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Meconium Atazanavir Concentrations and Early Language Outcomes in HIV-Exposed, Uninfected Infants with Prenatal Atazanavir Exposure

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Abstract

Objective—To investigate whether prenatal atazanavir (ATV) exposure, assessed by meconium antiretroviral quantification, predicts early child language outcomes. Prenatal ATV exposure previously was associated with poorer language development in one-year-olds.

Methods—Pregnant women with HIV and their uninfected infants enrolled in the SMARTT study. Meconium antiretroviral concentrations were quantified by liquid chromatography-tandem mass spectrometry. Language development at 1 year was assessed with MacArthur-Bates Communicative Development Inventory (CDI) and Bayley Scales of Infant and Toddler Development—Third Edition (Bayley-III). Late language emergence (LLE) was defined as ≥ one of four CDI scores ≤10th percentile for age. Associations between fetal ATV exposure timing and duration, meconium ATV concentration, and language outcomes were evaluated, adjusting for potential confounders.

Results—Through 2013, meconium samples were available from 175 of 432 infants with prenatal ATV exposure. Valid Bayley-III (n=93) and CDI (n=106) assessments also were available. After adjustment for potential confounders, higher ATV meconium concentrations were associated with lower LLE risk (P=0.04), and cumulative ATV exposure duration also was associated with

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higher Bayley-III Language scores (P=0.03). Maternal ATV duration and initiation week correlated with ATV meconium concentrations (positively and negatively, respectively).

Conclusions—Higher meconium ATV concentrations were protective against developmental language delays at 1 year, suggesting the importance of fetal ATV detoxification into meconium. This information supports ATV exposure safety for infant language development. ATV is a preferred ARV for pregnant women with HIV, suggesting the importance of ATV safety investigations. Additionally, further pursuit of the influences on language development in HEU infants is required.

Keywords

meconium; atazanavir; antiretroviral; language; HIV-exposed infants

INTRODUCTION

Antiretroviral therapy (ART) during pregnancy improves maternal health and reduces mother-to-child transmission of human immunodeficiency virus (HIV) to less than 2%[1] in the US. Such extensive ART use during pregnancy, however, requires careful monitoring for potential ART toxicity in prenatal HIV-exposed uninfected (HEU) children. The protease inhibitor atazanavir (ATV) is a preferred ART agent in pregnancy and is becoming one of the most commonly prescribed antiretroviral (ARV) drugs for pregnant women with HIV.[1,2] Recent Pediatric HIV/AIDS Cohort Study (PHACS) research demonstrated associations between fetal ATV exposure and poor early language development risk.[3,4] One-year-olds whose mothers were prescribed ATV during pregnancy had lower mean Bayley Scales of Infant and Toddler Development—Third Edition[23] (Bayley-III) Language domain scores compared to infants without ATV exposure.[3] In a separate investigation, prenatal ATV exposed one-year-olds also had increased late language emergence (LLE) risk, but language screening at age two showed no significant association between LLE and ATV exposure.[4] This association of intrauterine ATV exposure and lower performance on infant language development measures deserves further study.

Approximately 20% of maternal ATV in blood crosses the placenta, directly exposing the fetus.[5] ATV also inhibits uridine diphosphate glucuronosyltransferase (UGT) 1A1, the hepatic enzyme responsible for maternal and fetal bilirubin conjugation.[6] However, as fetal liver bilirubin conjugation capacity is limited, the fetus relies primarily on maternal hepatic bilirubin conjugation to prevent neurotoxic bilirubin exposure to the vulnerable fetal brain.[7,8] Neonatal unconjugated bilirubin exposure is associated with adverse neurologic and developmental outcomes, including language delays.[9–12] Because maternal ATV administration leads to higher maternal unconjugated bilirubin concentrations,[5] the fetus also may be exposed to elevated unconjugated bilirubin levels for a prolonged period.

Combination ART regimens are most effective at preventing mother-to-child transmission,[13–15] but concomitant medications affecting maternal ATV pharmacokinetics may also affect fetal ATV exposure. Tenofovir disproxil fumarate (TDF) co-administration reduces ATV trough concentrations;[16] similar ATV concentration effects were seen during pregnancy with third trimester TDF co-administration.[17]

Intrauterine ATV exposure may affect the fetus directly through transplacental drug transfer or indirectly through increased unconjugated bilirubin exposure, thus, accurate fetal ATV exposure quantification is critical to understanding infant and child outcomes. Previous fetal drug exposure research from terminated pregnancies demonstrated meconium, the first neonatal feces, begins to form early in the second trimester,[18,19] with meconium drug concentrations reflecting drug exposure during the third and perhaps second trimester.[20,21] We recently developed a novel meconium ARV drug assay enabling quantitative *in utero* ARV exposure assessment.[22] As previous studies reported associations between infant language development and intrauterine ATV exposure, we sought to quantify ARV meconium concentrations and investigate associations between ATV meconium concentrations, infant language measures, and maternal ATV medication history.

METHODS

Participants

The prospective PHACS Surveillance Monitoring of ART Toxicities (SMARTT) study enrolls pregnant women with HIV and their infants at 22 US sites to evaluate long-term prenatal ART exposure effects.[25] Infants enrolled between 22 weeks gestation and 1 week postnatal were included. Each site's Institutional Review Board approved the study and written informed consent was obtained. ARV exposure information, including start and stop dates, was abstracted from medical charts.

Meconium ARV quantification

Meconium was collected within 72 hours. Beginning in 2011, meconium was frozen immediately after collection; prior to 2011, meconium was refrigerated at study sites. Storage conditions were changed to ensure accurate analysis of alcohol use markers; unlike these other markers, meconium ATV concentrations proved equally stable under refrigerated and frozen conditions. Therefore, meconium ATV concentrations from both storage conditions were included. After laboratory receipt, all specimens were frozen (≤−20°C) until analysis (0–6 years). Meconium ARV drugs were quantified by our validated liquid chromatography tandem mass spectrometry method.[22] Sixteen parent ARVs and four metabolites were quantified in 0.25 g meconium with 10–500 ng/g quantification limits.[22] ATV linearity was 10–2,500 ng/g; inter-assay imprecision and accuracy were 3–5% and 85–119%, respectively.[22]

Language assessments

The Bayley-III[3] Language domain provides an age-referenced, standardized measure of language development from 1–42 months (mean score, SD; 100 ±15). The MacArthur-Bates Communicative Development Inventory (CDI)[4] provides gender-specific, age-adjusted percentile scores in four domains: Phrases Understood, Vocabulary Comprehension, Word Production, and Total Gestures. Validity of each Bayley-III assessment was determined by local examining psychologists; when needed, assessment results were reviewed by a study team member to resolve questions. When several CDI questionnaire items were omitted, scores were reviewed by a study team language expert. Both measures were administered at 9–15 months (the one-year study visit). Bayley-III scales were administered directly to

infants; the CDI was administered as a parent/caregiver interview utilizing the age-appropriate CDI Words and Gestures form. The Bayley-III is available only in English, while the CDI is available in English and Spanish. For this study, LLE was defined as a CDI score ≤10th percentile in one or more of the four domains.

Statistical analyses

ATV meconium concentration and language outcome distributions were inspected and appropriate transformations performed to achieve approximate normal distributions. Spearman correlations (ρ) of meconium ATV concentration with ATV exposure duration and timing were calculated. Infants whose mothers had interrupted ATV use during pregnancy (>three day gap between two regimens or stopped ATV use before delivery) were excluded from certain analyses, since maternal time off ATV can affect meconium drug concentrations. ATV meconium concentrations from infants whose mothers stopped ATV use before delivery were compared separately to those with uninterrupted intrauterine ATV exposure with the Wilcoxon rank sum test. A Jonckheere-Terpstra test assessed the trend between ATV meconium concentration and prenatal ATV exposure timing.

General linear and logistic regression models were built for continuous and binary language measures, respectively. Univariable analyses first identified potential confounders. For each outcome, a core model was obtained by identifying covariates associated with the outcome with $P<0.20$ in univariable models and retained with $P<0.10$ in multivariable models. Additionally, when an ATV exposure was added to the model, covariates not included initially were evaluated and those that changed ATV exposure estimates by at least 10% and were associated with the outcome ($P<0.10$) were included. Multivariable models were then built to estimate associations of adjusted ATV exposure with language outcomes, controlling for all identified covariates. Concomitant TDF was forced in multivariable models a priori. Maternal and infant characteristics evaluated as potential covariates are described in Table 1. Maternal CD4 and HIV RNA during pregnancy were excluded from adjusted models; most measurements were obtained after ATV initiation and therefore, we cannot control for medical reasons that may have indicated why a mother started ATV. These measurements could potentially be confounders or intermediates on the causal pathway between ATV exposure and language outcomes.

To evaluate maternal concomitant TDF impact on meconium ATV concentrations, we compared ATV concentrations from infants exposed to uninterrupted TDF+ATV+ritonavir to those from infants exposed to uninterrupted ATV+ritonavir with Wilcoxon's rank sum test. Group language measures were compared by a two-sample t-test or Fisher's exact test, as appropriate.

Sensitivity analyses evaluated the impact of adjusting for premature birth and low birth weight, covariates possibly on the causal pathway between ATV exposure and language outcomes.[23] Sensitivity analyses also adjusted for research site differences, using generalized estimating equations models considering individual language evaluations within sites as repeated measures. Unpaired t-tests, chi-square tests, and Fisher's exact test compared language scores and neonatal prophylaxis with previously published samples.

RESULTS

Participants

Of 1817 SMARTT Dynamic cohort infants enrolled through January 1, 2014, 432 (24%) were exposed to ATV during pregnancy. Meconium specimens were available for ARV quantification from 175 of these infants. Supplemental Table 1 compares maternal and infant demographic characteristics between these 175 and the other 257 infants. Demographic characteristics were similar between groups with a few exceptions; infants with meconium ATV results were less likely to be Black, preterm, have low birth weight or caregivers with English as their primary language, and they were more likely to have longer cumulative ATV exposure durations. Of our 175 ATV-exposed infants with meconium available, 93 had valid Bayley-III language scores, and 106 had valid CDI scores. Table 1 shows our study samples' maternal and infant demographic characteristics. The percentage of mothers with viral suppression (HIV RNA ≤400 copies/mL) increased from 48–53% at the earliest to 84–86% at the latest measure in pregnancy, while the percentage with CD4 ≥350 cells/mm^3 showed smaller increases from 70–72% at the earliest to 75–79% at later measures. Maternal substance use in pregnancy was similar to SMARTT overall.[24] Most infants in our analysis were born in 2010 or 2011. No infant demonstrated hearing loss, based on hearing screening or caregiver report.

Of the 175 meconium specimens with ARV quantification, 166 infants had uninterrupted ATV exposure (≤three day gap) in the second and third trimesters. Five mothers discontinued ATV before delivery; their infants' meconium ATV concentrations were considered separately when uninterrupted exposure was required. Four mothers had a >three day interruption in their ATV-containing regimen during the second or third trimester but returned to an ATV regimen pre-delivery; these infants were excluded from all analyses requiring uninterrupted exposure.

Maternal ATV duration and meconium ATV

Median (range) meconium ATV concentration in the 166 infants with uninterrupted second and third trimester ATV exposure was 16,929 ng/g (29–143,018). Many women were on ATV throughout pregnancy; median (range) uninterrupted ATV exposure duration, excluding first trimester ATV administration, was 24 weeks (2.6–28.1). Uninterrupted ATV exposure duration was positively correlated with ATV meconium concentrations (ρ=0.230, P=0.003).

Maternal ATV initiation and meconium ATV

Most women using ATV during pregnancy started ATV before pregnancy or in the first trimester. Gestational ATV initiation week for women who started ATV before pregnancy or in the first trimester was left-truncated to exclude the first trimester and set to 14.1 weeks, as meconium only begins to form at the beginning of the second trimester. Median (range) ATV initiation week during pregnancy was 14.1 (14.1–35.7). Meconium ATV concentrations were grouped by ATV initiation week (in or before early second trimester, ≤21 weeks; in late second, 21.1–28 weeks; early third, 28.1–34 weeks, or late third trimester, 34.1–42 weeks), revealing a trend toward decreasing median ATV meconium concentrations

with later maternal ATV initiation (P=0.07, Table 2). ATV initiation week negatively correlated with ATV meconium concentration (ρ=−0.215, P=0.01).

Five infants whose mothers discontinued ATV-containing regimens before delivery had significantly lower ATV meconium concentrations than the 166 infants whose mothers remained on ATV through delivery (P<0.001). These five mothers discontinued ATV 19–64 days before delivery, their ATV duration prior to cessation ranged from 4.1–21.6 weeks, and infant meconium ATV concentrations were 102–3,468 ng/g. These five infants' gestational ages at birth were 37–40 weeks and at the time ATV was discontinued, 28.9–35.3 weeks.

Language measures and meconium ATV concentrations

Among the 93 infants with Bayley-III data, the median (interquartile range) Language composite score was 94 (86–97) and ATV meconium concentrations ranged from 48–78,963 ng/g. Infant's cumulative ATV exposure duration, over the entire pregnancy, ranged from 4.1–42 weeks. Higher Bayley-III language scores were associated with longer cumulative ATV exposure duration (Supplemental Table 2 and Table 3). Meconium ATV concentrations were not associated with Bayley-III Language scores in unadjusted or adjusted models; additionally, mean Bayley-III scores were not significantly different between infants with the lowest (≤10th percentile) and highest (>90th percentile) ATV meconium concentrations.

Among the 106 infants with CDI data, median (interquartile range) percentile scores were Vocabulary Comprehension, 45 (20–70); Word Production, 50 (33–60); Phrases Understood 60 (35–75); Total Gestures, 52.5 (30–70); and mean CDI percentile scores across all domains, 50 (34.8–63.8). ATV meconium concentrations ranged from 48–85,166 ng/g and cumulative ATV duration from 4.1–41.6 weeks. Higher CDI Phrases Understood scores were associated with longer ATV durations, both for cumulative and uninterrupted ATV duration measures (Table 3). Meconium ATV concentrations were not associated with individual CDI domains or average CDI percentile scores (Supplemental Table 2 and Table 3). Mean CDI scores were not significantly different between infants with the lowest and highest decile ATV meconium concentrations.

Most (92%) CDI-evaluated infants received zidovudine-only neonatal prophylaxis; eight infants received zidovudine with other drugs (nevirapine, stavudine, or lamivudine +stavudine+nevirapine). Prophylaxis duration ranged from 1–66 days. There was no difference in the proportion of infants who received combination prophylaxis among those with LLE risk (4/25) and those without LLE risk (4/81, P=0.09); additionally, neonatal prophylaxis duration was not significantly different between the two groups.

Maternal concomitant ART medication

Most mothers (77% of the 166 with uninterrupted ATV exposure) were on a TDF-containing ATV regimen. Three infants exposed to other ATV regimens were excluded from this comparison. Figure 1 illustrates group differences by uninterrupted ATV exposure duration. Spearman correlations between ATV exposure duration and meconium concentration were ρ=0.212, P=0.01 overall; ρ=0.199, P=0.03 for TDF+ATV+ritonavir; and ρ=0.343, P=0.03 for ATV+ritonavir (Figure 1). Bayley-III and CDI scores and LLE risk prevalence were not significantly different between infants exposed to TDF+ATV+ritonavir and ATV+ritonavir.

The most prevalent maternal combination ART regimen in our sample was TDF +emtricitabine+ritonavir+ATV followed by zidovudine+lamivudine+ritonavir+ATV.

Sensitivity analyses

Similar results as the primary analyses were observed from sensitivity analyses. Adjusting for preterm birth and low birth weight, or accounting for within-site correlations, did not change the associations except significant associations of cumulative ATV exposure duration with Bayley-III language and CDI Phrases Understood scores were attenuated after accounting for differences within sites.

Sample comparisons

Mean Bayley-III Language scores obtained in this study (93.2 ± 10.7) were lower than mean scores from the Bayley-III standardization sample[25] (P<0.001). Infants in our study also had significantly higher mean Bayley-III Language scores, higher CDI Total Gestures percentile scores, and lower LLE risk incidence compared to those in previously published SMARTT studies,[3,4] excluding infants who contributed data to both (Bayley-III, n=16; CDI, n=30). The mean Bayley-III Language score from our unique infant cohort (n=77) was 93.1 (±10.7) compared to the mean, 88.8 (±14.2), of unique infants with ATV exposure (n=62) in the previously published cohort (P=0.04). Our unique CDI-evaluated cohort (n=76) obtained a higher Total Gestures domain percentile score compared to those in the previous report (53 versus 35, P=0.02); otherwise, there were no differences between cohorts across CDI domains. LLE risk incidence among infants with ATV exposure in our sample was lower than in the previous report (22% versus 37%, P=0.05).[4] Proportion of infants who received combination neonatal prophylaxis and duration of this treatment among those with and without LLE risk were not significantly different between the two unique ATV-exposed groups.[4]

DISCUSSION

In utero HIV exposure was previously associated with increased language impairment risk among 7–16 year-olds with prenatal HIV exposure (HIV-infected, 51%; HEU, 37%) compared to the general US population (16%).[26] Associations at one year of age between prenatal ATV exposure, lower Bayley-III Language scores,[3] and elevated LLE risk[4] also were identified. As ATV use during pregnancy becomes more widespread,[1,2] these associations warrant further monitoring.

In this investigation, longer ATV exposure durations were associated with higher ATV meconium concentrations, which were protective against LLE risk. Confirming this association was our finding that longer cumulative ATV exposure durations resulted in higher Bayley-III Language scores. These results differ from two previous SMARTT reports,[3,4] possibly due to sampling differences associated with small samples or random variability. There was minimal overlap between the three studies' samples; only 30 of our 106 CDI-evaluated infants and 16 of our 93 Bayley-evaluated infants were included in previous SMARTT studies. Infants in our study also had lower LLE risk and higher Bayley-III Language scores compared to those in our previous research; these improved outcomes

may have challenged confirmation of previous negative ATV exposure associations, or may be causative for the decreased LLE risk observed here compared to the previously seen increased LLE risk. Additionally, our results confirm previous PHACS research that showed increased LLE risk among HEU children; 24–42% of HEU children in our study and previous PHACS studies[4, 26] demonstrated risk of LLE or language impairment, compared to an expected prevalence of 16–20% among children in the general population.[26]

Changing ARV use during pregnancy trends, random sampling differences, and cohort effects may have contributed to differences among the studies. Healthier women are receiving ATV more often now than in previous years; additionally, pregnant women with HIV may be healthier now due to continuous use of well-tolerated medications with lower pill burdens.[1,2] Most infants included in this investigation were born later than infants in the previous SMARTT analyses; 78% of infants in the current study with Bayley-III data and 59% with CDI data were born after the earlier analyses' cutoff dates (5/1/2009 and 4/1/2010, respectively).[3,4] Further investigations must clarify these different results; however, the present findings confirm ATV exposure safety on infant language development, which supports the 2014 HHS recommendations[1] of ritonavir-boosted ATV as a preferred ART regimen for pregnant women with HIV. Further study also should determine if associations remain consistent as children age.

A potential mechanism for our observed lowered LLE risk may be ATV clearance into meconium. Higher ATV meconium concentrations are likely an indication of fetal ATV detoxification. ATV is primarily eliminated via hepatic pathways.[27] In adults, ATV is metabolized by CYP3A4 and CYP3A5,[28,29] mainly by oxidation, although several metabolites and metabolic pathways are known.[30,31] ATV metabolism varies widely as adults on steady-state ritonavir-boosted ATV showed cumulative metabolite concentrations from 4–32% of parent ATV concentrations.[31] Fetal ATV metabolism and clearance are unknown; however, published fetal liver CYP3A studies indicate fetal ATV metabolism is limited. Fetal CYP3A4 content and activity is <10% of adult levels,[32–34] and little is known about fetal CYP3A5 activity.[33,34] CYP3A7 accounts for 87–100% of total fetal liver CYP3A content, and shows high catalytic activity toward endogenous steroids but lower activity towards exogenous substances, traditional CYP3A4 substrates.[35,36] Our data suggest a potential mechanism for the lowered LLE risk via fetal ATV clearance capacity, although ATV clearance to meconium also may be affected by maternal and infant factors not investigated in this study.

Associations between infant meconium ATV concentrations, uninterrupted second and third trimester ATV exposure duration, and gestational ATV initiation week were significant. Meconium drug concentrations can be influenced by variations in maternal dosage, fetal exposure duration and timing, placental transfer, and maternal and fetal pharmacokinetics and metabolism. Our significant observations and wide ATV meconium concentration range among women with uninterrupted ATV exposure (29–143,018 ng/g) demonstrate the significance of these influencing factors.

Three potentially reactive CYP3A4-generated ATV metabolites (an aromatic aldehyde, alpha-hydroxyaldehyde, and hydrazine metabolite) were recently identified in human liver

microsome incubations.[37] Oral ATV administration to mice, however, produced no detectable urinary or fecal concentrations of these metabolites.[37] Potential metabolite toxicity is not yet known, though similar chemical structures are associated with glutathione and protein adducts, hepatic lesions, and neurotoxicity.[37–39] Initially, we hypothesized higher ATV meconium concentrations might indicate greater fetal exposure and therefore, predict lower language scores and higher LLE risk. Our findings now suggest higher ATV meconium concentrations likely indicate greater fetal ATV detoxification as there is less ATV available to form potentially toxic metabolites and hence better language outcomes. Additionally, lower ATV meconium concentrations suggest reduced ATV clearance, likely associated with higher fetal plasma ATV or metabolite concentrations throughout pregnancy, which might be correlated with increased LLE risk. Human plasma and meconium metabolite concentrations should be investigated, although commercial standards are currently unavailable. Further study into these metabolites' clinical implications is needed. However, as ritonavir is commonly co-administered, the in vitro metabolite study failed to investigate ritonavir's reduction of potentially toxic ATV metabolite formation, as seen with lopinavir.[40]

Associations between infant meconium ATV concentrations, uninterrupted second and third trimester ATV exposure duration, and gestational ATV initiation week were significant. Longer ATV exposure periods correlated with higher ATV meconium concentrations, and later ATV initiation resulted in lower ATV meconium concentrations. These correlations were weak ($\rho < \pm 0.25$), suggesting meconium ATV concentrations do not predict and only partially reflect maternal ATV duration or initiation timing.

TDF addition to a mother's ritonavir-boosted ATV therapy resulted in a decreasing meconium ATV concentration trend, as predicted from previous pharmacokinetic studies.[17] However, no group language differences were observed between infants exposed to ritonavir-boosted ATV regimens with and without TDF.

While our investigation was unique, it was limited by lack of information about neonatal and maternal bilirubin concentrations, maternal UGT1A1 polymorphisms, and infant phototherapy. Future studies could employ these additional tests to better understand factors contributing to ATV's impact on infant language development. Further investigation of pathophysiological and psychosocial influences on HEU infant language development is needed to develop targeted interventions to address potential language delays. Previous research indicated socioeconomic factors, genetic influences, and maternal HIV disease status may affect language acquisition.[4, 26, 41]

ATV meconium quantification provided a novel *in utero* ATV exposure assessment, and for the first time, demonstrated the value of including meconium ARV concentrations in longitudinal research on HIV. Higher meconium ATV concentrations were protective of LLE risk. Clinically, this information supports ATV safety during pregnancy for infant language development. Additionally, higher ATV meconium concentrations observed in our study may indicate greater fetal ATV detoxification as these concentrations protected against LLE risk. With ATV now a preferred ARV for pregnant women with HIV, our data add evidence to fetal ATV exposure safety for language development. Our ATV meconium research

findings will be helpful to medical professionals evaluating *in utero* ATV exposure safety for infant language development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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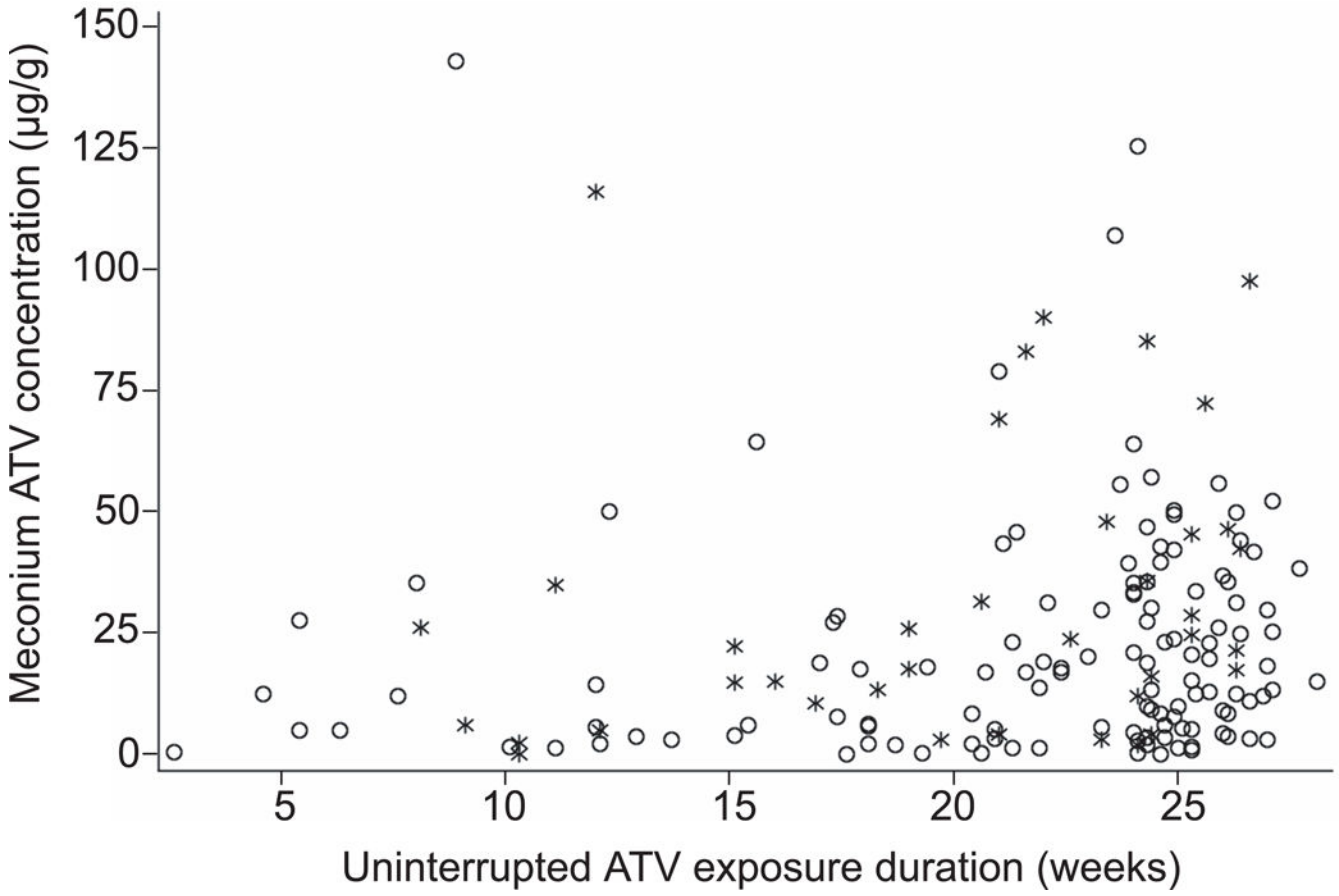

Figure 1.

Meconium ATV concentration by uninterrupted ATV exposure duration (from gestational week 14 through delivery) for infants exposed to TDF+ATV+ritonavir (n=125, astericks) and ATV+ritonavir (n=38, open circles). Exposure duration was determined by excluding first trimester ATV exposure (0–14 weeks), as meconium only begins to form at the beginning of the second trimester. Spearman correlations between ATV exposure duration and meconium concentration were ρ=0.212, P=0.01 overall; ρ=0.199, P=0.03 for TDF+ATV +ritonavir; and ρ=0.343, P=0.03 for ATV+ritonavir.

Table 1

Maternal, caregiver, and infant demographic characteristics among atazanavir (ATV)-exposed infants with meconium antiretroviral (ARV) quantification and completed language evaluations

Characteristic		Bayley-III (n=93); n (%)	CDI (n=106); n (%)
Maternal age at delivery ≥ 35 years		19 (20%)	21 (20%)
Tobacco use during pregnancy		16 (17%)	18 (17%)
Alcohol use during pregnancy		8 (9%)	10 (9%)
Illicit drug use during pregnancy		9 (10%)	9 (8%)
First absolute CD4 (cells/mm^3) during pregnancy[a]	< 200	11 (12%)	12 (11%)
	≥ 350	65 (70%)	76 (72%)
Last absolute CD4 (cells/mm^3) before delivery[a]	< 200	8 (9%)	8 (8%)
	≥ 350	70 (75%)	84 (79%)
First RNA (copies/mL) during pregnancy	≤ 400	45 (48%)	56 (53%)
	> 1000	42 (45%)	44 (42%)
Last RNA (copies/mL) before delivery	≤ 400	78 (84%)	91 (86%)
	> 1000	14 (15%)	14 (13%)
Concomitant TDF use with ATV regimen		79 (85%)	85 (80%)
Caregiver primary language	English	66 (71%)	67 (63%)
	English bilingual	21 (23%)	20 (19%)
	Spanish	4 (4%)	18 (17%)
	Other	2 (2%)	1 (1%)
Household annual income ≤ $10,000[b]		50 (54%)	57 (54%)
Caregiver education < high school		35 (38%)	37 (35%)
Live with partner/spouse		47 (51%)	59 (56%)
Sexually transmitted disease during pregnancy		45 (48%)	48 (45%)
Caregiver IQ[c]	Median (range)	85 (58–115)	85 (58–115)
Caregiver IQ[c] < 85		37 (40%)	35 (33%)
Caregiver: positive screen for any postpartum psychiatric syndrome[d]		27 (29%)	31 (29%)
Caregiver: positive screen for postpartum alcohol abuse[d]		2 (2%)	2 (2%)
Caregiver: positive screen for postpartum drug abuse[d]		3 (3%)	3 (3%)
Infant age (months) at time of language evaluation	Median (range)	13.0 (11.7 – 19.8)	12.8 (11.7 – 23.4)
Language evaluation version	English	93 (100%)	84 (79%)
	Spanish	Not applicable	22 (21%)
Infant sex	Male	54 (58%)	60 (57%)
Infant race	Black	73 (78%)	70 (66%)
	Puerto Rican	Not applicable	6 (6%)
	Caucasian/Other	20 (22%)	30 (28%)
Infant Hispanic ethnicity		20 (22%)	37 (35%)
Gestational age (weeks)	Median (range)	38.6 (33.0 – 41.9)	38.6 (33.0 – 41.4)
Preterm (< 37 weeks gestation)		10 (11%)	10 (9%)
Low birth weight (< 2500 g)		7 (8%)	8 (8%)

Characteristic		Bayley-III (n=93); n (%)	CDI (n=106); n (%)
Small for gestational age (< 10th percentile for gestational age)		5 (5%)	6 (6%)
Infant birth year	2007	3 (3%)	3 (3%)
	2008	13 (14%)	15 (14%)
	2009	13 (14%)	18 (17%)
	2010	22 (24%)	27 (25%)
	2011	28 (30%)	29 (27%)
	2012	14 (15%)	14 (13%)

Missing data: *Bayley-III*: maternal first/last CD4 count during pregnancy (n=1, 1%), maternal/caregiver IQ (n=17, 18%), maternal/caregiver positive screen for postpartum psychiatric syndrome, alcohol or drug abuse (n=3, 3%), and small for gestational age (n=1, 1%); *CDI*: household annual income (n=1, 1%), sexually transmitted disease during pregnancy (n=1, 1%), maternal/caregiver IQ (n=31, 29%), maternal/caregiver positive screen for postpartum psychiatric syndrome or alcohol or drug abuse (n=1, 1%), and small for gestational age (n=1, 1%). Tobacco, alcohol, and illicit drug use during pregnancy were determined from maternal self-report and validated through meconium analysis.[24] Maternal/caregiver cognition and maternal/caregiver mental health status were evaluated at the one-year-old study visit with the Wechsler Abbreviated Scale of Intelligence[42] and Client Diagnostic Questionnaire (CDQ),[43] respectively. CDI indicates the MacArthur-Bates Communicative Developmental Index.

Table 2

Atazanavir (ATV) meconium concentrations by gestational week of ATV initiation

Timing of ATV Exposure	n	Median (range) ATV meconium concentration (ng/g)[a]
Early second trimester (≤21 weeks)	130	17,840 (99 – 125,352)
Later second trimester (21.1–28 weeks)	24	13,510 (29 – 116,000)
Early third trimester (28.1–34 weeks)	10	9,026 (139 – 143,018)
Later third trimester (34.1–42 weeks)	2	6,447 (443 – 12,450)

[a] Jonckheere-Terpstra trend test, P=0.07

Table 3

Adjusted associations of atazanavir (ATV) exposure measures with language outcomes, among infants with meconium and completed language evaluations

Language Outcome	Exposure measures					
	ATV meconium concentration (square-root transformed ng/g)[a]		Uninterrupted ATV exposure duration in second and third trimester (weeks)[b]		Cumulative ATV exposure duration during pregnancy (weeks)[a]	
	Estimated coefficient[c] (95% CI)	P-value	Estimated coefficient (95% CI)	P-value	Estimated coefficient (95% CI)	P-value
Bayley-III Language composite score[d]	0.40 (−0.67, 1.46)	0.46	0.35 (−0.05, 0.75)	0.09	0.25 (0.05, 0.46)	**0.02**
CDI Vocabulary Comprehension[e]	0.44 (−2.12, 2.99)	0.74	0.34 (−0.74, 1.42)	0.54	0.31 (−0.21, 0.83)	0.25
CDI Word Production[f]	0.46 (−1.37, 2.28)	0.62	0.41 (−0.36, 1.19)	0.29	0.20 (−0.17, 0.56)	0.29
CDI Phrases Understood[g]	1.82 (−0.43, 4.08)	0.11	1.09 (0.13, 2.06)	**0.03**	0.50 (0.04, 0.97)	**0.03**
CDI Total Gestures[h]	1.45 (−0.88, 3.77)	0.22	0.36 (−0.63, 1.34)	0.47	0.21 (−0.28, 0.70)	0.39
Average CDI percentile score[i]	0.82 (−0.87, 2.50)	0.34	0.56 (−0.15, 1.27)	0.12	0.32 (−0.03, 0.67)	0.07
	Adjusted odds ratio (95% CI)	P-value	Adjusted odds ratio (95% CI)	P-value	Adjusted odds ratio (95% CI)	P-value
Late Language Emergence (CDI score ≤10th percentile in one or more domains)[j]	0.76 (0.57, 0.98)	**0.04**	0.92 (0.83, 1.02)	0.09	0.97 (0.92, 1.02)	0.24

[a] All ATV exposure durations during pregnancy are included, regardless of ATV therapy interruptions and ATV exposure timing.

[b] Excluding ATV exposure duration during the first trimester. Subjects with interrupted ATV exposure during the second or third trimester were excluded (Bayley-III, n=5; CDI and LLE, n=6)

[c] Coefficients represent changes in language score for each one unit increase in square-root transformed ATV meconium concentrations, week of either uninterrupted second and third trimester ATV exposure, or cumulative ATV exposure during pregnancy. Potential confounder identification is described in the Methods' Statistical Analysis section.

[d] Multivariable models adjusted for concomitant TDF use, maternal/caregiver positive screen for psychiatric syndrome, tobacco use during pregnancy, and maternal/caregiver primary language. The cumulative duration model also adjusted for infant age at testing.

[e] Multivariable models adjusted for concomitant TDF use, maternal/caregiver primary language, maternal age ≥35 at delivery, infant black race, and annual household income ≤ $10,000. The ATV meconium model also adjusted for illicit drug use during pregnancy, and the uninterrupted duration model included infant's birth year.

[f] Multivariable models adjusted for concomitant TDF use and infant age at language evaluation. The ATV meconium model also included infant sex, maternal/caregiver primary language, and illicit drug use during pregnancy. The uninterrupted duration model included maternal/caregiver primary language, and the cumulative duration model included the infant's sex.

[g] Multivariable models adjusted for concomitant TDF use and maternal age ≥35 at delivery.

[h] Multivariable models adjusted for concomitant TDF use, infant age at language evaluation, maternal age ≥35 at delivery, sexually transmitted disease during pregnancy, and infant black race. The uninterrupted duration model also adjusted for maternal/caregiver primary language and IQ.

[i] Average CDI percentile scores were calculated from the mean of all four individual domain scores. Multivariate models adjusted for concomitant TDF use, maternal age ≥35 at delivery, and maternal/caregiver primary language. The ATV meconium concentration model also adjusted for alcohol use during pregnancy.

[j] Multivariable models adjusted for concomitant TDF use, infant age at testing, maternal/caregiver primary language, and maternal age ≥35 at delivery. The ATV meconium concentration model also included infant black race, and the cumulative duration model included infant black race and Hispanic ethnicity.

CI indicates confidence interval and CDI indicates the MacArthur-Bates Communicative Developmental Index