Role of Membrane-associated Transporters in Modulating Fetal Drug Exposure: Relevance to Antiretroviral Drug Teratogenicity

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BACKGROUND

Combination antiretroviral (ARV) therapy (ART) during pregnancy is increasingly effective in reducing risk of vertical human immunodeficiency virus (HIV) transmission, however the fetal toxicity of widely administered ARV drugs remains poorly understood and existing literature often does not account for sex as a variable.

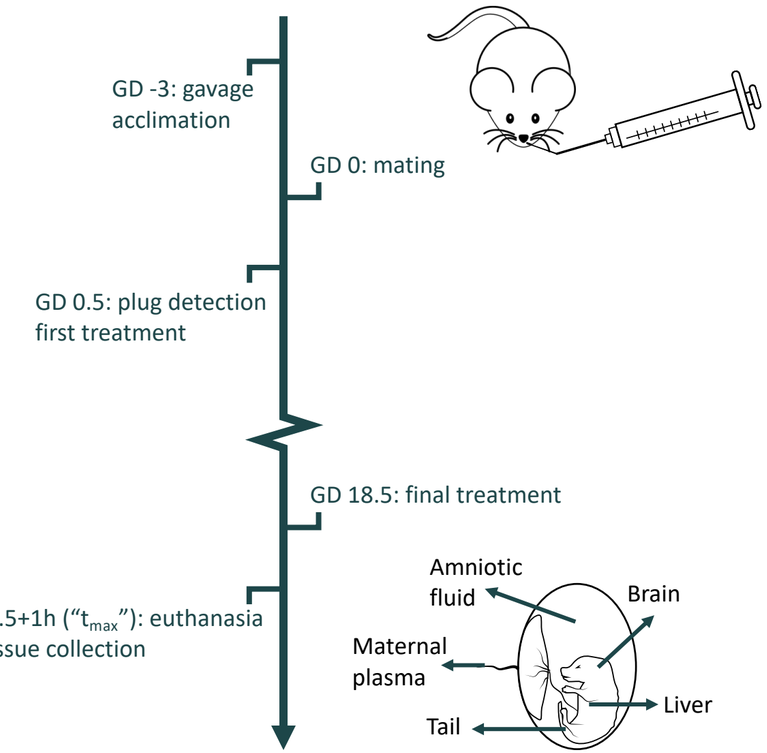
New evidence of sex-linked neurotoxicity in ARV exposed children suggests differential drug disposition.[1,2] These differences may be explained by sex-linked expression of membrane associated transporters from the ATP binding Cassette and Solute Carrier super-families, as well as other proteins which contribute to the *in utero* distribution of ARVs. Sex and age dependent transporter expression has been described previously in postnatal tissues, but characterization in fetal tissues is limited.[3]

Preliminary data from the lab of Dr. Lena Serghides (figure 1) has identified a link between ARV exposure and memory deficit in a mouse model of combination antiretroviral therapy, present in female but not male mice. These data further highlight the need for research into the sex-dependent toxicities of ARV exposure.

OBJECTIVE

The objective of this study was to assess the penetration of lamivudine/abacavir + atazanavir/ritonavir (3TC/ABC+ATV/r) into the fetal compartment, and to examine the effect of fetal sex and ARV exposure on the expression of transporters and other proteins in fetal brain, fetal liver and placenta, using a mouse model of pregnancy.

METHODS



Pregnant C57BL/6J mice were administered either 3TC/ABC+ATV/r (100/50 mg/kg/day + 50/16.6 mg/kg/day), or water by oral gavage (above). Fetal and maternal tissues were collected on gestational day (GD) 18.5. mRNA expression was assessed by qPCR. Fetal sex was determined by PCR amplification of the male specific sequence Sry. Drug concentrations were determined by LC/MS/MS.

RESULTS: characterization of mRNA expression in adult and fetal tissues

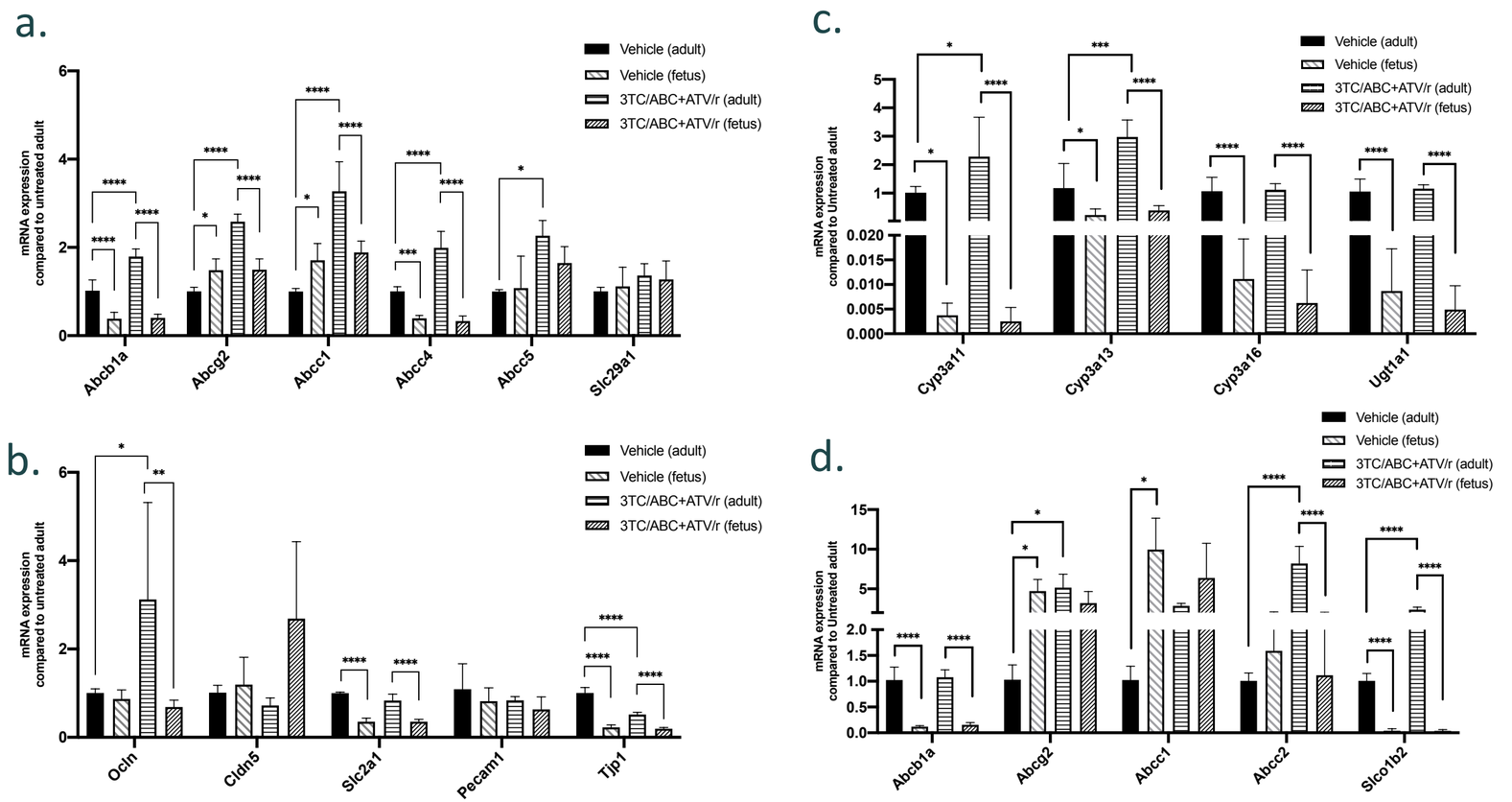


Figure 1 mRNA expression in adult and fetal brain and liver exposed to antiretroviral drugs

mRNA expression of: a) transporters and b) microvessel endothelial cell markers in GD18.5 fetal mouse brain, exposed to 3TC/ABC+ATV/r or vehicle control relative to expression in adult mouse brain (female), was assessed by qPCR. Expression of c) metabolic enzymes, and d) transporters was similarly assessed in adult and fetal liver. n = 6-13 fetuses from 6-8 unique dams. Differences between in 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.

Transporter and metabolic enzyme expression was generally greater in adult mice. Expression of ATP-binding cassette transporters was generally induced by ARV exposure in adults.

RESULTS: mRNA expression in fetal mouse brain and liver

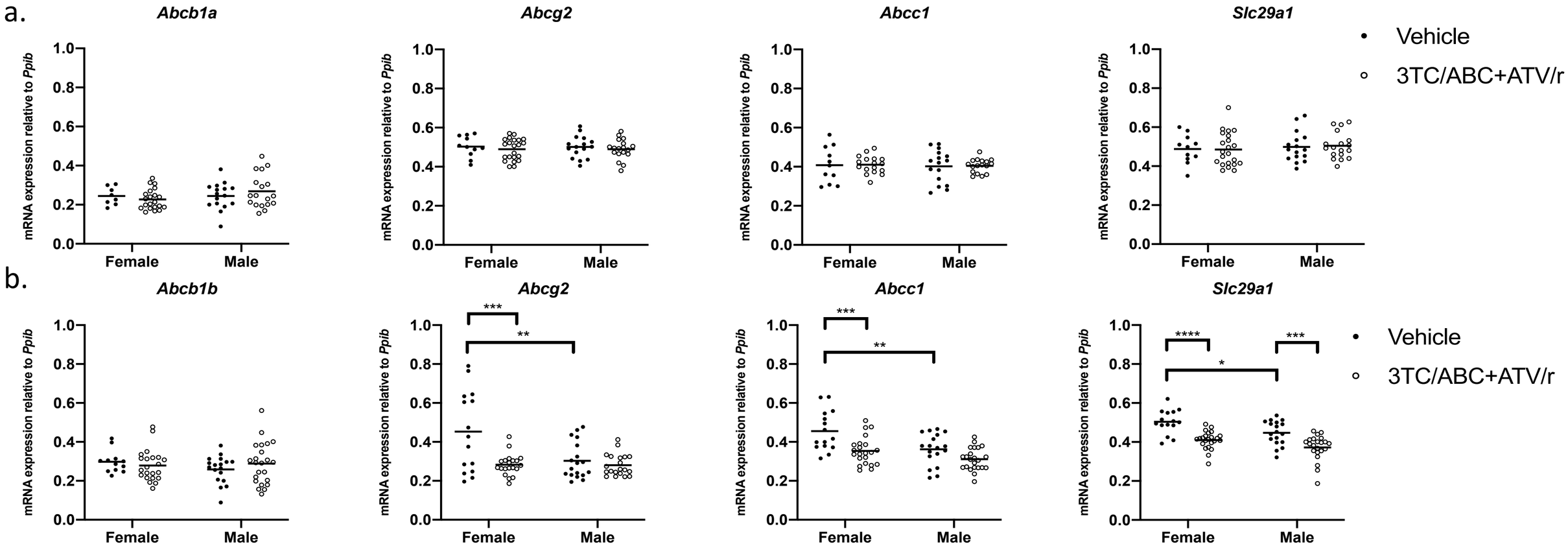


Figure 2: Effect of sex and ARV exposure on *Abcb1a/b, Abcg2, Abcc1* and *Slc29a1* mRNA expression in GD 18.5 mouse brain and placenta
Pregnant dams were exposed for 18.5 days following plug detection to 3TC/ABC+ATV/r, or vehicle control. mRNA expression of *Abcb1a/b, Abcg2, Abcc1* and *Slc29a1* was 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.

No impact of sex, or ARV exposure was identified in transporters of the brain. However, expression of placental *Abcg2*, *Abcc1* and *Slc29a1* was significantly reduced by exposure to antiretroviral drugs. In addition, expression of these three transporters was significantly greater in female compared to male placentas.

RESULTS: Drug penetration into maternal and fetal tissues

Drug \ Sample	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

Figure 4: Antiretroviral drug concentrations in maternal plasma, amniotic fluid and fetal brain at GD 18.5
We assessed the penetration of 3TC, ABC, ATV and RTV into fetal and maternal tissues by LC/MS/MS. 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 ARV was independently assessed in 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. ND = Not detected

Penetration of 3TC and ABC was identified in both amniotic fluid and fetal brain, with a modest accumulation of ABC in amniotic fluid, relative to maternal plasma. The HIV protease inhibitors ATV and RTV were not detected in fetal brain, suggesting limited penetration.

CONCLUSIONS

This study describes interactions between ARV exposure and transporter regulation in the context of ART during pregnancy.

- We characterized significant differences between adult and fetal transporter expression, consistent with literature describing the ontogeny of these proteins. Expression of metabolic enzymes *Cyp3a11* and *Ugt1a1* was particularly low in the fetal liver, relative to the adult liver, suggesting limited fetal contribution to the metabolism of circulating ARVs. We also identified a significant induction of transporter expression associated with ARV exposure in adults, particularly of transporters of the ATP-binding cassette family. This induction was not observed in the fetus.
- In fetal placenta, we identified a downregulation of *Abcg2, Abcc1* and *Slc29a1* associated with exposure to ARVs. In addition, we report greater expression of these transporters in female compared to male placenta, with potential bearing on the transport of ARVs and other molecules between mother and fetus. Transporters from the ATP-binding cassette superfamily are known to respond to sex-hormone exposure, and may be influenced by differences in the fetal environment[5].
- Finally, we characterized the penetration of ARVs into fetal tissues. In fetal brain, the HIV protease inhibitors ATV and RTV were undetectable, suggesting limited penetration perhaps related to interactions with transporters in the fetal brain.

Further research looking at drug concentrations in the brain will help in understanding the underlying causes of sex-differential outcomes in ARV exposed children, leading to safer and more effective HIV treatment during pregnancy.

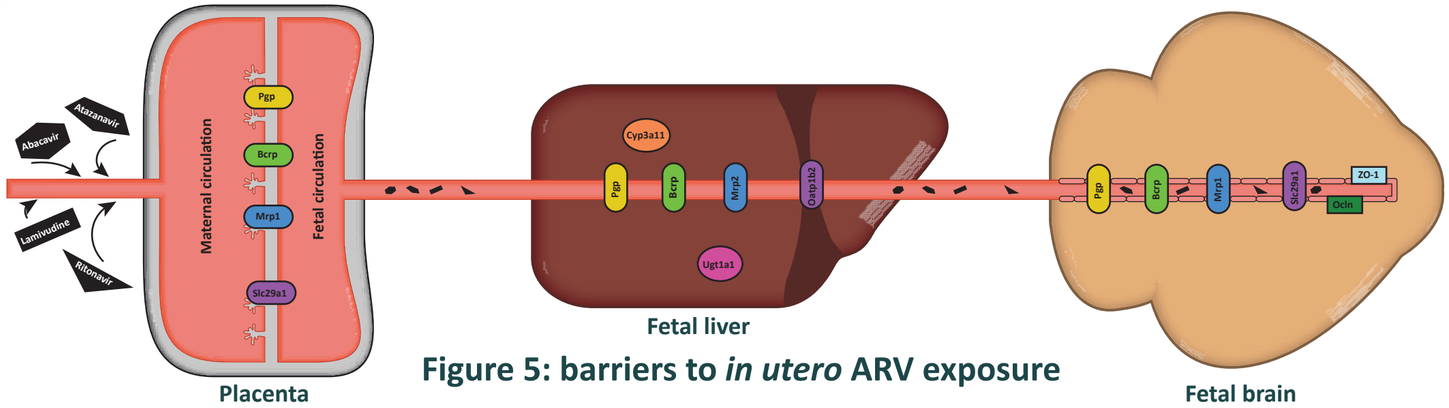


Figure 5: barriers to *in utero* ARV exposure

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