were found to harbor resistance to at least one ARV⁹. The increased risk of DRPs among those with MSM HIV acquisition risk in this study is noteworthy as this mode of transmission is responsible for increasing proportions of infections among behaviorally infected youth; and age bridging (having sex with older men) is a known risk factor for HIV acquisition.¹⁰ Many of these older individuals may have long treatment histories and thus antiretroviral exposure with risk of transmitting viral variants with resistance. The association of increasing viral load with DRPs in this study is of concern as it may reflect greater fitness of viral variants with resistance mutations, as well as higher or possibly more efficient risk of transmission of resistant variants in those individuals with increased viremia.¹¹ As this study was not designed to ascertain cause and effect behind this association, this instead may also reflect that viral quasiespecies with higher replication capacities are likely to be more error prone. Our study is limited by relatively small numbers, which diminishes our power to examine significant associations.

Primary drug resistance is commonly found among HIV-infected youth, with a significant proportion acquiring and harboring multi-drug resistant viruses. These findings are worrisome, underscoring the importance of genotypic testing prior to ARV initiation for treatment of HIV infection, particularly if ARV pre- and postexposure prophylaxis becomes more frequently prescribed as part of HIV prevention.¹² The significance of the high prevalence of DRPs is unclear and needs further study.

Acknowledgments

This work was supported by The Adolescent Trials Network for HIV/AIDS Interventions (ATN) from the National

Institutes of Health [U01 HD 040533 and U01 HD 040474] through the Eunice Kennedy Shriver National Institute of Child Health and Human Development (B. Kapogiannis)], with supplemental funding from the National Institutes on Drug Abuse (N. Borek) and Mental Health (P. Brouwers, S. Allison). The protocol was co-endorsed by the International Maternal Pediatric Adolescent AIDS Clinical Trials Group. Support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (NIAID), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the National Institute of Mental Health (NIMH) [U01 A1068632]. The study was scientifically reviewed by the ATN's Therapeutic Leadership Group. Network, scientific and logistical support was provided by the ATN Coordinating Center (C. Wilson, C. Partlow) at The University of Alabama at Birmingham. Network operations and analytic support was provided by the ATN Data and Operations Center at Westat, Inc. (J. Korelitz, B. Driver, G. Price).

The following ATN sites participated in this study: University of South Florida, Tampa (Emmanuel, Lujan-Zilberman, Julian), Children's Hospital of Los Angeles (Belzer, Flores, Tucker), University of Southern California at Los Angeles (Kovacs, Homans, Lozano), Children's National Medical Center (D'Angelo, Hagler, Trexler), Children's Hospital of Philadelphia (Douglas, Tanney, DiBenedetto), John H. Stroger Jr. Hospital of Cook County and the Ruth M. Rothstein CORE Center (Martinez, Bojan, Jackson), University of Puerto Rico (Febo, Ayala-Flores, Fuentes-Gomez), Montefiore Medical Center (Futterman, Enriquez-Bruce, Campos), Mount Sinai Medical Center (Steever, Geiger), University of California-San Francisco (Moscicki, Auerswald, Irish), Tulane University Health Sciences Center (Abdalian, Kozina, Baker), University of Maryland (Peralta, Gorle), University of Miami School of Medicine (Friedman, Maturo, Major-Wilson), Children's Diagnostic and Treatment Center (Puga, Leonard, Inman), St. Jude's Children's Research Hospital (Flynn, Dillard), and Children's Memorial (Garofalo, Brennan, Flanagan).

The following IMPAACT sites participated in the study: Children's Hospital of Michigan – Wayne State (Moore, Rongkavilit, Hancock), Duke University Medical Center Pediatric CRS (Cunningham, Wilson), Johns Hopkins University (Ellen, Chang, Noletto), New Jersey Medical School CTU/CRS (Dieudonne, Bettica, Monti), St. Jude/Memphis CTU/CRS (Flynn, Dillard, McKinley), University of Colorado School of Medicine/The Children's Hospital (Reirden, Kahn, Witte) University of Southern California Medical Center (Homans, Lozano), Howard University Hospital (Rana, Deressa).

Four of the ATN and IMPAACT sites utilized their General Clinical Research Center (GCRC)/Pediatric Clinical Research Center (PCRC) for the study. The centers were supported by grants from the General Clinical Research Center Program of the National Center for Research Resources (NCRR), National Institutes of Health, Department of Health and Human Services as follows: Children's National Medical Center, M01RR020359; Howard University Hospital, M01-RR010284; University of California at San Francisco, UL1 RR024131; and University of Colorado School of Medicine/Children's Hospital, UL1 RR025780. The University of Pennsylvania/Children's Hospital of Philadelphia utilized its Institutional Clinical and Translational Science Award Research Center (CTRC), supported by grant UL1 RR024134 from NCRR. The Tulane University Health Sciences Center utilized its Clinical and Translational Research Center (CTRC) for the study which was supported in whole or in part by funds provided through the Louisiana Board of Regents RC/EEP (RC/EEP-06).

We thank the ATN Community Advisory Board and the youth who participated in the study.

Author Disclosure Statement

No competing financial interests exist.

References

1. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. *PLoS One* 2009;4:e4724.
2. Johnson VA, Brun-Vezinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: December 2010. *Top HIV Med* 2010;18:156–163.
3. Viani RM, Peralta L, Aldrovandi G, et al. Prevalence of primary HIV-1 drug resistance among recently infected adolescents: A multicenter adolescent medicine trials network for HIV/AIDS interventions study. *J Infect Dis* 2006;194:1505–1509.
4. Ammaranond P, Cunningham P, Oelrichs R, et al. Rates of transmission of antiretroviral drug resistant strains of HIV-1. *J Clin Virol* 2003;26:153–161.
5. Tossonian HK, Raffa JD, Grebely J, et al. Primary drug resistance in antiretroviral-naïve injection drug users. *Int J Infect Dis* 2009;13:577–583.
6. Mellins CA, Tassiopoulos K, Malee K, et al. Behavioral health risks in perinatally HIV-exposed youth: Co-occurrence of sexual and drug use behavior, mental health problems, and nonadherence to antiretroviral treatment. *AIDS Patient Care STDs* 2011;25:413–422.
7. Hightow-Weidman LB, Hurt CB, et al. Transmitted HIV-1 drug resistance among young men of color who have sex with men: A multicenter cohort analysis. *J Adolesc Health* 2011;48:94–99.
8. Mitsch A, Hu X, McDavid Harrison K, Durant T; Division of HIV/AIDS Prevention, National Center for HIV AIDS Viral Hepatitis STD and TB Prevention CDC. Trends in HIV/AIDS diagnoses among men who have sex with men—33 states, 2001–2006. 2008. May 17, 2010.
9. Goldsamt LA, Clatts MC, Parker MM, Colon V, Hallack R, Messina MG. Prevalence of sexually acquired antiretroviral drug resistance in a community sample of HIV-positive men who have sex with men in New York City. *AIDS Patient Care STDs* 2011;25:287–293.
10. Hurt CB, Matthews DD, Calabria MS, et al. Sex with older partners is associated with primary HIV infection among men who have sex with men in North Carolina. *J Acquir Immune Defic Syndr* 2010;54:185–190.
11. Leigh Brown AJ, Frost SD, Mathews WC, et al. Transmission fitness of drug-resistant human immunodeficiency virus and the prevalence of resistance in the antiretroviral-treated population. *J Infect Dis* 2003;187:683–686.
12. Rudy BJ, Kapogiannis BG, Lally MA, et al. Youth-specific considerations in the development of preexposure prophylaxis, microbicide, and vaccine research trials. *J Acquir Immune Defic Syndr* 2010;54 Suppl 1:S31–S42.

Address correspondence to:

Allison Agwu, M.D., ScM.

Department of Pediatric Infectious Diseases

Johns Hopkins Medical Institution

200 North Wolfe Street

Room 3145

Baltimore, MD 21287

E-mail: ageorg10@jhmi.edu