Clinical Infectious Diseases

MAJOR ARTICLE

Maternal Human Immunodeficiency Virus (HIV) Drug Resistance Is Associated With Vertical Transmission and Is Prevalent in Infected Infants

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Background. We aimed to assess if maternal human immunodeficiency virus (HIV) drug resistance is associated with an increased risk of HIV vertical transmission and to describe the dynamics of drug resistance in HIV-infected infants.

Methods. This was a case-control study of PROMISE study participants. "Cases" were mother-infant pairs with HIV vertical transmission during pregnancy or breastfeeding and "controls" were mother-infant pairs without transmission matched 1:3 by delivery date and clinical site. Genotypic HIV drug resistance analyses were performed on mothers' and their infants' plasma at or near the time of infant HIV diagnosis. Longitudinal analysis of genotypic resistance was assessed in available specimens from infants, from diagnosis and beyond, including antiretroviral therapy (ART) initiation and last study visits.

Results. Our analyses included 85 cases and 255 matched controls. Maternal HIV drug resistance, adjusted for plasma HIV RNA load at infant HIV diagnosis, enrollment CD4 count, and antepartum regimens, was not associated with in utero/peripartum HIV transmission. In contrast, both maternal plasma HIV RNA load and HIV drug resistance were independent risk factors associated with vertical transmission during breastfeeding. Furthermore, HIV drug resistance was selected across infected infants during infancy.

Conclusions. Maternal HIV drug resistance and maternal viral load were independent risk factors for vertical transmission during breastfeeding, suggesting that nevirapine alone may be insufficient infant prophylaxis against drug-resistant variants in maternal breast milk. These findings support efforts to achieve suppression of HIV replication during pregnancy and suggest that breastfeeding infants may benefit from prophylaxis with a greater barrier to drug resistance than nevirapine alone.

Keywords. HIV; drug resistance; vertical transmission; prophylaxis; nevirapine.

Antiretroviral (ARV) therapy (ART) of pregnant women and ARV prophylaxis of infants have substantially reduced human immunodeficiency virus (HIV) vertical (ie, from mother-to-child) transmission rates from between 15% and 45% down to ≤2% [1, 2]. Since 2010, ART has decreased the incidence of global pediatric HIV infections by 48% to a low of 150 000 (95% confidence interval [CI], 94 000–240 000) [3]. This impressive decline has only been achieved in some countries [4]. Vertical transmission continues with a relatively high incidence of ~12% (95% CI, 9.8–15.2%) in 21 countries [5], far short of the UNAIDS Fast-Track target for 2020. The disparity in vertical transmission rates is likely due to multiple factors, including access to testing, care and ART, adherence to ART during pregnancy and breastfeeding, and potential HIV drug resistance.

Between 2014 and 2018, pretreatment drug resistance to nonnucleoside reverse transcriptase inhibitors (NNRTIs) among adults initiating first-line ART in resource-limited settings exceeded 10% [6]. This threatened the efficacy of NNRTI-based regimens, particularly among women of child-bearing potential with significantly greater rates of resistance compared to men [6, 7].

Whether NNRTI drug resistance in pregnant women and breastfeeding mothers increases the risk of HIV vertical transmission is not known. Our study aimed to assess if maternal plasma HIV drug resistance is associated with an increased risk of vertical transmission and to describe the dynamics (ie, acquisition and decay) of NNRTI resistance in HIV-infected infants.

METHODS

Study Design and Participants

We examined the genotypic HIV drug resistance profiles of HIV-infected mothers from the Promoting Maternal and Infant

Received 30 April 2021; editorial decision 23 August 2021; published online 1 September 2021.
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Clinical Infectious Diseases® 2021;XX(XX):0–0

https://doi.org/10.1093/cid/ciab744

Survival Everywhere (PROMISE) study who transmitted HIV (cases) versus mothers who did not transmit (controls) to their infants to assess the role of maternal HIV drug resistance in vertical transmission—both in utero and during breastfeeding. Each case was matched to 3 controls with the closest delivery date (within ≤91 days) at the same clinical site; with "ties" resolved by use of a least-absolute difference strategy.

PROMISE was a randomized, open-label strategy trial, conducted from 2011 to 2016 prior to universal ART. Asymptomatic HIV-infected pregnant women with relatively high CD4 counts (≥350 cells/uL) were enrolled to compare ARV strategies proven to reduce HIV vertical transmission (Figure 1). The trial was conducted at 14 sites in 7 countries: India, Malawi, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe. PROMISE randomized pregnant women (≥14 weeks of gestation) "antepartum", mothers (day 6–14 after delivery) "postpartum," and women after cessation of breastfeeding "maternal health" [8–11] (Figure 1). The current study of HIV drug resistance includes women participating in the antepartum and postpartum randomizations, and mother-infant pairs not eligible for postpartum randomization but who continued postpartum in observational follow-up and who received ART according to country guidelines. Over the course of PROMISE, country guidelines changed: ART was administered at CD4 levels <500 cells/uL and later to all women regardless of CD4 cell counts.

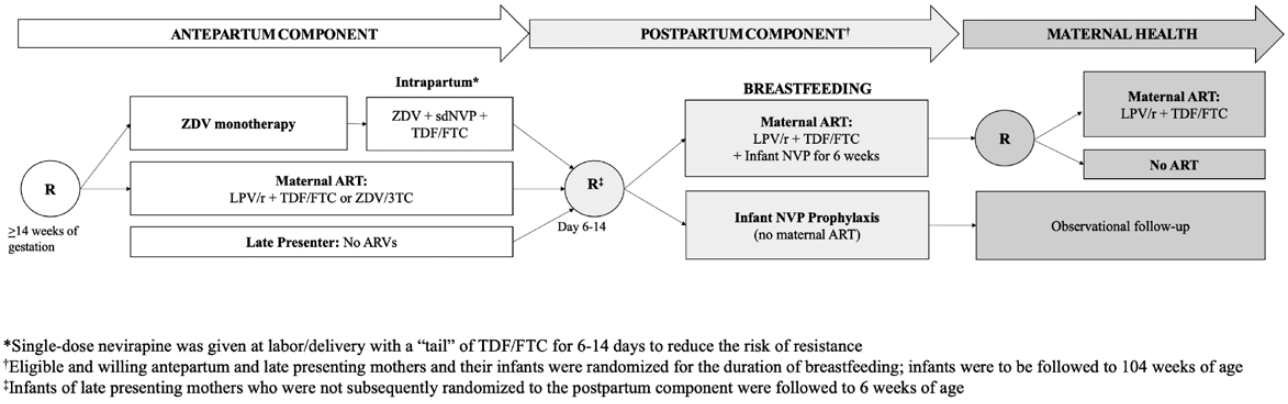
Vertical transmission was categorized as occurring "in utero/peripartum" if nucleic acids were detected in the infant within 2 weeks of birth or as "breastfeeding" if negative prior to day 14 of life and subsequently positive between 2 and 104 weeks of age. Genotypic HIV drug resistance was assessed in mothers' plasma nearest to the date of infant's HIV diagnosis (or similar postpartum time for matched control mothers) and infants' specimens at HIV diagnosis and longitudinally for infants to their last study visit. Associations between maternal drug resistance and vertical transmission were assessed in case versus control mothers. Drug resistance in infants was summarized by timing of HIV acquisition and over time.

Laboratory Methods to Evaluate HIV Genotypic Drug Resistance

HIV RNA was extracted from 0.14 to 1 mL of plasma using the QIAamp viral RNA mini kit (Qiagen, Valencia, California). Reverse transcription and polymerase chain reaction (PCR) amplification of human immunodeficiency virus type 1 (HIV-1) *pol* encoding protease and a portion of reverse transcriptase, sensitive to a single viral template, were carried out using PrimeScript One Step RT-PCR (Takara Bio USA, Inc., Mountain View, California), as described [12]. Amplicons underwent consensus sequencing (NCBI Genbank accession numbers: MZ706477 - MZ706941). Neighbor-joining phylogenetic trees of all sequences checked for potential specimen mix-ups or carry-over contamination (Geneious version 11.1.5). HIV genotypic drug resistance scores were determined by Stanford HIVdb Program (version 8.9–1, http://hivdb.stanford.edu/hivdb/by-sequences/) and categorized as wild-type or drug-resistant genotype based on major drug resistance mutations with a score ≥10.

Statistical Analysis

Univariate associations of maternal drug resistance, plasma HIV RNA load at infant HIV diagnosis, and enrollment CD4 count with HIV vertical transmission were examined with Fisher exact and Mann-Whitney tests. Conditional logistic regression was used for unadjusted and adjusted models of maternal genotype, plasma HIV RNA load, CD4 count, and PROMISE regimen. In these models, HIV RNA load and CD4 count were categorized above/below 4 $\log_{10}$ copies/mL or 500 cells/uL, the approximate observed medians. Analyses were performed separately among infants diagnosed in utero/peripartum and during the breastfeeding period. A sensitivity analysis used a more stringent definition of maternal drug resistance cutoff score (defined



*Single-dose nevirapine was given at labor/delivery with a "tail" of TDF/FTC for 6-14 days to reduce the risk of resistance
†Eligible and willing antepartum and late presenting mothers and their infants were randomized for the duration of breastfeeding; infants were to be followed to 104 weeks of age
‡Infants of late presenting mothers who were not subsequently randomized to the postpartum component were followed to 6 weeks of age

Figure 1. PROMISE trial randomization schema. Timing and antiretroviral drug randomizations for all 3 components of the PROMISE trial. Abbreviations: ART, antiretroviral therapy; ARV, antiretroviral drugs; LPV/r, lopinavir boosted with ritonavir; PROMISE, Promoting Maternal and Infant Survival Everywhere; sdNVP, single dose nevirapine; TDF/FTC, tenofovir and emtricitabine; ZDV, zidovudine; ZDV/3TC, zidovudine and lamivudine.

as having at least 1 mutation with a Stanford drug resistance score ≥30) to categorize participants. Drug resistance among infants categorized as in utero/peripartum transmission versus breastfeeding transmission was compared by Fisher exact test. Statistical significance was defined as a 2-sided *P*-value < .05 for all analyses. All statistical analyses were conducted using RStudio (version 1.1.442) [13].

RESULTS

HIV Drug Resistance as a Potential Risk Factor for HIV Vertical Transmission

Our analyses included 85 mothers-infant cases and 255 matched maternal controls. Of the cases, 21 were randomized antepartum and postpartum, and 64 were in observational follow-up. Slightly more infants were infected in utero/peripartum (n = 48) compared to breastfeeding (n = 37). HIV drug resistance genotypes were derived for 82 case mothers, 77 case infants (41 in utero/peripartum, and 26 breastfeeding transmission cases), and 226 control mothers; 24 plasma were missing and 18 did not amplify.

HIV drug resistance was greater in transmitting versus non-transmitting mothers at the time of infant HIV diagnosis (14.6% vs 6.7%, *P* = .039) (Figure 2A); detected in 12/82 (14.6%) mothers, including 11/12 (91.7%) transmitting during breastfeeding (Figure 2B).

The HIV drug resistance mutations by drug class (Table 1) observed K103N, a NNRTI-associated mutation, as most prevalent in both transmitting and nontransmitting mothers. Among mothers with HIV drug resistance, the one who transmitted during pregnancy was randomized antepartum to zidovudine monotherapy and had K219N conferring resistance to nucleoside reverse transcriptase inhibitors (NRTI), and 11 transmitted during breastfeeding, all with NNRTI resistance. Importantly, only 2/11 women were prescribed an NNRTI (nevirapine plus TDF/FTC tail) during PROMISE, both had wild-type virus, suggesting selection by the nevirapine taken at labor/delivery (Table 2).

Genotyping and phylogenetic analyses were performed on study enrollment specimens to assess the possibility of pretreatment HIV drug resistance. All but 1 of the enrollment specimens from the 9 mothers without reported exposure to NNRTIs but detectable NNRTI resistance at time of infant HIV diagnosis grouped with their specimens from the time of infant diagnosis (data not shown). One mother's enrollment specimen did not cluster with her and her infant's diagnostic and longitudinal (n = 2) specimens, which all clustered; this indicated that the maternal enrollment specimen was misattributed to this woman, and thus, it was excluded from analyses.

At study enrollment two women had NNRTI resistance (G190GA and K103KN) that persisted in later specimens (Table 2), indicating likely acquisition of the drug-resistant virus at the time of their becoming infected. A third woman's enrollment and only specimen had K103KN suggesting possible acquisition

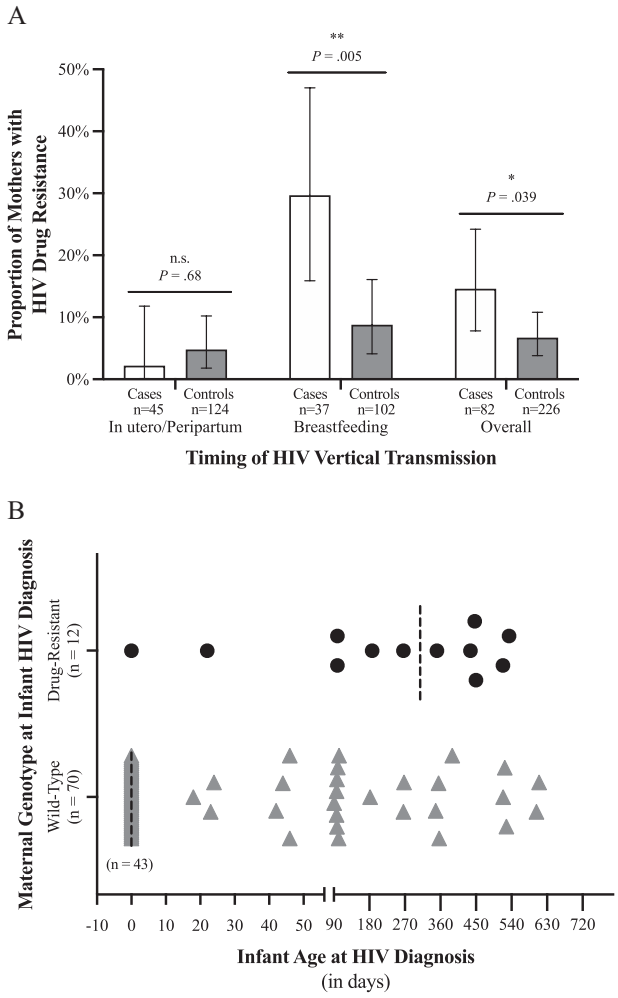


Figure 2. HIV drug resistance among HIV transmitting and control mothers by timing of transmission, and by the infant's age at time of diagnosis. *A*, Proportion of mothers with HIV drug resistance by timing of vertical transmission and overall; white bars show transmitting mothers and gray bars show non-transmitting (control) mothers. Bars indicate the 95% binomial exact confidence interval for each proportion. *B*, Timing of HIV vertical transmission (by infant age in days) by maternal genotype at infant diagnosis; gray triangles indicate wild-type genotype in mothers and black circles indicate drug-resistance mutations detected in mothers. Black dotted lines indicate the median age of infants within each grouping of maternal genotype. Abbreviation: HIV, human immunodeficiency virus.

at time of primary HIV infection. No drug resistance mutations were detected among the remaining five women, suggesting that their resistance detected at the time of infant HIV diagnosis (single K103K/N, a single V179VD, a single Y181C, or both Y188C and G190A) were from unrecorded administration of NNRTI.

Other Risk Factors for HIV Vertical Transmission

HIV transmitting mothers had higher plasma HIV RNA loads at time of infant diagnosis compared to non-transmitting mothers (medians 4.28 vs 3.86 $\log_{10}$ copies/mL, *P* < .0001; Figure 3A); all this difference is attributed to the breastfeeding transmission

Table 1. Human Immunodeficiency Virus (HIV) Drug Resistance Mutations by Antiretroviral Class Detected in Transmitting (Case) and Nontransmitting (Control) Mothers at Time of Infants' HIV Diagnosis

Drug Resistance Mutation	Number of Cases (%) n = 82	Number of Controls (%) n = 226
PI-associated		
M46I	0	2 (0.9%)
NRTI-associated		
M41L	0	1 (0.4%)
D67N	0	2 (0.9%)
K70R	0	1 (0.4%)
K219N	1 (1.2%)	0
NNRTI-associated		
A98G	0	1 (0.4%)
K101E	1 (1.2%)	2 (0.9%)
K103N	7 (8.5%)	6 (2.7%)
E138K	0	1 (0.4%)
V179D	1 (1.2%)	0
Y181C	1 (1.2%)	0
Y188C	1 (1.2%)	0
G190A/E	2 (2.4%)	2 (0.9%)
Total number of mothers with ≥1 drug resistance mutation (%, 95% CI)	12 (14.6%, 7.8–24.2%)	15 (6.6%, 3.8–10.7%)

Abbreviations: CI, confidence interval; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitor.

group (medians 4.73 vs. 3.89 $\log_{10}$ copies/mL, $P < .0001$). Transmitting mothers' enrollment CD4 counts were lower compared to nontransmitting (median 496 vs 539.5 cells/uL, $P = .028$; Figure 3B), also limited to the mothers transmitting during breastfeeding. The plasma RNA measurement analyzed for the in utero/peripartum transmitting cases and controls was from a median 1 day (interquartile range [IQR]: 1–3) and 0 days (IQR: −95 to 2), respectively, from delivery. Because vertical transmission may have occurred earlier during pregnancy, maternal HIV RNA loads

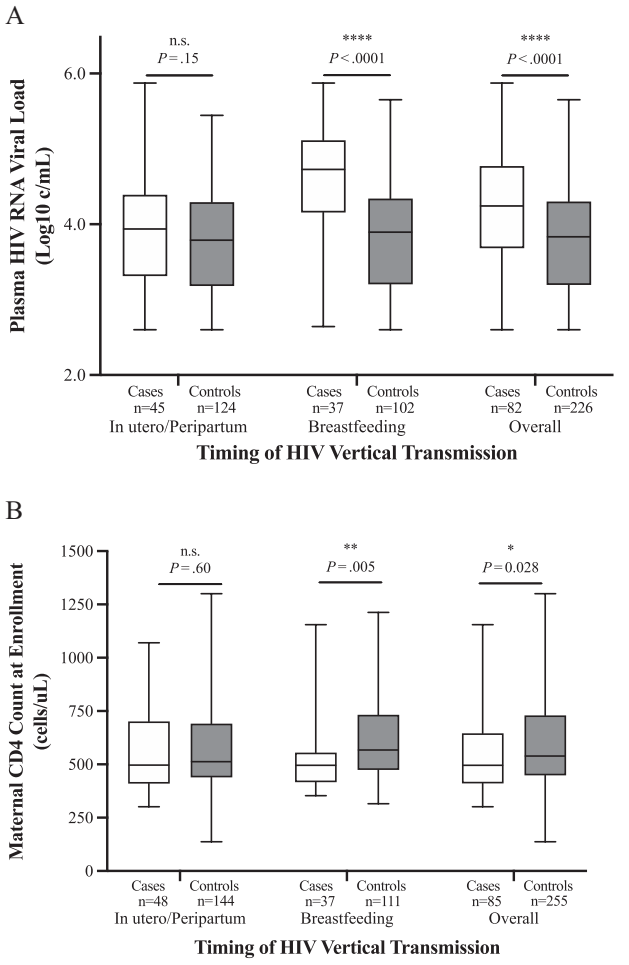


Figure 3. Plasma HIV RNA load at time of infant HIV diagnosis and CD4 T-cell counts at study enrollment of HIV transmitting and control mothers. *A*, Maternal plasma HIV RNA load by timing of vertical transmission and overall; white bars show transmitting mothers and gray bars show nontransmitting (control) mothers. *B*, Maternal enrollment CD4 count by timing of vertical transmission and overall; white bars show transmitting mothers and gray bars show nontransmitting (control) mothers. Abbreviation: HIV, human immunodeficiency virus.

Table 2. Antiretrovirals Administered to Transmitting Mothers With Human Immunodeficiency Virus (HIV) Drug Resistance Detected at Time of Infant HIV Diagnosis and Their HIV Drug Resistance Genotype at Enrollment

Mother	Antepartum Treatment Regimen	Postpartum Treatment Regimen	Maternal NNRTI Exposure During PROMISE	HIV Drug Resistance Mutations at Study Enrollment	HIV Drug Resistance Mutations at Time of Infant Diagnosis
M021	ZDV+NVP+TDF/FTC tail	Infant NVP	Yes	none	K103KN
M038	ZDV+NVP+TDF/FTC tail	No ARVs	Yes	none	K101KE, K103KN
M076	TDF+FTC+LPV/r	TDF+FTC+LPV/r	No	G190AE	G190GA
M035	ZDV+3TC+LPV/r	TDF+FTC+LPV/r	No	K103N	K103KN
M063	TDF+FTC+LPV/r	Infant NVP	No	none	K103KN
M037	ZDV+3TC+LPV/r	TDF+FTC+LPV/r	No	n/a[a]	K103KN
M012	ZDV+3TC+LPV/r	Infant NVP	No	none	V179VD
M085	TDF+FTC+LPV/r	No ARVs	No	K103KN	n/a[b]
M031	ZDV+3TC+LPV/r	No ARVs	No	none	K103N
M062	ZDV+3TC+LPV/r	Infant NVP	No	none	Y181YC
M069	ZDV+3TC+LPV/r	Infant NVP	No	none	Y188YC, G190GA

Abbreviations: LPV/r, ritonavir-boosted lopinavir; NNRTI, non-nucleoside reverse transcriptase inhibitors; NVP, nevirapine; PROMISE, Promoting Maternal and Infant Survival Everywhere; TDF/FTC, tenofovir and emtricitabine; 3TC, lamivudine; ZDV, zidovudine.

[a]Sample excluded as phylogenetic analyses indicated the specimen was likely not from the same individual.

[b]Specimen unavailable for genotypic testing.

at study entry were also compared with higher plasma HIV RNA loads observed in case compared to control mothers (medians 4.39 vs 3.96 $\log_{10}$ copies/mL, $P < .001$).

Adjusted Analyses

To assess whether factors were independently associated with vertical transmission, conditional logistic regression analyses were performed by the timing of transmission.

Table 3. Risk Factors for In Utero/Peripartum Human Immunodeficiency Virus (HIV) Transmission, a Multivariate Analysis

		In Utero/ Peripartum Mothers		Unadjusted Conditional Logistic Regression		Adjusted Conditional Logistic Regression	
Risk Factor	Total N = 191	Case N = 48	Control N = 143	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
Antepartum treatment regimen							
Triple ART (PI-based)	83	11	72	Reference		Reference	
Zidovudine + sdNVP +TDF/FTC tail	87	28	59	3.04 (1.41–6.55)	.005[a]	4.54 (1.70–12.1)	.003[a]
None (late presenter)	22	9	13	6.19 (1.82–21.1)	.004[a]	9.82 (2.07–46.7)	.004[a]
Genotype							
Wild-type	162	44	118	Reference		Reference	
Drug-Resistant	7	1	6	0.44 (.05–3.67)	.448	0.34 (.03–3.38)	.354
Missing genotype data	23	3	20	-		…	
HIV RNA load at infant dagnosis							
<4 log10 c/mL	98	24	74	Reference		Reference	
≥4 log10 c/mL	74	23	51	1.36 (.70–2.65)	.367	1.00 (.38–2.63)	.988
Missing VL data	19	1	18	…		…	
HIV RNA load at enrollment							
<4 log10 c/mL	96	16	80	Reference		Reference	
≥4 log10 c/mL	95	32	63	2.65 (1.31–5.35)	.007[a]	3.45 (1.24–9.57)	.018[a]
Enrollment CD4 count							
≥500 cells/uL	105	24	81	Reference		Reference	
<500 cells/uL	86	24	62	1.33 (.69–2.57)	.399	1.16 (.50–2.71)	.723

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; NVP, nevirapine; PI, protease inhibitor; TDF/FTC, tenofovir and emtricitabine.

[a] *P* value < .05.

Adjusted analyses of the in utero/peripartum cases indicated that HIV drug resistance, maternal plasma HIV RNA load at infant diagnosis, and enrollment CD4 count had no statistically significant associations with vertical transmission (Table 3). However, vertical transmission was associated with maternal plasma HIV RNA load at study enrollment (odds ratio [OR] 3.45, 95% CI: 1.24–9.57), and as observed in the larger study [8], antepartum treatment regimen, with ART reducing transmission compared to zidovudine monotherapy or no therapy (OR 4.54, 95% CI: 1.70–12.1 and OR 9.82, 95% CI: 2.07–46.6, respectively).

Adjusted analyses of the breastfeeding cases found that maternal drug resistance and high maternal HIV RNA load at infant diagnosis were independently associated with transmission (Table 4) (OR 4.45, 95% CI: 1.34–14.7 and OR 4.03, 95% CI: 1.43–11.4, respectively). Enrollment CD4 count and postpartum treatment regimen were not statistically associated with transmission.

A sensitivity analysis was performed with a Stanford HIV Database resistance score cutoff of ≥30 (mutations defined as having "intermediate-level resistance" or "high-level resistance") instead of ≥10 (includes "potential low-level drug resistance" mutations). The higher HIV drug resistance score in mothers remained associated with breastfeeding transmission (OR 5.24, 95% CI: 1.29–21.2).

Prevalence of HIV Drug Resistance Among the Infants

At HIV diagnosis, the frequency of drug resistance was significantly lower in infants with in utero/peripartum versus breastfeeding transmission (12.2% vs 52.8%, $P < .001$; Figure 4). Resistance mutations were detected in 5/41 in utero/peripartum infants and included single NNRTI mutations (V106I/M, V108I, Y181C, and G190E). In contrast, 19/36 infants with breastfeeding transmission had either single NRTI (M184I and M184V), single (K103N and Y181C), and multiple NNRTI (combinations of A98G, K103N, V106M, V179D, Y181C, Y188C, and G190A), or dual-class resistance mutations (M184I, K101E, Y181C, and G190A) (Table 5).

Every infant in PROMISE, including those categorized as acquiring HIV in utero/peripartum, were given 6 weeks of nevirapine prophylaxis. Thus, determining if an infant's resistance originated from their mother or the nevirapine administered to them directly was not possible. Instead, we compared the presence or absence of drug resistance mutations among mother-infant pairs (ie, concordance or discordance) at the time of infant HIV diagnosis. Concordance of wild-type or drug-resistant HIV was detected in 48/74 (64.9%) and 8/74 (10.8%), respectively. The remaining mother-infant pairs were discordant, with wild-type in mothers and drug-resistance in infants (16/18; 88.9%) or the reverse (2/18; 11.1%). Of these 16 mother-infant pairs, 11 (68.8%) were classified as breastfeeding transmission. Six of these 11 had <14 days between the maternal and infant specimen collection, but mothers' specimens were collected at a much earlier time for the other 5 pairs (median 205 days [range: 91–357]).

Table 4. Risk Factors for Breastfeeding Human Immunodeficiency Virus (HIV) Transmission, a Multivariate Analysis

Risk Factor	Total N = 148	Breastfeeding Mothers: Case N = 37	Breastfeeding Mothers: Control N = 111	Unadjusted Conditional Logistic Regression: Odds Ratio (95% CI)	Unadjusted Conditional Logistic Regression: *P* Value	Adjusted Conditional Logistic Regression: Odds Ratio (95% CI)	Adjusted Conditional Logistic Regression: *P* Value
Postpartum treatment regimen							
Infant NVP prophylaxis	52	10	42	Reference		Reference	
Maternal triple ART	52	10	42	0.98 (.38–2.52)	.965	1.05 (.36–3.03)	.934
Not randomized to postpartum Component[b]	44	17	27	4.28 (1.42–12.9)	.010[a]	2.95 (.74–11.8)	.127
Genotype							
Wild-type	119	26	93	Reference		Reference	
Drug-resistant (DR score ≥10)	20	11	9	3.89 (1.49–10.1)	.006[a]	4.45 (1.34–14.7)	.015[a]
Missing genotype data	9	0	9	…		…	
HIV RNA load at infant diagnosis							
<4 log10 c/mL	68	7	61	Reference		Reference	
≥4 log10 c/mL	71	27	44	4.70 (1.85–11.9)	.001[a]	4.03 (1.43–11.4)	.008[a]
Missing VL data	9	3	6	…		…	
Enrollment CD4 count							
≥500 cells/uL	92	18	74	Reference		Reference	
<500 cells/uL	56	19	37	2.46 (1.06–5.72)	.037[a]	1.33 (.48–3.64)	.580

Abbreviations: ART, antiretroviral therapy; ARV, antiretroviral drugs; CI, confidence interval; NVP, neveperine; VL, viral load.

[a]*P* value < .05.

[b]Women not randomized to the postpartum component had no additional randomization after the antepartum component and were observed by the study team while receiving ARV treatment per their countries' guidelines.

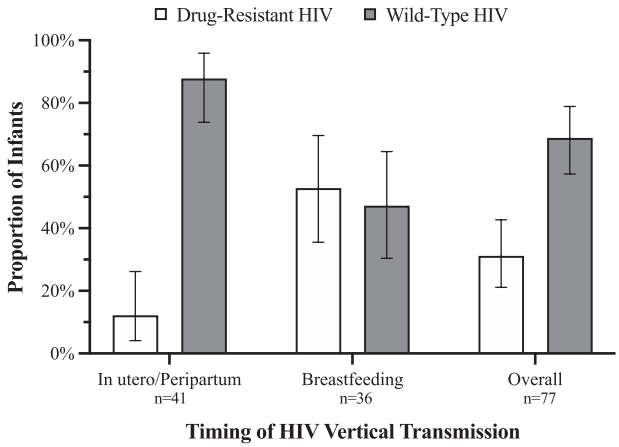


Figure 4. Infant's drug resistance genotypes at HIV diagnosis by timing of vertical transmission and overall. White bars indicate the proportion of infants with drug resistance at HIV diagnosis, and gray bars show the proportion of infants with wild-type HIV. Bars indicate the 95% binomial exact confidence intervals for each proportion. Abbreviation: HIV, human immunodeficiency virus.

Although the frequency of HIV drug resistance at diagnosis was greater among infants with breastfeeding versus in utero/peripartum transmission, resistance increased over time in all infants (Figure 5); from 12.2% to 55.5% in in utero/peripartum and 52.8% to 78.9% in the breastfed at last study visit.

DISCUSSION

The hypothesis that antiretroviral prophylaxis of infants against HIV infection is less effective in preventing vertical transmission when their mothers who harbor drug-resistant virus was evaluated by this study. Maternal HIV drug resistance was found to be independently associated with vertical transmission during breastfeeding, supporting our hypothesis. Among transmitting mothers with drug resistance, all had detectable NNRTI-associated resistance mutations, and infants appeared to acquire HIV during breastfeeding. HIV drug resistance was not associated with vertical transmission occurring in utero, potentially because women in this study were not administered NNRTI-based ART during pregnancy. However, those randomized to zidovudine during pregnancy were prescribed nevirapine with a tail of TDF/FTC, and all infants were prescribed ≥6 weeks of prophylactic nevirapine.

Our results are novel and add to previous studies of the impact of antiretroviral drug resistance on infant transmission [14, 15]. In a case-control study of in utero/intrapartum HIV transmission that detected HIV drug resistance in 63/606 (10.4%) of late-presenting, non-breastfeeding mothers, resistance did not appear to increase vertical transmission [14]. Rather, transmission was associated with maternal viral load and infants' prophylaxis (zidovudine alone vs zidovudine +3 doses of nevirapine vs zidovudine+nelfinavir+lamivudine). These observations are consistent with the findings of this study across the in utero/peripartum timeframe.

Our finding that maternal plasma HIV RNA load was independently associated with HIV vertical transmission concurs with other studies [16–20]. In our adjusted analyses by timing of transmission, high HIV RNA load (≥4log$_{10}$ copies/mL) at infant diagnosis was not associated with in utero/peripartum transmission; however, high HIV RNA load at study enrollment was significantly associated with transmission. This suggests that vertical transmission may have taken place during pregnancy prior to ARV reduction of plasma RNA load. HIV drug

Table 5. Human Immunodeficiency Virus (HIV) Drug Resistance in Infants at Time of Diagnosis by Antiretroviral Class and Timing of Vertical Transmission

Number and Antiretroviral Drug Class With Resistance Mutations Detected	In utero or Peripartum Vertical Transmission n = 41 (53.3%)	Breastfeeding Vertical Transmission n = 36 (46.7%)
NRTI		
Single	…	3 (8.3%)
Multiple	…	…
NNRTI		
Single	5 (12.2%)	8 (22.2%)
Multiple	…	7 (19.4%)
NRTI and NNRTI	…	1 (2.8%)
Total number (%; 95% CI)	5 (12.5%; 4.1%, 26.2%)	19 (52.8%; 35.5%, 69.6%)

Abbreviations: CI, confidence interval; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors.

resistance, however, was not associated with in utero transmission cases. Conversely, among cases of breastfeeding transmission both a higher maternal HIV viral load at infant diagnosis and HIV drug resistance were independently associated with vertical transmission. This latter association highlights our supposition that more potent ART regimens may be superior to infant nevaripine in preventing breastfeeding transmission, particularly in regions with a high prevalence of maternal pretreatment drug resistance to NNRTI [6]. Additionally, our finding that all case women with NNRTI resistance transmitted during breastfeeding further suggests that infant prophylactic ARVs without NNRTI cross-resistance, optimally long-acting injectables, could further reduce vertical transmission.

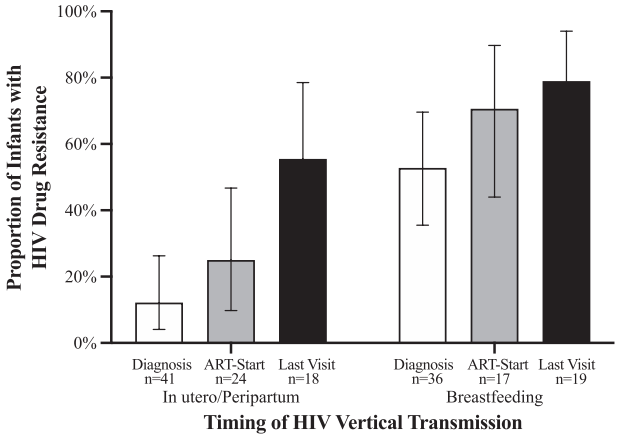


Figure 5. Proportion of infants with HIV drug resistance detected in longitudinal specimens by timing of vertical transmission. White bars indicate the proportion of infants with drug resistance at HIV diagnosis, gray bars show the proportion of infants with drug resistance at ART initiation, and dark gray bars show the proportion of infants with drug resistance at their last study visit. Bars indicate the 95% binomial exact confidence intervals for each proportion probability. For infants with in utero/peripartum HIV transmission the median age at HIV diagnosis was 2 days (IQR: 0–10), at ART initiation was 44 days (IQR: 31.75–63.5), and at last study visit was 683 days (IQR: 197–708.5). For infants with breastfeeding HIV transmission, the median age at HIV diagnosis was 182 days (IQR: 96–433), at ART initiation was 307 days (IQR: 125.3–423.5), and at last study visit was 681 days (IQR: 528.5–687.5). Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range.

Our analyses suggest that both pretreatment NNRTI-associated drug resistance and administration of nevirapine in labor likely contributed to maternal NNRTI resistance among transmitting mothers. Although the combination of single-dose nevirapine with 6–14 days of TDF/FTC should minimize NNRTI resistance based on earlier studies [21, 22], poor adherence could reduce effectiveness and select NNRTI resistance [23, 24]. Genotypic and phylogenetic analyses indicated that more than a fourth of these mothers had transmitted HIV drug resistance, which is consistent with the prevalence of pretreatment HIV drug resistance surveillance data between 2014 and 2016 in Eastern and Southern Africa (22–35%) [25]. Among those without pretreatment resistance at study enrollment, a minority were randomized to an antepartum regimen that included single-dose nevirapine at onset of labor, which often selects NNRTI resistance [26–34]. Medical records of those with newly detected NNRTI resistance during our study did not reveal prescribed NNRTIs, and our review of their chromatograms confirmed the absence of NNRTI mutations at enrollment. These findings suggest that unrecorded NNRTIs were administered during the current pregnancy or delivery, perhaps as part of routine clinical care. During the PROMISE trial, the administration of single-dose nevirapine at the onset of labor was routine care for prevention of HIV vertical transmission. Dedicated staff at the clinical sites may have administered single-dose nevirapine to PROMISE participants randomized to ART, although per protocol only those randomized to zidovudine should have received it. Additionally, women not randomized to a study regimen during breastfeeding may have been prescribed ART, likely efavirenz-based ART, by their clinician. Alternatively, NNRTI mutations may have been present at entry at a frequency below the sensitivity of consensus sequencing, then selected during breastfeeding due to linkage to other favored genotypes.

HIV drug resistance was less prevalent at diagnosis among infants with in utero/peripartum as compared to breastfeeding vertical transmission; however, over time drug resistance emerged in both groups. It is likely that administration of nevirapine to the mother at delivery and/or to the infant during breastfeeding contributed to the emergence of resistance. The majority of mother-infant case pairs with genotype discordance at diagnosis included maternal wild-type HIV and transmission during breastfeeding. Thus, these infants were likely infected with wild-type HIV and shortly thereafter drug resistance mutations were selected by nevirapine prophylaxis, which were detected in their diagnostic specimen. However, the interval between specimen collection for some mothers versus their infant limits our interpretation of these data. Our observation that NNRTI resistance increased

during nevirapine prophylaxis of breastfeeding infants is consistent with other studies [35–38]. Nevirapine has a low genetic barrier to resistance, and therefore short-term exposure to the drug can select resistant variants. The selection of NNRTI-associated mutations during early infancy generally persists into childhood [39–41] and thus can preclude the utility of this drug class.

Our study has some limitations. The women enrolled in PROMISE had asymptomatic HIV infection (96% World Health Organization [WHO] Clinical Class I) with relatively high CD4 cell counts and are not representive of all pregnant women with HIV. The PROMISE trial did not utilize NNRTI in the antepartum ART regimens, so we could not evaluate whether the prevalent NNRTI resistance would lessen the prophylactic effect of maternal use of NNRTI-containing ART such as tenofovir/lamivudine/efavirenz. Additionally, all infants received nevirapine prophylaxis within 48 hours of birth and for at least 6 weeks. Thus, we were not able to evaluate if resistance detected in infants was transmitted or selected for by infant exposure to nevirapine. However, in some instances, the selection of drug resistance in infants could be inferred as their mothers had wild-type HIV at the same timepoint. Another limitation was that our ability to assess associations between postpartum study treatment regimen and maternal drug resistance was limited because many mother-infant pairs we analyzed were followed observationally during breastfeeding in PROMISE.

Our observation that maternal HIV drug resistance to NNRTI which includes nevirapine currently used for infant prophylaxis [42] was an independent risk factor for vertical transmission during breastfeeding is novel. This combined with our observations of an increased prevalence of infant NNRTI-resistance during breastfeeding suggests that nevirapine may both fail to prophylax infants and have a role in selecting resistance. Indeed, our results support the recommendation to replace nevirapine prophylaxis with regimens that have a greater barrier to drug resistance and would retain NNRTI susceptibility in infected infants.

Notes

Acknowledgments. The authors acknowledge the contributions of the study participants, site investigators, and staff, and IMPAACT central resources who supported the 1077BF study.

Financial support. Support for this analysis and overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (NIAID) with cofunding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Crossref Funder Registry and the National Institute of Mental Health (NIMH), all components of the National Institutes of Health (NIH), (under award numbers UM1AI068632 [IMPAACT LOC], UM1AI068616 [IMPAACT SDMC], and UM1AI106716 [IMPAACT LC]) and by NICHD (award number HHSN275201800001I). Additional support came from the Molecular Profiling and Computational Biology Core of the University of Washington Fred Hutch Center for AIDS Research (award number P30 AI027757), NICHD (award number HHSN275201300003C), and NIAID (award number T32 AI007509). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Potential conflicts of interest. C. L. B. reports receiving New Investigator Scholarship 2019 for travel support to Conference on Retroviruses and Opportunistic Infections, outside the submitted work. P. D. reports employment with/support from Harvard TH Chan School of Public Health, Center for Biostatistics in AIDS Research (CBAR), during the conduct of the study. P. M. F. reports grant support for conducting clinical trial from NIH-JHU, during the conduct of the study. M. G. F. and L. M. F. report ongoing grants paid to their institutions, during the conduct of the study. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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