



Research Article

Theme: Celebrating Women in the Pharmaceutical Sciences

Guest Editors: Diane Burgess, Marilyn Morris and Meena Subramanyam

Role of HIV and Antiretroviral Therapy on the Expression of Placental Transporters in Women with HIV

Dea Kojovic,¹ Ragia H. Ghoneim,² Lena Serghides,^{3,4} and Micheline Piquette-Miller^{1,5}

Received 12 July 2020; accepted 28 September 2020; published online 25 October 2020

Abstract. Treatment guidelines recommend continuation of combination antiretroviral therapy (cART) throughout pregnancy for all women living with human immunodeficiency virus (HIV). Many of these drugs are substrates of transporters expressed in the placenta and therefore play a role in fetal exposure. As placental transporters can be impacted by both HIV infection and drug therapy, our objective was to explore the impact of HIV infection and cART on transporter expression. Drug transporter expression was examined in human placental samples collected from women with HIV ($n = 25$) and from healthy HIV(-) controls ($n = 23$). The effect of exposure to drugs commonly used in cART during pregnancy was examined *in vitro* in placental villous explants obtained from healthy women. Gene expression was measured via qRT-PCR. Several ABC (ABCG2, ABCC1,2,4) and SLC (SLC21A9, SLC22A1,3,11) transporters were significantly downregulated in placentas isolated from HIV(+) women as compared with HIV(-) controls ($p < 0.05$ – 0.001), while ABCB1 and SLC21A12 were significantly upregulated ($p < 0.001$). Twenty-four to 48-h exposure of human placental explants to agents used in cART resulted in significant upregulation of ABCB1 and downregulation of SLC22A11. Our findings suggest that transplacental transport may be compromised during HIV infection due to altered expression of clinically important transporters. Furthermore, *in vitro* results indicate that cART imposes significant alterations in placental transporters but not all changes are consistent with findings in the placenta from HIV(+) women, indicating disease effects. As this may impact in utero-fetal exposure to clinically used medications, further studies are needed to determine the overall impact on maternal-fetal transfer.

KEY WORDS: transporters; placenta; HIV; antiretroviral agents; pregnancy.

Guest Editors: Diane Burgess, Marilyn Morris and Meena Subramanyam

Electronic supplementary material The online version of this article (<https://doi.org/10.1208/s12248-020-00516-2>) contains supplementary material, which is available to authorized users.

¹ Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, Ontario M5S 3M2, Canada.

² Pharmacy Practice Department, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.

³ Toronto General Hospital Research Institute, University Health Network, Toronto, Ontario, Canada.

⁴ Department of Immunology and Institute of Medical Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada.

⁵ To whom correspondence should be addressed. (e-mail: m.piquette.miller@utoronto.ca)

INTRODUCTION

Human immunodeficiency virus (HIV) treatment guidelines recommend combination antiretroviral therapy (cART) in all individuals living with HIV. In 2018, there was an estimated 18.8 million women living with HIV, most of whom were of child-bearing age (1). Continuation of cART is further recommended during pregnancy, and thus, most pregnant women living with HIV are on a number of antiviral agents throughout the course of gestation. Optimal therapeutic management in this patient population is twofold, to continually treat HIV infection in the mother by suppressing viral load and concurrently prevent vertical transmission. The US Department of Health and Human Services recommend a dual nucleoside reverse transcriptase inhibitor (NRTI) backbone used in combination with a ritonavir-boosted protease inhibitor (PI) or an integrase strand transfer inhibitor (2). While these regimens have been successful and have reduced the incidence of vertical transmission to the fetus (<2%), drug toxicities, adverse pregnancy outcomes, and

neurobehavioral deficits remain a concern with in utero exposure to HIV and cART.

The placenta expresses numerous ATP-binding cassette (ABC) efflux transporters within both trophoblast membranes as well as in developing fetal organs. These transporters are essential in maintaining a thriving environment for fetal development as they play a role in the active efflux of endogenous substances, waste products, and xenobiotics out of the fetal compartment and/or fetal tissues. Within the placenta, the key members of this group of transporters are the breast cancer resistance protein (BCRP, *ABCG2*), P-glycoprotein (PGP, *ABCB1*), and the multidrug resistance family of proteins (MRPs, *ABCCs*).

The placenta is also essential in the uptake of nutrients, steroids, and other components essential for the maintenance of healthy pregnancy. The solute carrier uptake (SLC) transporters are a group of membrane transporter proteins that play an important role in the transfer of substrates across the placenta. Expressed at both the apical (maternal to fetal) and basolateral (fetal to maternal) membranes, they are crucial in the cellular uptake of substrates from the maternal to fetal circulation or in the removal or transfer of substrates from the fetus to mother. Some of the key SLC drug transporters found in the placenta are the organic anion transporting polypeptides (OATPs), the organic cation transporters (OCTs), organic anion transporters (OATs), and the equilibrative nucleoside transporters (ENTs). Furthermore, SLC transporters can also be found within fetal tissues, where they facilitate the uptake of nutrients and essential compounds for fetal development.

As there is significant substrate overlap between these two groups of transporters, the ABC and SLC transporters are important to consider together as they work in tandem to support the transplacental transfer of their substrates as well as fetal homeostasis (3). At term, the most highly expressed drug transporters within the placenta are OCT3, OAT4, BCRP, and OATP2B1, in the order from highest to lowest (4). The localization and directionality of transporters studied in this manuscript can be found in Fig. 1.

To date there are few studies that have studied placental transporter expression in the context of HIV. To our knowledge, expression of just PGP has been previously described in the placenta from HIV(+) women. Camus *et al.* found that PGP levels were significantly upregulated in the placentas of women living with HIV (5). On the other hand, a number of studies have shown changes in the basal dysregulation of transporters in HIV-transgenic rats in several tissues including the placenta (6–10). Other studies using rodent models of bacterial and viral infection corroborate these findings, depicting disease-mediated downregulation of placental transporters, ultimately leading to changes in fetal exposure to their substrates (11,12). These changes were also accompanied by increased levels of pro-inflammatory cytokines. Furthermore, clinical studies in placental samples obtained from women with chorioamnionitis, a bacterial infection of the placental chorion and amnion, portrayed the same trends with several ABC and SLC transporters dysregulated in the placenta along with the increases in placental levels of pro-inflammatory cytokines (13). Cytokine-treated primary cultures of human term trophoblasts also revealed significant decreases in the mRNA expression of PGP and BCRP (14). The impact of these changes can ultimately alter drug disposition and influence transplacental transfer of substrates. Furthermore, many drugs prescribed as

components of cART are known to be substrates of and/or interact with placental transporters. Moreover, as the substrate profile of many ABC and SLC transporters overlap, numerous HIV antiretrovirals (ARVs) have been shown to interact with both families of transporters (10). This is not limited to xenobiotics, as transporters also play an essential role in the transplacental uptake of endogenous compounds important for the maintenance of healthy pregnancy. Notably, sex hormones, which are produced by the placenta and crucial in the maintenance and progression of pregnancy (15), are transported in and out of placental cells by ABC and SLC transporters and are known to regulate their expression (14–16).

Drug therapy is continued throughout the course of pregnancy in mothers living with HIV. However, little is known about the effects of HIV and cART on placental transporters in a clinical setting. As many of the prescribed ARVs are substrates for transporters found in the placenta, understanding the potential interactions between ARVs and placenta transporters in the context of HIV infection is urgently needed. This will enable us to better understand the impact on transplacental transfer of these agents, fetal exposure to potentially toxic agents, as well as imbalance of essential endogenous substances. As the expression of placental transporters can be impacted by HIV infection and drug therapy or both, our objective was to examine the expression of key drug transporters in the placenta from women living with HIV on cART and to further explore the role of cART in the regulation of these transporters in cultured human placental explants.

MATERIALS AND METHODS

Human Placental Sample Acquisition. Human placental tissue samples from women with or without HIV were obtained from the Angiogenesis and Adverse Pregnancy Outcomes in Women with HIV (AAPH) Biobank in Toronto (LS lead PI) and from the Research Center for Women's and Infant's Health (RCWIH) BioBank program at Mount Sinai Hospital (MSH), Toronto, Canada. Participants were recruited over the period of 2010–2015. All control samples were obtained from term pregnancies, and 22 out of 25 HIV-infected placentas were obtained from term pregnancies while only three were considered preterm (30, 31, and 35 weeks). Inclusion and exclusion criteria for the AAPH Biobank have been previously published (17). All AAPH cohort participants gave informed consent for future use of their samples, and REB approval was obtained for this study. RCWIH Biobank samples were acquired in accordance with the policies of the MSH Research Ethics Board (Protocol Reference # 28225) and following the tenets of the Declaration of Helsinki.

Sample acquisition and processing are detailed on the RCWIH Biobank website (<http://biobank.lunenfeld.ca>), and inclusion and exclusion criteria are listed in Table S1. A similar sample acquisition was used for the AAPH samples as well.

In order to determine the appropriate sample size for our study, a power analysis was conducted for a desired power of 0.80 and 40% effect size (difference between groups), and a sample size of 25–30 in each group was predicted. Placental samples were collected from women who were clinically diagnosed with HIV ($n=25$) and control placental samples ($n=23$) were collected from healthy pregnancies matched for gestational age. Although our sample size was below the

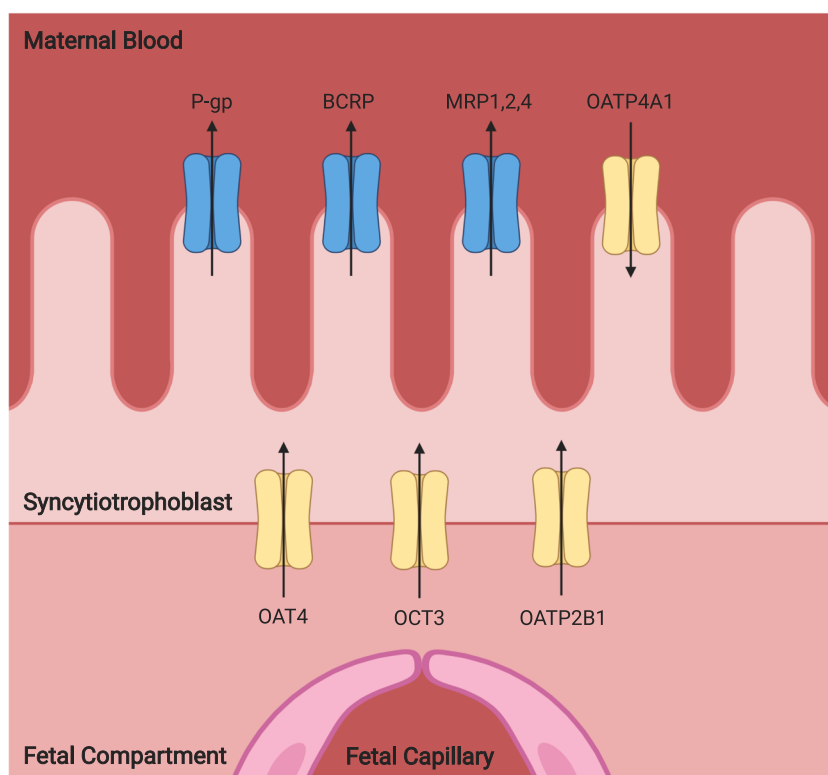


Fig. 1. Localization of placental transporters. Schematic depiction of selected ABC and SLC transporter localization and direction of transfer across the placenta. Created with [BioRender.com](https://www.biorender.com)

predicted required sample size in our control group, upon post hoc power calculations, it was determined that the power remained above 0.80 for all groups as a higher % change between groups was seen. Clinical data that were extracted from patient charts was collected by the RCWIH BioBank program of MSH and was made available to this study and extensively reviewed.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Analysis. The mRNA levels were determined using qRT-PCR as previously described (18). Briefly, RNA was extracted from 50 to 100 mg of placental tissue using Trizol (Invitrogen, Carlsbad, CA) and concentrations as well as RNA purity was determined using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Contaminating genomic DNA was removed by treatment with DNase (Invitrogen, Carlsbad, CA) and samples were subsequently reverse transcribed (RT) using a high-capacity cDNA RT kit (Applied Bio Systems, Waltham, MA). Real-time quantitative PCR was performed using power SYBR green detection system (ABI HT-7900). Relative gene expression was assessed using the $[\Delta\Delta c(t)]$ method normalized to the means of two housekeeping genes, Topoisomerase I (TOPO) and zeta polypeptide (YWHAZ). Primer sequences for the genes analyzed are listed in Supplementary Table S2.

Western Blotting. Crude membrane fractions were isolated from 300 mg of human placental tissue as previously described (13). Protein concentrations were measured using the Bradford assay with BSA standards. Isolated protein samples (30 μ g) in

Laemmli sample loading buffer were heated at 37 °C for 20 min, separated via 10% SDS-PAGE, and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, ON, Canada). Membranes were incubated overnight at 4 °C with a primary antibody in 2% nonfat milk (TBST). The antibodies included were anti-ABCG2 mouse monoclonal antibody (BXP-21 clone, 1:500, Abcam, Inc., Cambridge, MA) and anti-OCT3 (anti-SLC22A3 Q306 clone, Bioworld St Louis Park, MN), while anti- β -actin mouse monoclonal antibody (AC15 clone 1:75000, Sigma-Aldrich, Oakville, ON Canada) was used as a loading control. Membranes were then washed with TBST and incubated with a horseradish peroxidase-labeled secondary antibody (anti-mouse 1:3000, or anti-rabbit 1:10,000, Sigma-Aldrich, Oakville, ON Canada) in 2% nonfat milk TBST. Optical densities for bands of interest were visualized using a FluorChem Xplor imager (Alpha Innotech, San Leandro, CA) and quantified using Alpha Ease FC imaging software. Due to limited sample quantities of placentas obtained from HIV(+) women, BCRP, PGP and OCT3 were the only protein targets probed.

Human Placental Explant Culture. Villous explant cultures were obtained from caesarian section at term (38.4–39 weeks). All three women were Caucasian, had normal BMI (25.6 ± 3.1), and were not taking any prescribed medications. Neonatal sex was two males and one female while birth weights were 7.82 ± 1.5 lbs. Each placenta served as its own control to account for inter-placental differences and no differences were seen between male and female results.

Small fragments (~30 mg) of placental villi were dissected from the placenta and placed in 24-well plates.

Explants were cultured in Dulbecco's Modified Eagle Medium/F12, supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA), 0.1% non-essential amino acids (NEAA) (Thermo Fisher Scientific, Waltham, MA), 0.1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA), 0.1% insulin, (Thermo Fisher Scientific, Waltham, MA), and 0.1% Normocin (Invivogen). Explants were cultured at 37 °C under an atmosphere containing 5% CO₂ for 24 h prior to treatment with the ARVs, individually and in combination, or with vehicle as controls (0.0025% dimethyl sulfoxide (DMSO)). Explants from a single placenta were cultured in triplicate for 4, 24, or 48 h, and each experiment was repeated three times using placentae obtained from different donors. Lopinavir (LPV), ritonavir (RTV), atazanavir (ATV), lamivudine (3TC), and zidovudine (ZDV) (National Institutes of Health AIDS Reagent Program) were dissolved in SO in 100 mM stocks and stored at -20 °C until use. The concentration of DMSO in the final medium was <0.1%. Therapeutically relevant concentrations were used (10X MEC) and can be found in Supplementary Table S4. After the treatment period, explants were removed from media containing drug treatments and snap frozen in liquid nitrogen. Pre- and post-treatment media were collected in order to assess cell viability via the LDH assay (data not shown). While it is recognized that newer ART drug combinations exist to date, the selection of ART and their combinations were based on the ART drug combinations that the HIV + pregnant women were on during the study period. Hence the drugs were chosen based on their clinical use during the study period.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (San Diego, CA). Human data was log-transformed to conform to normality. For comparison between two groups, we used an unpaired *t* test with Welch's correction. Significance was defined as $p < 0.05$. Human explants were normalized to control values. Statistical comparisons were performed using one-way ANOVA with Dunnett's post-test. Correlations were assessed using the Spearman correlation coefficient.

RESULTS

Clinical Patient Characteristics

We collected 23 control placentas from the RCWIH Biobank and 25 HIV(+) placentas from the AAPH Biobank in Toronto. Placental control samples were collected from normal pregnancies that were not associated with any pathological abnormalities upon histological examination, while HIV placentas were obtained from women who consented to participate in a biobank program supporting research relevant to HIV infection during pregnancy (17). There was no statistically significant difference in maternal weight or gestational age between the two groups; however, the neonatal birth weight was significantly lower in the HIV(+) group compared with the control group (Table I). All HIV(+) subjects were receiving cART, 20 of which were

on treatment at conception and five that initiated therapy during pregnancy. All subjects were receiving two reverse transcriptase inhibitors, in combination with either a ritonavir-boosted PI (22 of 25), a non-NRTI (NNRTI) (2 of 25), or an integrase strand transfer inhibitor (INSTI) (1 of 25). Viral load for most women (22 of 25) were below 40 copies per mL (Table I).

Altered Expression of Placental Transporters in HIV Group

The expression of highly expressed, clinically relevant ABC and SLC transporters was examined in placentas from women with HIV and compared with placentas obtained from the control, HIV(-) women. Numerous transporters were dysregulated in the placenta obtained from women with HIV as compared with controls (Fig. 2). Within the ABC transporter family, transcript levels of BCRP and MRPs 1, 2, and 4 were significantly downregulated by 38–72%, while PGP mRNA expression was significantly upregulated 5.5-fold in the HIV(+) group as compared with the controls. Within the SLC transporter group, OATP2B1, OCT1, OCT3, and OAT4 were significantly downregulated by 85–99%, while there was a twofold induction of OATP4A1 in the HIV(+) group as compared with the controls.

Altered Protein Expression of Placental Transporters in HIV Group

Protein expression of BCRP, PGP, and OCT3 was analyzed as they are some of the most highly expressed drug transporters in the placenta, and transcript levels indicated pronounced differential expression in the HIV(+) group (Fig. 3). Consistent with mRNA changes, protein expression of OCT3 was significantly downregulated by 50% in the HIV(+) group compared with the controls ($p < 0.01$), while there was a pronounced trend towards upregulation of PGP ($p = 0.11$). On the other hand, while a pronounced decrease in BCRP mRNA was seen in the HIV(+) group, protein levels were not significantly changed. Rather, a trend towards increased protein expression was seen in the HIV(+) group compared with the controls ($p = 0.063$).

Altered Expression of Inflammatory Cytokines in HIV Group

Transcript levels of IL-1 β and TNF- α were significantly lower while levels of IL-8 were significantly higher in the placentas of women with HIV as compared with the controls (Fig. 4). There was no change in the mRNA levels of IL-6 in the HIV(+) group as compared with the controls.

Exposure to ARVs Alters Expression of Transporters in Human Placental Villous Explants

The expression of a variety of ABC and SLC transporters was examined in healthy term placental explants, after treatment with clinically relevant ARVs, individually, as well as in combination at 10 times the minimum effective concentrations (see methods) (Fig. 5). Although these concentrations are more reflective of maximal (C_{max}) rather than trough (C_{min}) concentrations, there was no observed

Table I. Clinical Patient Characteristics

Characteristic	HIV- (<i>n</i> = 23)	HIV+ (<i>n</i> = 25)
Maternal age, mean (SD)	31.81 (3.7)	33.75 (5.3)
Ethnicity, <i>n</i> (%)		
Caucasian	17 (74%)	4 (16%)
Asian	3 (13%)	1 (4%)
Black	0	17 (74%)
Hispanic	0	1(4%)
East Indian	3 (13%)	0
BMI, mean (SD)	24.58 ± 5.10	24.55 ± 5.77
Mode of delivery, <i>n</i> (%)		
Vaginal	7 (30%)	15 (60%)
C-section	16(70%)	10 (40%)
Gestational age	39.14 ± 0.92	38.22 ± 2.45
Neonatal birth weight	3470 ± 413	2981 ± 703*
Infant sex		
Male	13 (56%)	15 (60%)
Female	10 (44%)	10 (40%)
Birth Percentile		
< 50 <= 90	16 (70%)	8(32%)
> 10 and <= 50	6(26%)	9(36%)
> 5 and <= 10	1 (5%)	8(32%)
Median viral load (copies/mL)	N/A	<40
Detectable viral load at recruitment, <i>n</i> (%)	N/A	5 (20%)
Viral load above 1000, <i>n</i> (%)	N/A	1 (4%)
ARV regime		
PIs		22 (88%)
Lopinavir/ritonavir	N/A	11 (44%)
Atazanavir/ritonavir	N/A	8 (32%)
Darunavir/ritonavir	N/A	3 (12%)
NNRTIs	N/A	2 (8%)
INSTIs		1 (4%)

BMI body mass index, HIV human immunodeficiency virus, NNRTI non-nucleoside reverse transcriptase inhibitor, INSTI integrase strand transfer inhibitor, PI protease inhibitor. Statistical analysis results are presented as mean ± SD. All other results are presented as frequency (% of total). Statistical comparisons were performed using Student’s *t* test, where → indicated significantly different from controls (*P* < 0.01)

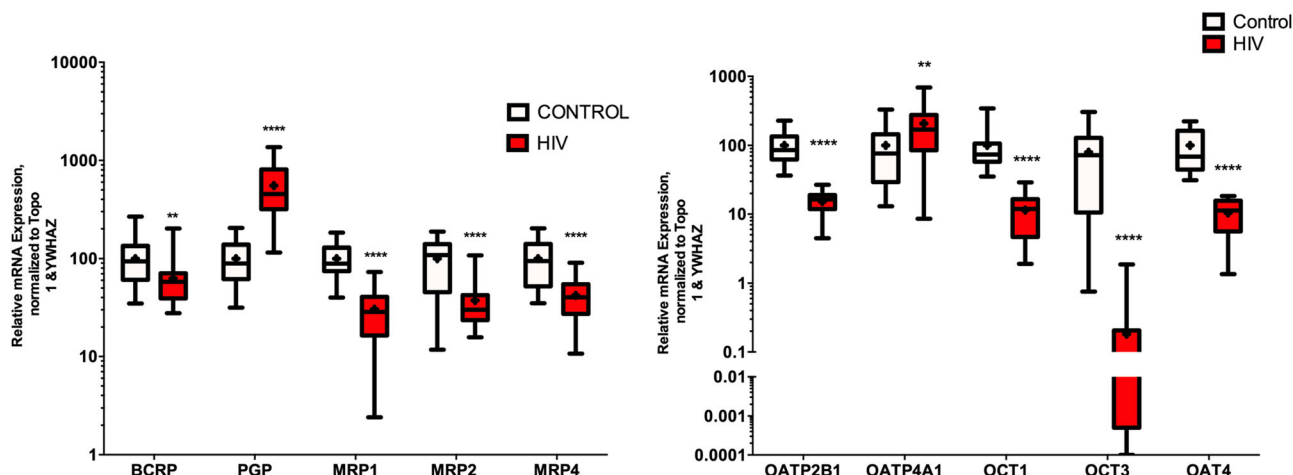


Fig. 2. Alterations of mRNA expression of placental transporters in HIV(+) women. The distributions of transporter mRNA expression in control (*n* = 23) vs. HIV (*n* = 25) groups are shown using a Box and whisker plot as a percentage of the mean indicated by “+.” The dark line represents the median, the box represents the interquartile range, and the whiskers represent the min to max. Statistical comparisons were performed using unpaired *t* test with Welch’s correction. Data are normalized to housekeeping genes and are log-transformed to conform to normality where **P* < 0.05, ***P* < 0.01, *****P* < 0.001

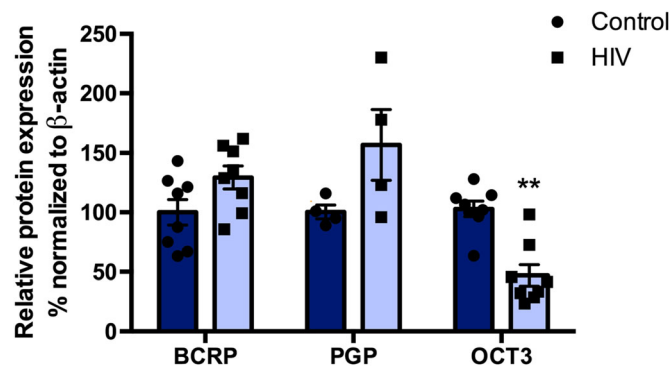


Fig. 3. Alterations of protein expression placental transporters in HIV(+) women. Data are presented as a percentage of the mean \pm S.E.M ($n = 4-8$ per group). Individual data points are depicted by black circles and $**P < 0.01$. Statistical comparisons were performed using unpaired t-test with Welch's correction

cytotoxicity to the placental explants. Transcript levels of PGP were elevated 1.6- to 2.5-fold by individual treatments of ATV, RTV, and LPV after 24-h exposure (Fig. 5a). Likewise, transcript levels of BCRP were elevated 1.6- to 1.9-fold after 24-h exposure to combination treatments of ZDV + 3TC, ATV + RTV, as well as ZDV + 3TC + ATV+ RTV (Fig. 5b). In contrast to the ABC transporters, OATP4A1 was significantly downregulated by 38% after 24-h exposure to RTV, by 50–60% with exposure to LPV at both 24 and 48 h, and by 80% with exposure to ZDV + 3TC + ATV+ RTV after 48 h (Fig. 5d). OAT4 was significantly downregulated after 48 h of treatment with ATZ, RTV, and LPV individually and by all combination treatments tested (Fig. 5f). While there are trends towards a downregulation of OCT3 with ARV treatments at 48 h, these results are not significant due to a high degree of variance within the vehicle-treated controls.

Estradiol Levels in Women with HIV Correlate with PGP and MRP1 mRNA Expression

As many of the placental transporters examined facilitate the transfer of endogenous substances including steroid hormones, we investigated the association between maternal and cord E2 levels at delivery and placental transporter transcript levels (Fig. 6). Levels of maternal and cord E2 for the AAPH cohort have been previously published (19), and the relevant subset of data were used in these analyses. Overall, maternal E2 levels significantly correlated with log-transformed values of HIV placental mRNA expression of PGP ($r = 0.533$, $p = 0.0187$) and MRP1 ($r = 0.4754$, $p = 0.0461$) (Fig. 6a and b). No significant correlations were observed with cord E2. Surprisingly, we did not observe any correlations between steroid hormones and several transporters for which they are substrates (BCRP, OAT4, and OATP2B1).

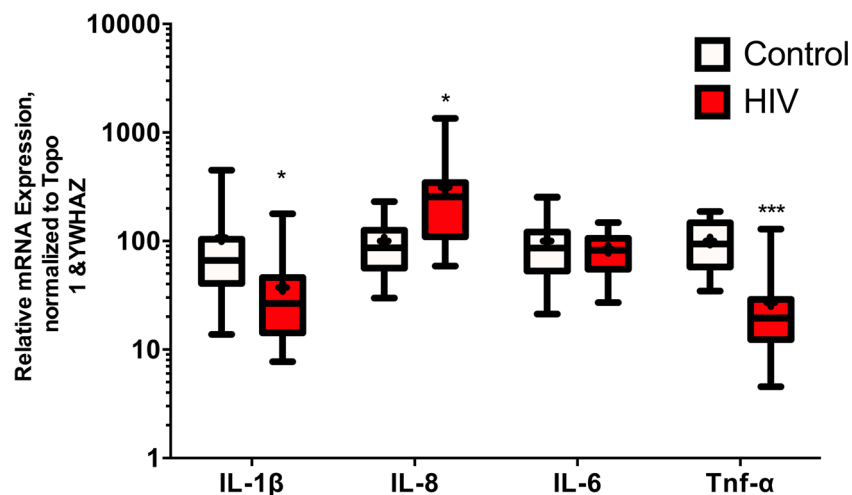


Fig. 4. Alterations of mRNA expression of pro-inflammatory cytokines in HIV(+) women. The distribution of mRNA levels in control ($n = 23$) vs. PE ($n = 25$) groups is shown using a Box and whisker plot as a percentage of the mean indicated by "+." The dark line represents the median, the box represents the interquartile range, and the whiskers represent the min to max. Statistical comparisons were performed using unpaired t test with Welch's correction. Data are normalized to housekeeping genes and log-transformed to conform to normality where $*P < 0.05$

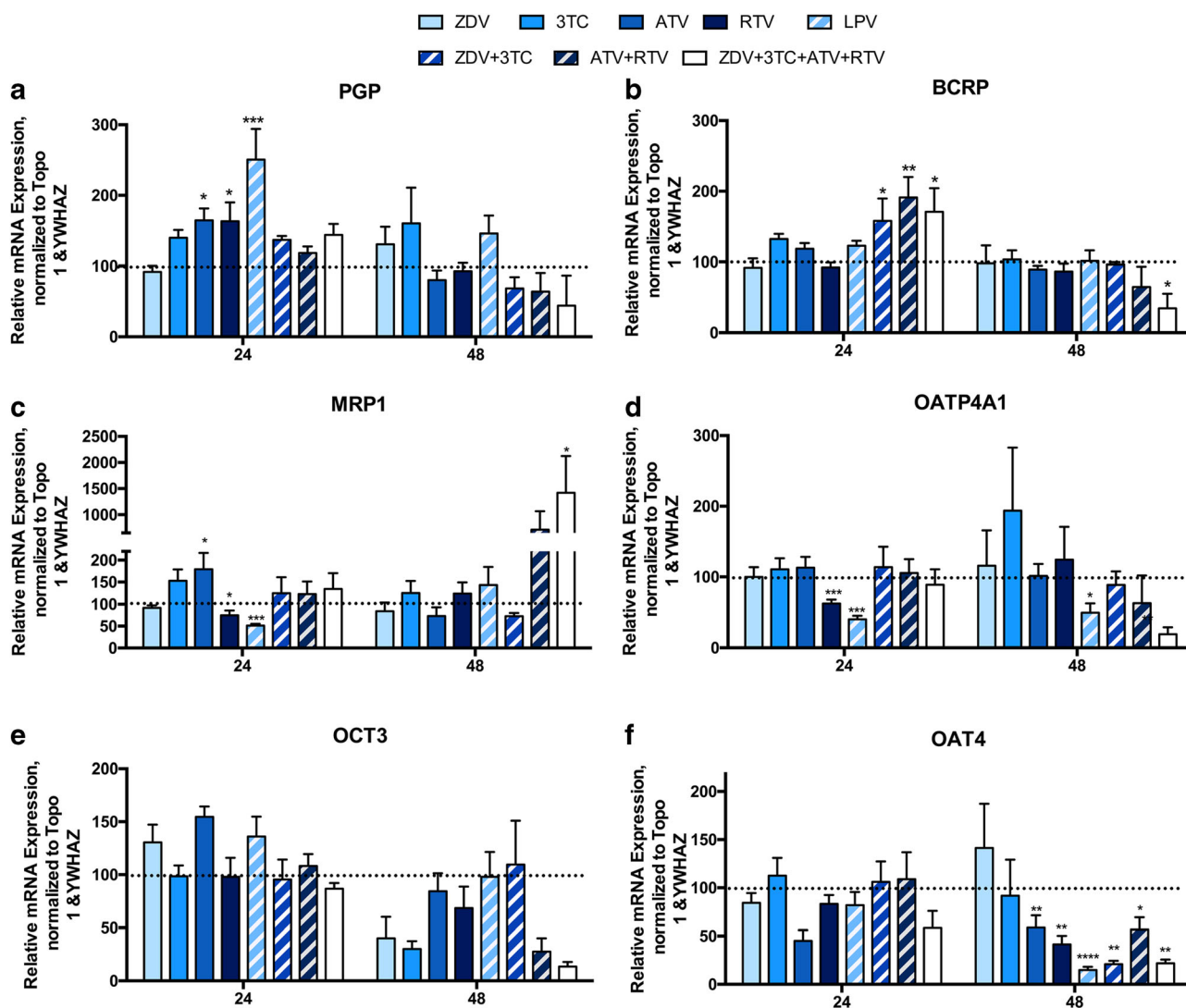


Fig. 5. Exposure to ARVs alters expression of **a** PGP, **b** BCRP, **c** MRP1, **d** OATP4A1, **e** OCT3, and **f** OAT4 in human placental villous explants. Data were normalized to vehicle-treated controls (represented by the horizontal dotted line) and are presented as a percentage of the mean \pm SEM of three different placentas with $n = 3$ separate samples per experiment. Statistical comparisons were performed using one-way ANOVA with Dunnett’s post-test. ZDV, zidovudine; 3TC, lamivudine; ATV, atazanavir; RTV, ritonavir; LPV, lopinavir

DISCUSSION

The placenta expresses many clinically important drug transporters which we found to be altered in the placenta of HIV(+) women on cART. We observed altered expression of several ABC efflux transporters, including BCRP, PGP, and MRPs, which are important in preventing fetal exposure to their drug substrates. Likewise, significant changes in the expression of the SLC uptake transporters, including OATP2B1, OATP4A1, OCT3, and OAT4, were seen. In addition to their involvement in drug transport, these transporters are important in the homeostasis of many sex steroids as well as other essential nutrients. Although elevated systemic levels of IL-6 have been reported in people with HIV, transcript levels of this cytokine were not changed in placenta tissues. However, increased transcript levels were seen with IL-8, while transcript levels of TNF- α and IL-1 β were decreased. As all women within the HIV group were

currently on cART, we further explored the effects of these drugs on transporters in cultured placental villous explants.

We observed a dramatic decrease in transcript levels of OCT3 in the placenta from women with HIV, with mRNA levels being 99% lower than the control group. Likewise, protein levels were significantly decreased. OCT3 is the most abundantly expressed SLC uptake transporter in term placenta and has been shown to significantly influence fetal accumulation of the substrate drug metformin (20). OCT3 plays an important role in the transfer of monoamine neurotransmitters, hormones, and steroids (21). Levels of OCT1 were also decreased in placentas from the HIV+ group, while OCT2 was undetectable in placental tissue from both groups indicating that compensatory substrate transport by OCT1 and 2 is unlikely. Although not significant, there was a pronounced trend of decreased OCT3 after 48 h in explants treated with cART containing RTV. There was also a pronounced decrease in the placental expression of OAT4 in the

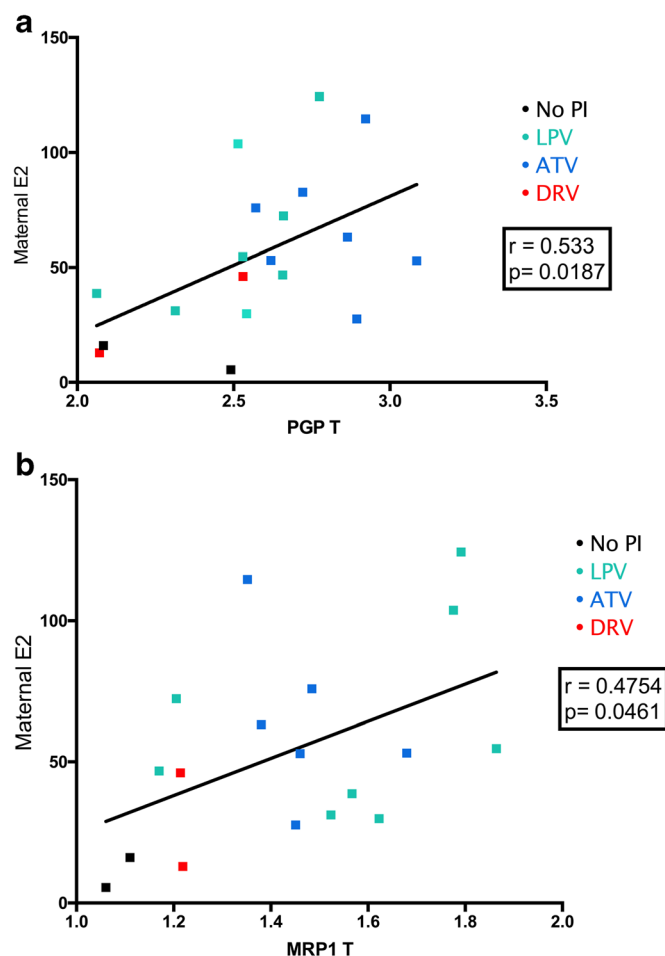


Fig. 6. Maternal estradiol levels in HIV-infected patients correlate with PGP and MRP1 mRNA expression. Correlation between placenta expression level of **a** PGP and **b** MRP1 (log-transformed) and maternal estradiol from HIV placentas. Individual data points are colored to represent different protease inhibitor therapies patients were prescribed. Correlation assessed by Spearman r

placenta from the HIV(+) group compared with the controls. OAT4 is located on the basolateral membrane and plays a role in the transport of numerous drugs as well as endogenous steroids and urates (22). We found that 48-h treatments of explants with atazanavir, RTV, and LPV alone or as cART regimens caused significant decreases in OAT4 expression. This suggests that antiretroviral therapy may play a substantial role in the regulation of OAT4 in the placenta of women with HIV.

Altered transcript levels of BCRP were seen both in the placenta obtained from the HIV(+) group and in cART-treated placental explants. BCRP is the most abundantly expressed ABC efflux transporter in term placenta and has been shown to have a pronounced influence on fetal accumulation of its substrates. Its substrates include many clinically important drugs as well as endogenous hormones. Although transcript levels of BCRP were significantly decreased in the placenta of women with HIV, there was a trend of increased protein levels. Interestingly, studies have suggested that BCRP is subject to post-translational modifications in response to drugs in order to enhance drug resistance and/or efflux (23). As all of our HIV(+) subjects were therapeutically managed with cART during pregnancy, this may explain the

discrepancy we see between the mRNA and protein levels of BCRP. Furthermore, transporters exhibit sensitivity to the exposure of their substrates, and thus it is also possible that BCRP as well as PGP are upregulated as a result of increased fetal accumulation of endogenous and exogenous compounds. This is corroborated by our findings in human placental explants which demonstrate an upregulation of PGP and BCRP as a result of exposure to the numerous ARVs that were used in the HIV(+) subjects. Likewise, Pfeifer *et al.* reported increased expression of PGP and BCRP in the placentas of hepatitis C-infected women, all of whom were on antiviral therapy (24). Although PGP expression was upregulated, it is unlikely that it is able to fully compensate for the widespread decreases seen within the other transporters tested. It is possible that an upregulation of both PGP and BCRP, while decreasing perinatal HIV transmission, may actually be protective in minimizing fetal exposure to these drugs.

It is well recognized that while ABC and SLC transporters display substrate overlap, they also work in tandem to facilitate the transcellular transfer of substrates across the polarized placental membranes (25–27). Expression of

transporters on either the apical or basolateral membranes enables coordination in the transfer of substrates from maternal to fetal circulation and vice versa. Therefore, it is not only important to look at each transporter individually, but the global picture and widespread dysregulation. We saw decreases in numerous SLC transporters that are expressed at the fetal facing membrane, and with ABC efflux transporters known to be expressed at the maternal facing, this indicates that this population of pregnant women may be at greater risk for fetal accumulation of both endogenous and exogenous substrates. For example, OATs mediate urate uptake from fetus into placental cells at the basolateral surface, while BCRP, found on the apical side, facilitates excretion into the maternal circulation (28). Within our sample pool of HIV(+) individuals, we saw significant downregulation of OAT4 and BCRP transcripts. This could possibly lead to the fetal accumulation of urate, its derivatives, as well as other co-transported substrates. Dysregulation of BCRP and OAT4 placental transporters has also been seen in women with preeclampsia (29), a condition which is associated with high fetal levels of uric acid (30,31). Additionally, while the transplacental transfer of BCRP substrates is often coordinated with OATP2B1 due to their significant substrate overlap, it is important to note that OATP2B1 expression was likewise decreased in the placenta from women with HIV. Therefore an increased placental or fetal accumulation of substrates could result. Despite the presence of ethnic differences between HIV(+) subjects and control subjects in our study, it is unclear whether this would have impacted our findings. To date, associations have not been found between ethnicity or race on mRNA expression of BCRP (32). The Q141K variant, which is associated with reduced BCRP protein but not mRNA expression, is relatively rare in African Americans and thus would not explain the lower transcript and protein expression seen in our HIV(+) group. In general, pharmacogenetics with respect to transporters is still in its infancy and it is unknown whether it may alter the expression of placental drug transporters.

The coordinated efforts of ABC and SLC transporters are also very important in the context of transport and regulation of steroid hormones. Sex steroids as well as their precursors are fundamental in establishing and maintaining a healthy pregnancy. At the same time, multiple hormones and growth factors are involved in the regulation of placental transporters throughout gestation (16,33). The placenta itself is an important contributor of progesterone and estrogens throughout gestation while the estrogen precursors, 16 α -OH-DHEAs and DHEAs, are largely derived from the fetus (34). OATP2B1 and OAT4 facilitate the placental uptake of both estrogen precursors essential for downstream synthesis of estrogens as well as their conjugates. Progesterone, estrone, estradiol, and their sulfate conjugates are synthesized within the placenta and are then transported into the maternal circulation by ABC transporters (15). We found that maternal estradiol levels significantly correlated with the mRNA expression of PGP and MRP1, both of which are involved in the transport of sex steroids and their conjugates. Therefore, the transporter changes that we observed in the HIV(+) subjects and in cART-treated placental explants would likely affect maternal and fetal levels of their steroid substrates. Indeed, it has been shown that women with HIV on protease inhibitor containing regimens have elevated serum levels and increased fetal cord blood concentrations of estradiol and DHEAS concentrations

(19,35). Our data also indicates that within our HIV group, the type of protease inhibitor influences the correlation of maternal estradiol levels and transporter mRNA levels. Moreover, dysregulation of sex hormones due to antiretroviral agents could further impact placental transporters as estrogen and progesterone have been shown to alter expression of transporters such as BCRP and PGP (14). While there are undoubtedly numerous factors influencing the regulation of sex steroids, the widespread dysregulation of transporters on both apical and basolateral membranes cannot be ignored. Although we did not look at the expression of the enzymes involved in steroid synthesis, it is well known that infection, inflammation, as well as protease inhibitors can broadly alter the expression of metabolic enzymes in a wide variety of tissues (36,37). Taken together, these data suggest that women on PI-based cART regimens are potentially at a greater risk for pregnancy complications and adverse fetal outcomes due to suboptimal production, regulation, and transport of placentally derived hormones.

The impact of these changes on neonatal outcomes has yet to be determined. However, our clinical data did show that birth weight was significantly reduced in the HIV(+) group compared with the control group. Furthermore, 32% of the neonates were below the 10th percentile and considered small for gestational age. Indeed, a meta-analysis of cohort studies in HIV patients demonstrated that women with HIV were at higher risk for having low birth weight or premature infants (38). This may be due to factors such as changes in placental transfer of hormones and nutrients due to dysregulation of transporters. However, the impact of ethnicity cannot be ruled out as we had a higher percentage of Black women in the HIV(+) group, and previous studies have reported higher rates of low birth weight within this population (39).

Over the last several decades, it has been well documented that pro-inflammatory cytokines have the ability to modulate the expression of ABC transporters in various tissues including the placenta (10,18,40–44). HIV infection leads to a robust increase in serum levels of pro-inflammatory cytokines (45–47). However, reports on the levels of these cytokines throughout pregnancy in women living with HIV are infrequent as a high percentage of pregnant women with HIV are on fully suppressive cART which can attenuate cytokine production (44). We found that transcript levels of TNF- α and IL-1 β were significantly lower in the HIV(+) group compared with the controls. This was not completely surprising considering the fact that all women in our HIV(+) group were on cART throughout gestation and the majority had undetectable viral loads. Moreover, we found that treatment of placental explants with many ARVs resulted in significant downregulation of TNF- α . Nevertheless, as only placental transcript levels were measured, it remains possible that circulating serum levels of pro-inflammatory cytokines are elevated in the HIV(+) group, thereby influencing transporter regulation. Indeed, previous studies by Osuji *et al.*, found that despite a prolonged treatment with cART, TNF- α and TGF- β remain significantly elevated in patient serum. Interestingly, we found that local expression of IL-8 was significantly upregulated in HIV(+) placentas. IL-8 is a chemokine that plays a role in the migration of white blood cells to sites of infection. This may indicate a degree of immune activation despite adequate viral suppression. On the other hand, IL-8 is known to be increased in oxidative stress situations, which could occur due to either HIV or drug therapy (48,49). However, these changes in the

expression of IL-8 did not significantly correlate with any observed changes in transporters.

It has been well documented that several antiretroviral agents can modulate the expression of drug transporters (7,47,48). Likewise, we found that numerous transporters were dysregulated in human placental explants upon exposure to various ARVs. Protease inhibitors are known to induce PGP, which we saw in the placenta from the HIV(+) group, most of which were on a PI-based regimen, as well as in PI-treated explants. Furthermore, OAT4 was consistently downregulated in explants after treatment with ARVs, as well as in our tissues obtained from women with HIV on PI-based cART. Overall, results obtained from explants can provide some insights and explanations for the changes seen in clinical samples. While drug therapy likely plays a role, these changes more likely stem from a complex interplay of the disease, medications, and other environmental or physiological parameters.

Our findings are not without their limitations as there are inherent challenges in studying the effects of HIV and cART on transporters in a clinical setting. The women in our HIV(+) group were mostly on a PI-based cART regimen, so we are not able to distinguish class effects for ARVs. Thus, many of the conclusions we have drawn are speculative given the broad dysregulation of transporters. Moreover, as cART treatment strategies for pregnant HIV(+) women continue to evolve, these novel therapeutic regimens may differentially regulate placental transporters. Lastly, while our sample size was sufficiently powered, our two groups were not matched for ethnicity (Table I).

CONCLUSION

In summary, our results demonstrate that women with HIV on PI-based cART have altered expression of clinically important drug efflux and uptake transporters in the placenta. To our knowledge, this is the first study demonstrating widespread dysregulation of both ABC and SLC transporters in the placenta from women infected with HIV. These transporters play an important role in maintaining a healthy pregnancy through the uptake and efflux of essential endogenous substrates while also providing necessary expulsion of waste products and harmful agents. As cART use in pregnancy is needed for the health of the mother and to prevent vertical transmission of HIV to the fetus, this population of pregnant women is at greater risk for altered accumulation and/or subtherapeutic levels of ARVs leading to unpredicted fetal outcomes due to altered transplacental pharmacokinetics. As there are over 2 million women of child-bearing age living with HIV worldwide and as transporters are involved in numerous uptake and efflux processes at the placental barrier, understanding the regulation of placental transporters in the context of HIV infection and cART use is important in predicting fetal drug exposure and optimizing therapeutic regimens for safety and management of the disease during pregnancy.

FUNDING

This work was supported by operating grants from the Canadian Institutes of Health Research [MOP 13346, MOP-

130398, PJT-148684]. D.K was a recipient of an Ontario Graduate Scholarship.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest Micheline Piquette-Miller, Ragia H Ghoneim, and Dea Kojovic have no conflicts of interest to disclose pertaining to this project. Lena Serghides reports personal fees from ViiV Healthcare for participation in a Women and Transgender Think Tank.

REFERENCES

1. amfAR :: Statistics: Women and HIV/AIDS :: The Foundation for AIDS Research :: HIV / AIDS Research (n.d.). <https://www.amfar.org/About-HIV-and-AIDS/Facts-and-Stats/Statistics%2D%2DWomen-and-HIV-AIDS/> (accessed July 2, 2020).
2. Pregnant Women Living with HIV Who Have Never Received Antiretroviral Drugs (Antiretroviral Naive) | Recommendations for Use of Antiretroviral Drugs During Pregnancy | Perinatal | AIDSinfo, (n.d.). <https://aidsinfo.nih.gov/guidelines/html/3/perinatal/156/pregnant-women-living-with-hiv-who-have-never-received-antiretroviral-drugs%2D%2Dantiretroviral-naive-> ().
3. Dallmann A, Liu XI, Burckart GJ, den Anker J. Drug transporters expressed in the human placenta and models for studying maternal-fetal drug transfer. *J Clin Pharmacol*. 2019;59:S70–81. <https://doi.org/10.1002/jcph.1491>.
4. Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metab Pharmacokinet*. 2005;20:452–77. <http://www.ncbi.nlm.nih.gov/pubmed/16415531>.
5. Camus M, Deloménie C, Didier N, Faye A, Gil S, Dauge M, et al. Increased expression of MDR1 mRNAs and P-glycoprotein in placentas from HIV-1 infected women. *Placenta*. 2006;27:699–706. <https://doi.org/10.1016/j.placenta.2005.08.001>.
6. Ghoneim RH, Kojovic D, Piquette-Miller M. Impact of endotoxin on the expression of drug transporters in the placenta of HIV-1 transgenic (HIV-Tg) rats. *Eur J Pharm Sci*. 2017;102:94–102. <https://doi.org/10.1016/j.ejps.2017.03.004>.
7. Ghoneim RH, Piquette-Miller M. Endotoxin-mediated downregulation of hepatic drug transporters in HIV-1 transgenic rats. *Drug Metab Dispos*. 2016;44:709–19. <https://doi.org/10.1124/dmd.115.067827>.
8. N.K. Pour, M. Piquette-Miller, Endotoxin modulates the expression of renal drug transporters in HIV-1 transgenic rats, *J. Pharm. Pharm. Sci.* 21 (2018) 117s–129s. doi:<https://doi.org/10.18433/jpps30017>.
9. Kis O, Robillard K, Chan GNY, Bendayan R. The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends Pharmacol Sci*. 2010;31:22–35. <https://doi.org/10.1016/j.tips.2009.10.001>.
10. Alam C, Whyte-Allman SK, Omeragic A, Bendayan R. Role and modulation of drug transporters in HIV-1 therapy. *Adv Drug Deliv Rev*. 2016;103:121–43. <https://doi.org/10.1016/j.addr.2016.05.001>.
11. Petrovic V, Wang J-H, Piquette-Miller M. Effect of endotoxin on the expression of placental drug transporters and glyburide disposition in pregnant rats. *Drug Metab Dispos*. 2008;36:1944–50. <https://doi.org/10.1124/dmd.107.019851.very>.
12. Petrovic V, Piquette-Miller M. Impact of polyinosinic/polycytidylic acid on placental and hepatobiliary drug transporters in pregnant rats. *Drug Metab Dispos*. 2010;38:1760–6. <https://doi.org/10.1124/dmd.110.034470>.
13. Petrovic V, Kojovic D, Cressman A, Piquette-Miller M. Maternal bacterial infections impact expression of drug transporters in human placenta. *Int Immunopharmacol*. 2015;26:349–56. <https://doi.org/10.1016/j.intimp.2015.04.020>.
14. Evseenko DA, Paxton JW, Keelan JA. Independent regulation of apical and basolateral drug transporter expression and function in placental trophoblasts by cytokines, steroids, and

- growth factors. *Drug Metab Dispos.* 2007;35:595–601. <https://doi.org/10.1124/dmd.106.011478>.
15. W. Chatuphonprasert, K. Jarukamjorn, I. Ellinger, Physiology and pathophysiology of steroid biosynthesis, transport and metabolism in the human placenta. *Front. Pharmacol.* 9 (2018). doi:<https://doi.org/10.3389/fphar.2018.01027>.
 16. Coles LD, Lee IJ, Voualalas PJ, Eddington ND. Estradiol and progesterone-mediated regulation of P-gp in P-gp overexpressing cells (NCI-ADR-RES) and placental cells (JAR). *Mol Pharm.* 2009;6:1816–25. <https://doi.org/10.1021/mp900077q>.
 17. Papp E, Mohammadi H, Loutfy MR, Yudin MH, Murphy KE, Walmsley SL, et al. HIV protease inhibitor use during pregnancy is associated with decreased progesterone levels, suggesting a potential mechanism contributing to fetal growth restriction. *J Infect Dis.* 2015;211:10–8. <https://doi.org/10.1093/infdis/jiu393>.
 18. Abualsunun WA, Piquette-Miller M. Involvement of nuclear factor κ B, not Pregnane X receptor, in inflammation-mediated regulation of hepatic transporters. *Drug Metab Dispos.* 2017;45:1077–83. <https://doi.org/10.1124/dmd.117.076927>.
 19. Balogun KA, Guzman Lenis MS, Papp E, Loutfy M, Yudin MH, Macgillivray J, et al. Elevated levels of estradiol in human immunodeficiency virus-infected pregnant women on protease inhibitor-based regimens. *Clin Infect Dis.* n.d.;420:66–427. <https://doi.org/10.1093/cid/cix761>.
 20. Lee N, Duan H, Hebert MF, Liang CJ, Rice KM, Wang J. Taste of a pill: organic cation transporter-3 (OCT3) mediates metformin accumulation and secretion in salivary glands. *J Biol Chem.* 2014;289:27055–64. <https://doi.org/10.1074/jbc.M114.570564>.
 21. Wu X, Kekuda R, Huang W, Fei YJ, Leibach FH, Chen J, et al. Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. *J Biol Chem.* 1998;273:32776–86. <https://doi.org/10.1074/jbc.273.49.32776>.
 22. Hagos Y, Stein D, Ugele B, Burckhardt G, Bahn A. Human renal organic anion transporter 4 operates as an asymmetric urate transporter. *J Am Soc Nephrol.* 2007;18:430–9. <https://doi.org/10.1681/ASN.2006040415>.
 23. Xie Y, Xu K, Linn DE, Yang X, Guo Z, Shimelis H, et al. The 44-kDa Pim-1 kinase phosphorylates BCRP/ABCG2 and thereby promotes its multimerization and drug-resistant activity in human prostate cancer cells. *J Biol Chem.* 2008;283:3349–56. <https://doi.org/10.1074/jbc.M707773200>.
 24. Pfeifer E, Parrott J, Lee GT, Domalakes E, Zhou H, He L, et al. Regulation of human placental drug transporters in HCV infection and their influence on direct acting antiviral medications. *Placenta.* 2018;69:32–9. <https://doi.org/10.1016/j.placenta.2018.07.005>.
 25. Nishikawa M, Iwano H, Yanagisawa R, Koike N, Inoue H, Yokota H. Placental transfer of conjugated bisphenol A and subsequent reactivation in the rat fetus. *Environ Health Perspect.* 2010;118:1196–203. <https://doi.org/10.1289/ehp.0901575>.
 26. Ahmadimoghaddam D, Zemankova L, Nachtigal P, Dolezelova E, Neumanova Z, Cerveny L, et al. Organic cation transporter 3 (OCT3/SLC22A3) and multidrug and toxin extrusion 1 (MATE1/SLC47A1) transporter in the placenta and fetal tissues: expression profile and fetus protective role at different stages of gestation. *Biol Reprod.* 2013;88:55. <https://doi.org/10.1095/biolreprod.112.105064>.
 27. Hemauer SJ, Nanovskaya TN, Abdel-Rahman SZ, Patrikeeva SL, Hankins GDV, Ahmed MS. Modulation of human placental P-glycoprotein expression and activity by MDR1 gene polymorphisms. *Biochem Pharmacol.* 2010;79:921–5. <https://doi.org/10.1016/j.bcp.2009.10.026>.
 28. Wu W, Dnyanmote AV, Nigam SK. Remote communication through solute carriers and ATP binding cassette drug transporter pathways: an update on the remote sensing and signaling hypothesis. *Mol Pharmacol.* 2011;79:795–805. <https://doi.org/10.1124/mol.110.070607>.
 29. Kojovic D, Workewych NV, Piquette-Miller M. Role of elevated SFLT-1 on the regulation of placental transporters in women with pre-eclampsia. *Clin Transl Sci.* 2020;13:580–8. <https://doi.org/10.1111/cts.12742>.
 30. Bainbridge SA, Roberts JM. Uric acid as a pathogenic factor in preeclampsia. *Placenta.* 2008;29:67–72. <https://doi.org/10.1016/j.placenta.2007.11.001>.
 31. Paula LG, Pinheiro da Costa BE, Hentschke MR, Antonello IC, Luz JH, da Cunha Filho EV, et al. Increased proteinuria and uric acid levels are associated with eclamptic crisis. *Pregnancy Hypertens.* 2019;15:93–7. <https://doi.org/10.1016/j.preghy.2018.12.003>.
 32. Bircsak KM, Moscovitz JE, Wen X, Archer F, Yuen PYS, Mohammed M, et al. Interindividual regulation of the breast cancer resistance protein/abcg2 transporter in term human placentas. *Drug Metab Dispos.* 2018;46:619–27. <https://doi.org/10.1124/dmd.117.079228>.
 33. H. Wang, L. Zhou, A. Gupta, R.R. Vethanayagam, Y. Zhang, J.D. Unadkat, Q. Mao, R. Robert, Regulation of BCRP/ABCG2 expression by progesterone and 17 β -estradiol in human placental BeWo cells, (2008) 798–807. doi:<https://doi.org/10.1152/ajpendo.00397.2005>.
 34. Kaludjerovic J, Ward WE, Alexander B. The interplay between estrogen and fetal adrenal cortex. *J Nutr Metab.* 2012;2012:1–12. <https://doi.org/10.1155/2012/837901>.
 35. McDonald CR, Conroy AL, Gamble JL, Papp E, Hawkes M, Olwoch P, et al. Estradiol levels are altered in human immunodeficiency virus-infected pregnant women randomized to efavirenz-versus lopinavir/ritonavir-based antiretroviral therapy. *Clin Infect Dis.* n.d.;428:66–436. <https://doi.org/10.1093/cid/cix772>.
 36. Morgan ET. Impact of infectious and inflammatory disease on cytochrome P450-mediated drug metabolism and pharmacokinetics. *Clin Pharmacol Ther.* 2009;85:434–8. <https://doi.org/10.1038/clpt.2008.302>.
 37. Griffin L, Annaert P, Brouwer KLR. Influence of drug transport proteins on the pharmacokinetics and drug interactions of HIV protease inhibitors. *J Pharm Sci.* 2011;100:3636–54. <https://doi.org/10.1002/jps.22655>.
 38. Xiao PL, Zhou YB, Chen Y, Yang MX, Song XX, Shi Y, et al. Association between maternal HIV infection and low birth weight and prematurity: a meta-analysis of cohort studies. *BMC Pregnancy Childbirth.* 2015;15:246. <https://doi.org/10.1186/s12884-015-0684-z>.
 39. Catov JM, Lee M, Roberts JM, Xu J, Simhan HN. Original contribution race disparities and decreasing birth weight: are all babies getting smaller? *Am J Epidemiol.* 2016;183:15–23. <https://doi.org/10.1093/aje/kwv194>.
 40. D. Kojovic, M. Piquette-Miller, Regulation of drug transporters by inflammation, in: *Drug Metab. Dis.*, Elsevier Inc., 2017: pp. 59–89. doi:<https://doi.org/10.1016/B978-0-12-802949-7.00003-1>.
 41. Abualsunun WA, Piquette-Miller M. STAT3 is involved in IL-6-mediated downregulation of hepatic transporters in mice. *J. Pharm. Pharm. Sci.* 2018;21:325. <https://doi.org/10.18433/jpps30241>.
 42. Y. Lin, K.M. Bircsak, L. Gorczyca, X. Wen, L.M. Aleksunes, Regulation of the placental BCRP transporter by PPAR gamma, *J. Biochem. Mol. Toxicol.* 31 (2017). doi:<https://doi.org/10.1002/jbt.21880>.
 43. Liptrott NJ, Penny M, Bray PG, Sathish J, Khoo SH, Back DJ, et al. The impact of cytokines on the expression of drug transporters, cytochrome P450 enzymes and chemokine receptors in human PBMC. *Br J Pharmacol.* 2009;156:497–508. <https://doi.org/10.1111/j.1476-5381.2008.00050.x>.
 44. Liptrott NJ, Owen A. The role of cytokines in the regulation of drug disposition: extended functional pleiotropism? *Expert Opin Drug Metab Toxicol.* 2011;7:341–52. <https://doi.org/10.1517/17425255.2011.553600>.
 45. Maharaj NR, Phulukdaree A, Nagiah S, Ramkaran P, Tiloke C, Chuturgoon AA. Pro-inflammatory cytokine levels in HIV infected and uninfected pregnant women with and without preeclampsia. *PLoS One.* 2017;12:e0170063. <https://doi.org/10.1371/journal.pone.0170063>.
 46. Roberts L, Passmore JAS, Williamson C, Little F, Bebell LM, Mlisana K, et al. Plasma cytokine levels during acute HIV-1

- infection predict HIV disease progression. *AIDS*. 2010;24:819–31. <https://doi.org/10.1097/QAD.0b013e3283367836>.
47. F.N. Osuji, C.C. Onyenekwe, J.E. Ahaneku, N.R. Ukibe, The effects of highly active antiretroviral therapy on the serum levels of pro-inflammatory and anti-inflammatory cytokines in HIV infected subjects 11 Medical and Health Sciences 1103 Clinical Sciences 11 Medical and Health Sciences 1107 Immunology, *J. Biomed. Sci.* 25 (2018). doi:<https://doi.org/10.1186/s12929-018-0490-9>.
48. Verhasselt V, Goldman M, Willems F. Oxidative stress up-regulates IL-8 and TNF-alpha synthesis by human dendritic cells. *Eur J Immunol.* 1998;28:3886–90. [https://doi.org/10.1002/\(SICI\)1521-4141\(199811\)28:11<3886::AID-IMMU3886>3.0.CO;2-M](https://doi.org/10.1002/(SICI)1521-4141(199811)28:11<3886::AID-IMMU3886>3.0.CO;2-M).
49. Popoola TD, Awodele O. Interplay between antiretroviral therapy and oxidative stress in HIV seropositive patients. *Afr J Med Med Sci.* 2016;45:5–21.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.