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Impact of *in utero* antiretroviral drug exposure on expression of membrane-associated transporters in mouse placenta and fetal brain

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Abstract

Objective: Although antiretroviral therapy (ART) during pregnancy is effective in limiting vertical HIV transmission, adverse outcomes persist amongst uninfected children exposed to antiretroviral drugs *in utero*. Membrane-associated drug transporters, metabolic enzymes and tight junction proteins play important roles in adult antiretroviral drug disposition and toxicity, however, the fetal expression of these proteins in the context of ART, and their impact on *in utero* antiretroviral drug distribution remain poorly understood. This study aimed to characterize the role of these proteins in modulating *in utero* antiretroviral drug exposure.

Methods: Pregnant mice were exposed to an ART regimen consisting of lamivudine,

abacavir, atazanavir and ritonavir, at clinically relevant doses. Fetal brain, liver, placenta amniotic fluid and maternal plasma were collected on gestational day 18.5 and concentration of antiretroviral drugs in fetal tissues was measured by LC/MS/MS, while transporter expression was assessed by qPCR.

Results: Abacavir and lamivudine were detected in fetal brain and amniotic fluid, while atazanavir and ritonavir were detected in amniotic fluid only. Robust mRNA expression of key transporters was observed in adult and fetal tissues, and sex differences were identified in the expression of *Abcc1* and *Slc29a1* in the placenta. Antiretroviral drug exposure was associated with a reduction in relative placental *Abcg2, Abcc1* and *Slc29a1* expression.

Conclusions: These findings identify a novel effect of fetal sex and antiretroviral drug treatment on the expression of placental transporters in a mouse model, and characterize the penetration of lamivudine and abacavir into fetal brain, uncovering a potential role of transporters in modulating fetal exposure to antiretroviral drugs.

Keywords: HIV, antiretroviral drugs, pregnancy, drug transport, placenta, sex differences

Introduction

Understanding mechanisms of drug distribution between mother and fetus is crucial for designing and optimizing antiretroviral therapy (ART) regimens for use during pregnancy. Modern ART effectively reduces maternal viral load, while penetration of antiretroviral drugs into the fetus provides prophylaxis in the perinatal period. However, *in utero* antiretroviral drug exposure is associated with developmental toxicity leading to adverse pregnancy outcomes and adverse developmental outcomes in neonates and children, including growth delay and neurodevelopmental metabolic complications [1–3]. Neurodevelopmental deficits have also been reported in older children, including evidence of late language development, associated with *in utero* protease inhibitor exposure [4–7]. The increased use of ART during pregnancy has led to a rapid growth in the population of antiretroviral drug-exposed children [8], and a deeper understanding of the effects of *in utero* antiretroviral drug exposure in this population is required to mitigate toxicity of current and future treatment regimens. Presently, little evidence exists to explain sex differences in *in utero* antiretroviral drug toxicity described in the clinical literature [6,9–14].

The placenta, which is the main interface between fetal and maternal circulation, plays a critical role in limiting fetal exposure to antiretroviral drugs. Membrane-associated transporter expression at the placenta regulates transplacental permeability of xenobiotics [15–17]. Transporters of the ATP-binding cassette superfamily including P-glycoprotein (P-gp), Breast Cancer Resistance Protein (Bcrp) and the multidrug resistance-associated proteins (Mrps) as well as members of the solute carrier (SLC) superfamily play an important role in the distribution of antiretroviral drugs across the placenta. Exposure to antiretroviral drugs

also impacts the activity of several transporters through direct inhibition, and indirectly through altered regulation [18–22].

The barriers of the brain including the blood-brain and blood-cerebrospinal fluid barriers, demonstrate expression of several efflux transporters, notably P-gp, Bcrp, Mrp1, Mrp4 and Mrp5 [23,24], which along with other barrier mechanisms such as microvessel endothelial cell tight junctions, play a role in limiting antiretroviral drug penetration into the brain in the context of ART [25–27]. As in adults, efflux transporter expression at the immature blood-brain barrier of the fetus, in concert with efflux activity at the placenta, may contribute to a limitation of fetal brain antiretroviral drug exposure. Importantly, sex differences in the expression of transporters in the brain, described in adult human and rodent models [28,29] and to some extent in fetuses [30], may underlie clinical sex differences observed in the fetal neurotoxicity of antiretroviral drugs. For example, neurotoxicity observed in cases of neonatal hyperbilirubinemia, linked to atazanavir (ATV) exposure, displays sex differences in occurrence, raising questions regarding ATV interactions with bilirubin transporters and metabolic enzymes in the fetal and neonatal liver [31,32]. To date, limited research has focussed on understanding the expression of transporters and metabolic enzymes in the fetal brain and liver as well as placenta in the context of ART [33,34], with little consideration of fetal sex as a variable.

Lamivudine (3TC), abacavir (ABC), ATV and ritonavir (RTV) comprise an ART regimen (3TC/ABC+ATV/r) used in pregnancy, with each of these drugs also recommended by the WHO as part of other first and second line regimens [35]. Previous work in rodents links this regimen to concerning findings of lower birth weight and length, as well as delayed reflex ontogeny and impaired olfaction, with some effects present through adulthood [36]. The objective of this study was to assess the penetration of these drugs into the fetal compartment, and to examine the effect of sex and antiretroviral drug exposure on the expression of ATP-binding cassette and SLC transporters in the antiretroviral drug-exposed fetal brain, fetal liver and placenta, using a mouse model of pregnancy. Comparison of transporter expression between fetal and adult tissues was also performed.

Methods

Animals and tissue collection

All animal studies were approved by the University Health Network Animal Care Committee and were performed following national guidelines of the Canadian Council for Animal Care. Experimental procedures have been previously described [36]. In brief, following plug detection (gestational day (GD) 0.5) pregnant C57BL/6J mice (Jackson Laboratories) were randomly assigned to either a treatment or control arm, and administered 3TC/ABC+ATV/r (100/50 mg/kg/day + 50/16.6 mg/kg/day), or water respectively by oral gavage. Antiretroviral drug dosing was previously established in previous studies [37], demonstrating maternal plasma concentrations comparable to those achieved with human ART. Pregnant animals were euthanized by CO_2 asphyxiation on GD 18.5, one hour following final treatment

administration when antiretroviral drug plasma concentration is expected to be at a maximum. Maternal plasma, placenta, fetal (GD18.5) and adult (10-12 week) brain, liver and fetal body were flash-frozen in liquid nitrogen and stored at -80°C. Fetal sex was determined by PCR amplification of the male specific sequence Sry, as well as the autosomal gene IL3 (as a control), as described previously [38]. DNA oligonucleotide primers for *Sry* and *IL3* (see Table S1, Supplemental Digital Content, http://links.lww.com/QAD/C231) were purchased from Sigma Aldrich.

RNA isolation, reverse transcription, and quantitative real-time polymerase chain reaction (qPCR)

Fetal brain, fetal liver, and placental tissue were homogenized on ice. RNA isolation and cDNA synthesis were performed as previously described [39]. In brief, total RNA was extracted from tissue homogenate using TRIzol reagent (Invitrogen) according to manufacturer's instructions. RNA concentration and purity were assessed by absorbance at 260 nm and absorbance ratio 260/280 respectively, using a UV-Vis spectrophotometer. RNA was treated with DNase I (4U/μL) to eliminate genomic DNA. cDNA was synthesized from 2 μg of RNA using a high-capacity reverse transcription cDNA kit (Applied Biosystems), according to manufacturer's instructions. mRNA expression of target genes was assessed by qPCR using a Mastercycler ep Realplex 2S thermal cycler (Eppendorf), using validated primers and probes (see table S2, Supplemental Digital Content, http://links.lww.com/QAD/C231) designed by Life Technologies for use with TaqMan qPCR mastermix. Each reaction contained either 50ng or 100ng of cDNA, 1 μL of TaqMan primer, and 10 μL of TaqMan MasterMix and was run in triplicate. Analysis was performed on the average of triplicate values for each sample using the comparative threshold cycle (ΔCT) method. Expression was normalized using the housekeeping gene *Ppib*. Results are expressed as mean relative expression to *Ppib* (ΔCT) or fold change compared to adult (ΔΔCT) ± SD.

Sample preparation and drug quantification

Flash frozen fetal brain tissue at GD 18.5 was homogenized in water at 2.5 μL per mg of tissue. Antiretroviral drug standards (National Institutes of Health AIDS Research and Reference Reagent Program) were prepared in pooled adult brain tissue (processed as above), amniotic fluid, and maternal plasma that had not been exposed to ART, to produce concentrations of 20,000 ng/mL, 15,000 ng/mL, 10,000 ng/mL, 5000 ng/mL, 1000 ng/mL, 500 ng/mL, 250 ng/mL, 100 ng/mL, 75 ng/mL, 25 ng/mL, 5 ng/mL, 1 ng/mL for ABC, 3TC, ATV and RTV. A set of internal quality controls (QC) used for the validation of the bioassay were also separately prepared with different concentrations (low, medium and high) of antiretroviral drug in blank brain homogenate, amniotic fluid and maternal plasma. The frozen brain homogenate, amniotic fluid, and maternal plasma analysis samples, standards and QCs were transferred to the Clinical Investigation Unit at the Ottawa Hospital for antiretroviral drug quantification.

The drug concentrations of ABC, 3TC, ATV and RTV were determined by using a validated liquid chromatography mass spectrometry/mass spectrometry (LC/MS/MS) method. Briefly, all samples (standards, QCs and unknowns) were first spiked with 6,7-dimethyl-2,3-di(2-pyridyl)-quinoxaline (Sigma Aldrich) as an internal standard (IS) and subjected to protein precipitation with acetonitrile (1:3) followed by centrifugation at 5,000 X g for 5 minutes. 10 µL of clear supernatant was used for LC/MS/MS analysis. The LC/MS/MS system consists of an ACCELA LC system (Thermo Fisher Scientific) with a 1.9 µm C18 column (Hypersil Cold; 2.1 x 50 mm, Thermo Fisher Scientific) equipped with a 2-cm precolumn packed with the same material. Detection was done by tandem mass spectrometer (TSQ Quantum Access MAX, Thermo Fisher Scientific) with atmospheric pressure chemical ionization in positive mode. LC was performed at 40°C with a gradient elution of acetonitrile- 0.1% (v/v) formic acid in water at a flow rate of 300 µL/min. MS was quantified using electrospray multiple reaction monitoring (MRM). Xcalibur and TSQ Tune master software (Thermo Fisher Scientific) were used as the system controller and integrator. The MRM transitions were m/z 287.2 to 91.2, 230.1 to 112.0, 705.1 to 168.2, 721.2 to 197.0 and 313.1 to 246.1 for ABC, 3TC, ATV, RTV and IS, respectively. All within-day and between-day coefficients of variation (CV) were below 15% and the recovery rates were between 80%-95%. The effective linear range was 1-5,000ng/mL for homogenized brain tissue, and 5-15,000ng/mL for amniotic fluid, and maternal plasma.

Data analysis

All experiments were repeated at least three times using tissues from a minimum of 3 dams per treatment arm with at least 2 fetuses per litter. Statistical analysis was performed using Prism 9 software (GraphPad Software). Statistical significance between two groups was assessed by two-tailed Student's *t*-test for unpaired experimental values. Comparison between multiple groups was performed using one-way analysis of variance (ANOVA), while multi-factor comparisons (treatment, fetal sex) were performed using two-way ANOVA, both with the Bonferroni post-hoc correction for multiple comparisons. Results are presented as mean ± SD.

Results

Differential effects of ART in pregnancy on mRNA expression of transporters and endothelial cell markers in adult vs. fetal mouse brain

We first sought to screen the mRNA expression of membrane associated-transporters, and microvessel endothelial cell markers in adult and fetal mouse brain, liver and placenta, in the context of ART. Pregnant mice were treated throughout gestation with clinically relevant doses of 3TC/ABC+ATV/r, or to a vehicle control. Genes of interest were selected due to evidence of interactions with ABC, 3TC, ATV or RTV. Expression for all genes was normalized to the housekeeping gene *Ppib,* and additionally to expression in vehicle treated pregnant dams.

Amongst vehicle-treated dams and fetuses, expression of *Abcb1a* and *Abcc4* was significantly lower in fetal compared to adult brain (figure 1a). In contrast, expression of *Abcg2* and *Abcc1* was significantly greater in fetal compared to adult brain. Antiretroviral drug treatment significantly induced the expression of all five ATP-binding cassette transporters (ie. *Abcb1a, Abcg2, Abcc1, Abcc4* and *Abcc5*) in adult but not fetal brain. As a result, ATP-binding cassette transporter expression was greater in adult compared to fetal brain amongst antiretroviral drug-exposed animals. In addition to membrane associated transporters, endothelial cell junctions of the blood-brain barrier are critical in excluding xenobiotics from the brain parenchyma. As such, we next measured the expression of a panel of microvessel endothelial cell markers, associated with blood-brain barrier development, in order to assess differences between adult and fetal barrier integrity in the context of ART. We observed significantly lower expression of the glucose transporter *Slc2a1* (GLUT1), and the tight-junction protein *Tjp1* (ZO-1) in fetal compared to adult brain, amongst both vehicle and antiretroviral drug-treated animals (figure 1b). mRNA expression of the tight-junction associated protein *Ocln* (occludin) was induced in antiretroviral drug-treated compared to vehicle-treated dams.

Differential effects of ART in pregnancy on mRNA expression of metabolic enzymes and transporters in mouse liver and placenta

As in humans, the immature mouse liver is known to express relatively low levels of most transporter and metabolic enzyme mRNA, with rapid increases beginning in the final days of gestation and into the postnatal period [40]. Accordingly, we identified low expression of transporters and metabolic enzymes in fetal compared to adult liver amongst both antiretroviral drug-treated and vehicle-treated animals, with the exception of *Abcg2* and *Abcc1* which displayed significantly greater expression in the fetal liver compared to adult liver amongst vehicle-treated animals (figure 2a,b). Similarly to transporters in the adult brain, exposure to antiretroviral drugs was associated with an induction major metabolic enzymes *Cyp3a11* and *Cyp3a13* (homologous to human *CYP3A4/5*), as well as the ATP-binding cassette transporters assessed in the adult liver. Expression of the transporter *Slco1b2* and the enzyme *Ugt1a1,* involved in bilirubin metabolism and elimination, was considerably greater in adult compared to fetal liver. *Abcc2,* which is involved in bilirubin transport across the canalicular membrane, was expressed at comparable levels in vehicle-treated adult and fetal liver, but was induced 8-fold in antiretroviral drug-treated adult liver, with no corresponding induction in antiretroviral drug-treated fetal liver.

In GD 18.5 mouse placenta, expression of ATP-binding cassette and SLC transporters was characterized, with *Abcb1b, Abcg2, Abcc1, Abcc5* and *Slc29a1* being most highly expressed amongst the panel of transporters relevant to antiretroviral drug distribution in the model (figure 3). Expression of placental *Cyp3a11* was low or undetectable.

Differential effects of fetal sex and ART exposure on transporter mRNA expression in fetal mouse brain and placenta

We next sought to further investigate the expression of four transporter genes, *Abcb1a/b*, *Abcg2*, *Abcc1* and *Slc29a1,* in fetal brain and placenta by qPCR. These genes were selected for further investigation based on findings from the preliminary screen, as well as on their relevance to antiretroviral drug disposition. In order to more deeply investigate subtle differences in transporter mRNA expression, we applied the qPCR gene expression assay to an larger cohort of 56 fetuses from 10 dams, with data stratified by fetal sex and antiretroviral drug exposure. In fetal brain, transporter mRNA expression did not differ by antiretroviral drug exposure or by fetal sex (figure 4a). In the placenta, antiretroviral drug exposure was associated with a reduction in mean relative expression of *Abcc1* from 0.46 ± 0.10 to 0.36 ± 0.07 amongst female fetuses. A similar reduction was observed in expression of *Abcg2*. In addition, antiretroviral drug exposure was associated with a reduction in mean relative *Slc29a1* expression from 0.50 ± 0.06 to 0.41 ± 0.05 amongst female fetuses, and a reduction in mean relative expression of *Slc29a1* from 0.45 ± 0.06 to 0.37 ± 0.06 amongst male fetuses (figure 4b). Male sex was also associated with lower expression of *Abcc1* and *Slc29a1* in vehicle-treated male compared to female fetuses.

Antiretroviral drug concentration in maternal plasma, amniotic fluid and fetal brain

Characterization of transporter function at the brain and placenta was further examined through an assessment of antiretroviral drug penetration into the fetal compartment. Concentrations of 3TC, ABC, ATV and RTV at GD18.5 in maternal plasma, amniotic fluid and fetal brain were quantified by LC/MS/MS, one hour after administration of the final antiretroviral drug dose to the dam. Maternal plasma concentrations of each drug was found to be to be within the range of human therapeutic concentrations (table 1) [41–43]. Late gestation amniotic fluid is composed primarily of fetal urine and secretions, and as such, can be reflective of fetal exposure to antiretroviral drugs in maternal circulation. 3TC, ABC, ATV and RTV were detected in the amniotic fluid at mean concentrations of 662.0 ± 359.1 ng/mL, 7575.8 ± 2325.5 ng/mL, 413.1 ± 450.5 ng/mL, 16.1 21.7 ng/mL respectively (table 1), and with amniotic fluid to maternal plasma ratios of 0.269 ± 0.144, 1.217 ± 0.610, 0.087 ± 0.059 and 0.071 ± 0.075 respectively. *in utero* antiretroviral drug exposure has been linked to neurodevelopmental toxicity, particularly amongst children exposed to ATV [6,7]. As such, we next investigated antiretroviral drug concentrations in the fetal brain, and detected 3TC and ABC at concentrations of 12.8 ± 2.6 ng/mg and 434.6 ± 126.6 ng/mg respectively, normalized to brain wet-weight (table 1). ATV and RTV were detected in maternal plasma and amniotic fluid but not in fetal brain, suggesting limited penetration. No significant sex differences were identified in the penetration of 3TC/ABC+ATV/r into either the fetal brain or amniotic fluid compartments (see figure S1, Supplemental Digital Content, http://links.lww.com/QAD/C231).

Discussion

Ensuring the safety of ART administration during pregnancy requires extensive understanding of the fetal distribution and potential toxicity of drugs in the regimen. The contribution of transporters to antiretroviral drug distribution, while described extensively in

adult tissues, is relatively unknown in the fetus. This study joins a growing body of literature in characterizing fetal expression of transporters, and their role in modulating *in utero* antiretroviral drug exposure [24,30].

Major transporters of the fetal brain including *Abcb1* and *Abcg2* were detected at the mRNA level in this study, consistent with existing literature [24,44,45]. We also identified a robust induction of ATP-binding cassette transporter expression in antiretroviral drug-exposed adult brain, consistent with evidence of altered transporter regulation in the context of antiretroviral drug exposure, through xenobiotic nuclear receptors such as the pregnane X receptor and constitutive androstane receptor [18,46–49]. Studies investigating the ability of antiretroviral drugs to regulate blood-brain barrier transporter expression were reported in *in vitro* and *in vivo* models by our group and others [46,50–54]. Interestingly, *in utero* antiretroviral drug exposure was not associated with changes in ATP-binding cassette transporter expression in fetal brain, suggesting limited penetrate on of these drugs into the fetal brain. This is consistent with the LC/MS/MS analysis, in which ATV and RTV were not detected in the fetal brain. Limited penetration of antiretroviral drugs, and particularly protease inhibitors into the fetal brain is also consistent with literature that describes low CNS permeation of these drugs due to their large size, high plasma protein binding and interactions with membrane-associated transporters [55,56]. As a result antiretroviral drug concentrations in the fetal brain were likely too low to elicit a detectable effect on transporter expression. Our group previously described an *in vitro* induction of P-gp at 10 μM ATV and 15 μM ABC [50,51], concentrations much greater than ones observed in the fetal brain in the present study. *In utero* exposure to ATV has been associated with neurodevelopmental delays in clinical studies [4,6,7], and recently, *in utero* exposure to 3TC/ABC + ATV/r was linked to delays in rodent neurodevelopment [36]. In contrast to ATV and RTV, the NRTIs were detectable, but at subtherapeutic concentrations in fetal brain, suggesting a weaker prophylactic effect in the context of perinatal HIV exposure.

Limited penetration of ATV into the fetal brain suggests that ATV-associated neurotoxicity, and associated sex differences, may arise from toxicity at a peripheral site. Due to the established link between neonatal hyperbilirubinemia and neurodevelopmental deficits [57,58], our group and others have hypothesized that a dysregulation of bilirubin metabolism and elimination at the liver may underlie ATV-associated neurotoxicity [2,5]. Although expression of the important bilirubin transporter *Slco1b2* (homologous to human SLCO1B3), and enzyme *Ugt1a1*, was negligible in fetal compared to adult liver, *Abcc2* expression in fetal liver was comparable to expression in antiretroviral drug-unexposed adult liver in this model. This robust expression of fetal *Abcc2* suggests a potential role of the fetal liver in the development of sex differences in ATV-associated neonatal hyperbilirubinemia and neurotoxicity, though an investigation of bilirubin transport and metabolism in the fetal liver would be required to substantiate this finding. Sex differences in Mrp2 function were not investigated in this study, but have been previously reported [59].

In contrast to findings in the fetal brain, antiretroviral drug exposure was associated with a modest reduction of placental *Abcg2, Abcc1* and *Slc29a1* mRNA expression. This

finding of *Abcg2* and *Abcc1* downregulation is contrary to induction of ATP-binding cassette transporter expression commonly associated with antiretroviral drug exposure in *in vitro* models [60]. Reduced expression of *Abcc1*/Mrp1 as described in our model, could limit the efflux of substrates including ATV and RTV [22], leading to increased accumulation in the fetus, while changes in *Slc29a1*/Ent1 expression are linked to mitochondrial toxicity through altered uptake of NRTIs at the mitochondrial membrane [61].

Data investigating potential sex differences in transporter regulation in the placenta are very limited, despite well documented evidence of sex differences in placental gene expression, and fetal endocrine environment [62,63]. Higher relative expression of *Abcg2*, *Abcc1* and *Slc29a1* in the placentas of female compared to male fetuses was identified at the mRNA level in this model, emphasizing the role of sex in modulating transporter expression. Sex differences in ATP-binding cassette transporter expression have been described elsewhere [30,64], and are well established in adult rodent and human tissues (though not in the placenta), often demonstrating higher transporter expression and activity in females compared to males for rodent efflux transporters including P-gp [65], and Mrps [59,64]. *In vitro* modulation of placental transporter expression by sex hormones such as 17β-estradiol has also been demonstrated for P-gp and BCRP [66–68], however, findings of sex-differential transporter expression in an *in vivo* model of antiretroviral therapy is novel, and in the context of existing research in *in vitro* systems, suggests that differences in transporter expression may be influenced by sex differences in fetal endocrine environment.

Overall, we have characterized the expression of several membrane-associated transporters, metabolic enzymes and endothelial cell markers in the context of ART, demonstrating novel effects of both sex and antiretroviral drug exposure on the expression of transporters in the murine placenta. These results are also the first, to our knowledge, to quantify the penetration of 3TC, ABC, ATV and RTV into the fetal mouse brain, revealing important information about *in utero* antiretroviral drug exposure. The strengths of this animal model allow for a preliminary investigation into the role of transporters in antiretroviral drug-associated toxicity in pregnancy, though future work in clinical studies will be important to verify these findings. This study and others continue to establish the importance of considering fetal sex as a variable in studies of antiretroviral drug safety, as efforts continue to improve health outcomes of mothers and children exposed to HIV.

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Figure Legends

Figure 1: mRNA expression of transporters and endothelial cell markers in adult and GD18.5 fetal brain exposed to antiretroviral drugs

mRNA expression of: a) transporters and b) microvessel endothelial cell markers in GD18.5 fetal mouse brain (males and females combined), exposed to 3TC/ABC+ATV/r or vehicle control relative to expression in adult mouse brain (female), was assessed by qPCR. n = 6-13 fetuses from 6-8 unique dams. Differences between fetal and adult brain expression were determined by applying one-way ANOVA with Bonferroni's correction for multiple comparisons. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

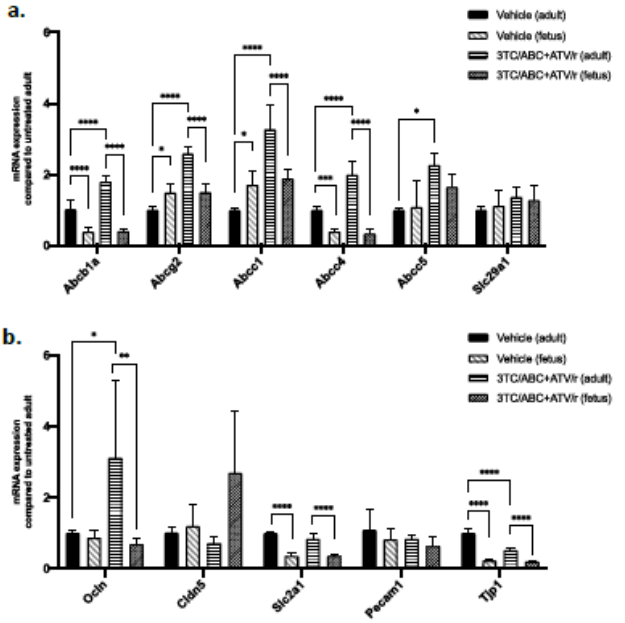

Figure 2: mRNA expression of metabolic enzymes and transporters in adult and GD18.5 fetal liver exposed to antiretroviral drugs

mRNA expression of: a) metabolic enzymes and b) transporters in GD18.5 fetal mouse liver (males and females combined), exposed to 3TC/ABC+ATV/r or vehicle control relative to expression in adult mouse liver (female), assessed by qPCR. n = 6-8 fetuses from 6-8 unique dams. Differences between fetal and adult brain expression were determined by applying one-way ANOVA with Bonferroni's correction for multiple comparisons. *p<0.05, ***p<0.001, ****p<0.0001.

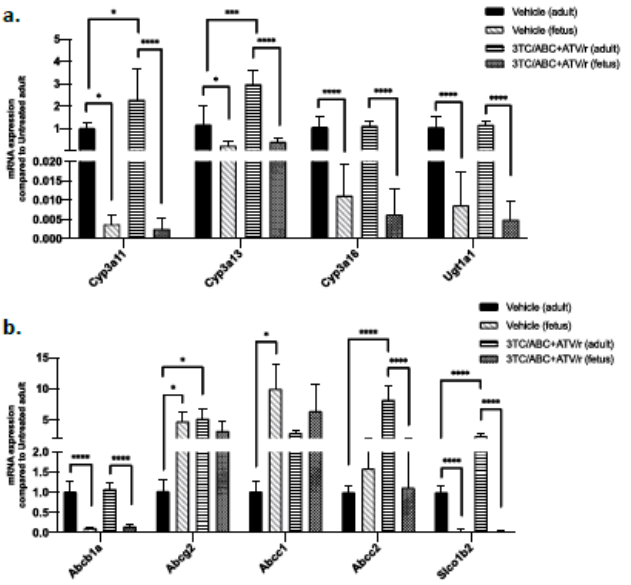

Figure 3: mRNA expression of transporters in GD18.5 placenta exposed to antiretroviral drugs

mRNA expression of key antiretroviral drug transporters in GD18.5 placenta (male and female combined), exposed to 3TC/ABC+ATV/r or vehicle control relative to expression of the housekeeping gene *Ppib* was assessed by qPCR. n = 6-8 fetuses from 6-8 unique dams. Differences between treated and untreated placental transporter expression were determined by applying Student's t-test; no statistically significant differences were observed between the groups.

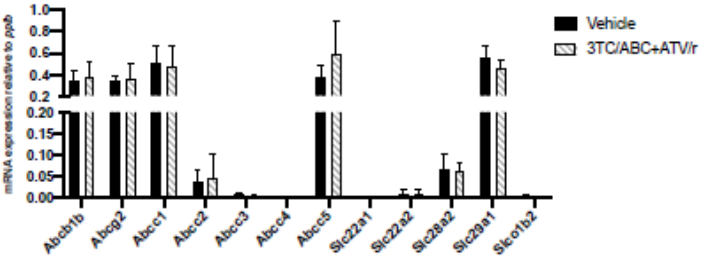

Figure 4: Effect of sex and antiretroviral drug exposure on *Abcb1a/b, Abcg2, Abcc1* and *Slc29a1* mRNA expression in GD 18.5 mouse brain and placenta

Pregnant dams were exposed for 18.5 days following plug detection to 3TC/ABC+ATV/r, or vehicle control. mRNA expression of *Abcb1a/b, Abcg2, Abcc1* and *Slc29a1* was assessed by qPCR in GD18.5 a) fetal brain and b) placenta. Expression is plotted relative to housekeeping gene *Ppib*. n=40 treated (6 dams) and 27 untreated (4 dams). Differences in expression between male and female, treated and untreated fetuses was determined by applying 2-way ANOVA with Bonferroni's correction for multiple comparisons *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

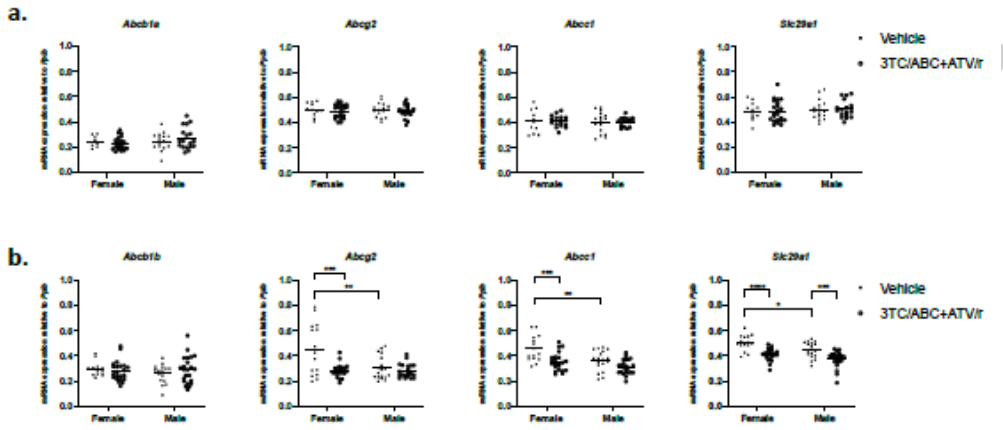

Table 1: Antiretroviral drug concentrations in maternal plasma, amniotic fluid and fetal brain at GD 18.5

Pregnant dams (n = 7) were treated by oral gavage with the ART regimen 3TC/ABC+ATV/r for 18.5 days following plug detection. Concentration of each antiretroviral drug was independently assessed in a) maternal plasma (n = 7) and amniotic fluid of each fetus (n = 56, 19 male, 37 female) at GD18.5, as well as in GD18.5 fetal brain (n = 56, 19 male, 37 female), normalized to brain wet-weight.

	Maternal plasma (ng/mL)	Amniotic fluid (ng/mL)	Fetal brain (ng/mg normalized to brain wet weight)	Amniotic fluid to maternal plasma ratio	Fetal brain to maternal plasma ratio
3TC	2696.6 ± 855.3	662.0 ± 359.1	12.8 ± 6.9	0.269 ± 0.144	0.005 ± 0.004
ABC	7264.7 ± 2656.1	7575.8 ± 2325.5	434.6 ± 126.6	1.217 ± 0.610	0.071 ± 0.036
ATV	5293.0 ± 4158.6	413.1 ± 450.5	ND	0.087 ± 0.059	ND
RTV	334.7 ± 363.0	16.1 ± 21.7	ND	0.071 ± 0.075	ND

ND = not detected