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ROLE OF PLACENTAL ATP-BINDING CASSETTE (ABC) TRANSPORTERS IN ANTIRETROVIRAL THERAPY DURING PREGNANCY

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Abstract

Highly Active Anti-Retroviral Therapy (HAART) is used to treat HIV-infected patients and involves administration of multiple antiretroviral drugs acting at different steps of the HIV life cycle. In treating HIV-infected pregnant patients, the aim of therapy is not only to treat the mother but also to prevent the transmission of the virus to the fetus. Among the antiretroviral drugs used, there are differences in the extent of transfer of these drugs across the placenta; HIV protease inhibitors are particularly poorly transferred. Activities of ABC transporters expressed in the human placenta as well as differences in plasma protein binding may account for the poor transplacental transfer of certain drugs. This review discusses factors affecting the extent of placental transfer of antiretroviral drugs during pregnancy. These issues may also apply to drugs in other therapeutic categories.

Keywords

ABC transporters; P-glycoprotein; Multidrug resistance-associated proteins; Placenta; Pregnancy; Transporters; Active transport; Efflux pumps; Pharmacokinetics/pharmacodynamics; Multidrug resistance transporters

INTRODUCTION

The placenta is a complex organ that forms an interface between the maternal and the fetal circulation during the gestation period. It functions in the exchange of nutrients, respiratory gases, and metabolic waste, and is also a source of hormones. Additionally, it may limit fetal exposure to various xenobiotics in the maternal circulation. Drug concentrations achieved in the fetus depend on the maternal drug concentration, kinetics of drug transfer across the placenta, and placental and fetal drug metabolism. The rate and extent of drug transfer depends on physicochemical properties of the drugs and the physiological characteristics of the maternal-placental-fetal unit.¹ The properties of drugs that may affect the extent of placental transfer include their molecular weight, pKa, lipid solubility, and plasma protein binding. Also, several types of influx and efflux transporters are present on the maternal facing (brush-border or apical) as well as the fetal facing (basolateral) side of syncytiotrophoblast, which potentially modulate the drugs intended to treat the mother, the fetus or both. Among them are the ATP Binding Cassette (ABC) transporters, which may limit the passage of some drugs intended for

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maternal or fetal treatment and thus, alter drug exposure. These transporters utilize the energy of ATP hydrolysis to transfer substances out of the cells usually toward maternal circulation.

Another factor that needs to be considered is that pregnancy results in physiologic changes that not only concern the mother but also the fetus and the placenta. Besides changes observed throughout the pregnancy in the cardiovascular system, respiratory system, GI system, kidneys, and the uterus, there are a number of significant changes that occur in the placenta during gestation. Some of these physiological changes are summarized below in Table 1.^{2,3}

Thus, these physiological changes, which may affect all four pharmacokinetic processes, may result in altered drug concentrations during pregnancy. As can be seen from the table above, these changes may also affect the expression of ABC transporters present in the placenta and as a result, may affect the extent of placental drug transfer.

This review discusses the placental transfer of antiretroviral drugs, specifically protease inhibitors. These drugs are known to be substrates for the ABC transporters⁴ and we presently review how these placental transporters affect the transfer of these drugs. We will also discuss the effect of changes during pregnancy on the expression of placental ABC transporters, and how those changes affect the transfer of the antiretroviral drugs. There are several articles reviewing ABC transporters present at the maternal-fetal interface in general.^{1,5} However, this review will focus on P-glycoprotein, Multidrug Resistance Proteins (MRPs), and Breast Cancer Resistant Proteins (BCRP) and their role regarding the transfer of antiretroviral drugs across the placenta.

PLACENTAL ABC TRANSPORTERS

Ceckova-Novotna et al. have reviewed the expression, localization and function of P-glycoprotein (P-gp) in the placenta.⁶ P-gp, encoded by the multi drug resistance 1 (MDR1/ABCB1) gene, is present on the apical (brush-border) syncytiotrophoblast membrane and actively extrudes many substrates, including a wide range of drugs. The efflux of lipophilic or slightly charged compounds from the cell takes place with the utilization of energy from ATP hydrolysis. Rhodamine 123 (Rho123), a well known P-gp substrate, has a higher maternal-to-fetal transplacental passage in the presence of P-gp inhibitors, PSC833, cyclosporine A, quinidine, and chlorpromazine. The fetal-to-maternal passage of Rho123 decreases in the presence of PSC833, cyclosporine A, and quinidine.⁷ These data indicate that the transplacental passage of Rho123 is in part directed by P-gp. Atkinson et al. carried out RT-PCR and western blotting studies in placenta, primary cytotrophoblast cell cultures and BeWo, JAr, and JEG choriocarcinoma cell lines.⁸ They found MDR1 to be absent or expressed minimally in BeWo and JEG cell lines. In the syncytiotrophoblast, P-gp was found to be present predominantly on the apical side (maternal-facing). The low level of MDR1 in BeWo cells they observed was contrary to the higher level observed by Ushigome et al. and Utoguchi et al.^{9,10} Ceckova-Novotna et al. mention that expression of P-gp seemed to differ among particular clones of BeWo cells, as they also observed an undetectable expression of MDR1 mRNA when they used BeWo cells from the same source as Atkinson et al.⁶ Western blotting analysis carried out by Nagashige et al., with human placental membrane vesicles indicated the localization of P-gp on the apical membrane.¹¹ In comparison to ABCB1, MDR3/ABCB4 gene product transports a limited number of substrates. The expression of MDR3 in human placenta has been reported,¹² however, its functional significance in the placenta is unknown. It is reported to be present in the syncytial basolateral membrane and cells lining the fetal capillaries,¹³ and was found to be up regulated four fold in third trimester placenta compared to first trimester,¹² in contrast to MDR1, which is expressed more abundantly during the early gestation.¹⁴

Breast Cancer Resistance Protein (BCRP, ABCG2) confers resistance to hydrophobic/anionic xenobiotics like mitoxantrone, topotecan derivatives, and anthracyclines. It is present on the

apical side of the syncytiotrophoblast and its substrates include a wide variety of anticancer agents, organic cations and lipophilic conjugates.⁵ BCRP is known as a “half-transporter” as it is reported to be composed of just one transmembrane region and one ATP-binding domain compared to other ABC transporters which are composed of two transmembrane regions and two ATP-binding domains.¹⁵ Mao et al. have reviewed the role of BCRP in multi-drug resistance and drug disposition.¹⁶ Functional evidence of Bcrp1 activity in the mouse placenta was established by Jonker et al.¹⁷ They showed that treatment with a BCRP inhibitor GF120918, which also inhibits P-gp, decreases plasma clearance and hepatobiliary excretion of topotecan and increases absorption of this anticancer drug from the small intestine in P-gp knockout mice. In pregnant GF120918 treated P-gp deficient mice, the relative fetal concentration of topotecan was found to be 2-fold higher than that in pregnant vehicle treated mice.¹⁷ The authors explained that topotecan was a weaker substrate for P-gp and suggested the increase in bioavailability was due to inhibition of BCRP by GF120918, resulting in increased intestinal absorption and decreased biliary excretion of the agent. Also, inhibition of BCRP in the placenta may have resulted in higher fetal topotecan concentrations.

Evseenko et al. have reviewed different ABC transporters involved in the active transport across the human placenta.⁵ The MRP family of drug transporters has nine members. They are organic anion transporters; however, MRP1, MRP2, and MRP3 also transport certain neutral drugs. Their substrate range is much narrower compared to P-gp, and are reported to be involved in the efflux of toxic fetal metabolites, in particular unconjugated bilirubin and bile acids. They have also been shown to be involved in the removal of glutathiolated, glucuronidated, or sulfated metabolites from cells. St-Pierre et al. examined if members of the MRP family were expressed in term placenta.¹⁸ Immunofluorescence and immunoblotting studies carried out by the authors showed that MRP2 was localized to the apical syncytiotrophoblast membrane, whereas MRP1 and MRP3 were predominantly expressed in blood vessel endothelia with some evidence for expression in the apical syncytiotrophoblast.¹⁸ However, MRP1 is also reported to be localized on the basolateral side of the placental membrane. Atkinson et al. carried out RT-PCR and western blotting studies in placenta, primary cytotrophoblast cell cultures and BeWo, JAr, and JEG choriocarcinoma cell lines, and found MRP1 to be expressed ubiquitously.⁸ In the syncytiotrophoblast, MRP1 was found to be present on the basolateral side. Western blotting analysis carried out by Nagashige et al., with human placental membrane vesicles indicated the localization of MRP1 on the basolateral membrane.¹¹ Langmann et al. carried out real-time reverse transcription-PCR expression profiling of the complete human ABC transporter superfamily in various tissues and have documented the MRP4, -5, and -7 mRNA expression in the placenta in addition to the above mentioned MRP transporters.¹⁹ Schuetz et al. have investigated if overexpression and amplification of the MRP4 gene was correlated with ATP-dependent efflux of some dideoxynucleosides, which are a common component of Highly Active Anti-Retroviral Therapy.²⁰ The two were found to be correlated and overexpression of MRP4 mRNA and protein severely impaired the antiviral efficacy of the studied drugs. Higher resistance to the studied compounds and amplification of the MRP4 gene was correlated with enhanced drug efflux.²⁰ MRP5 mRNA as well as protein expression in the human placenta has been reported and its functional activity has been measured in the placental basal membrane vesicles and fetal endothelium.²¹ However, localization and physiological role of MRP7 in pregnancy still remains obscure.⁵ Although the MRP family plays important roles in other barrier epithelia, their impact and role in the human placenta is not as clearly established.

REGULATION OF ABC TRANSPORTER EXPRESSION

Expression of placental ABC transporters has been observed to vary with gestational age. In rodents, two genes, *mdr1a* and *mdr1b*, encode for P-gp. The expression of *mdr1a* and *mdr1b* genes was observed on the 11th gestation day and increased with advancing gestation.²² In

contrast, *mdr1a* and *mdr1b* mRNA expression were found to progressively decrease towards term in case of mice.²³ Expression of MDR1 mRNA and protein in human placenta has been observed to decrease with increase in gestational age.²⁴ In a different study, expression of P-gp and BCRP in human placentae of different gestational ages has been reported.²⁵ The authors determined the placental expression of pregnane X receptor (PXR), constitutive androstane receptor, Vitamin D receptor and hepatocyte nuclear factor-4 α which have been reported to affect the expression of P-gp. P-gp expression was found to be significantly higher (44.8-fold as protein; 6.5-fold as mRNA) in early gestational age human placentae (60–90 days) vs. term placentae. They found a decrease in hCG transcription with gestational age which was highly correlated with P-gp transcription. However, the link between hCG and P-gp expression is unknown.

The BCRP expression has also been examined in the human placenta and was highly variable without any gestational age dependence.²⁵ In another study, mRNA levels of BCRP in the human placenta did not change significantly as gestation progressed; however, protein levels increased towards the end of gestation.²⁶ A reduced expression of ABCG2 mRNA was found in the group of term placentae (39 ± 2 weeks) compared to early (28 ± 1 week) or late preterm (35 ± 3 weeks) placentae. A reduction of the protein expression of ABCG2 in the group of human term placentae was also observed.²⁷ It has also been reported that BCRP expression in the human placenta peaks at mid-gestation.^{28,29} These studies use relatively small numbers of tissue samples. The gestational regulation of expression of BCRP has been reviewed,²⁸ and while more studies with higher numbers of tissue samples are required, it appears that BCRP expression is under tight control.

Effect of gestational age on Bcrp1 expression in the mouse placenta has also been investigated.³⁰ Bcrp1 protein levels peaked at gestation day 15 in case of mice (term in mice is approximately 20–21 days). Immunohistochemistry data showed that cellular localization of Bcrp1 in placenta was not influenced by pregnancy. To see if nuclear receptors had an effect on the regulation of Bcrp1 gene during pregnancy, the authors examined the mRNA levels of aryl hydrocarbon receptor (AhR), hypoxia-inducible factor 1 α (HIF1 α), estrogen receptor α (ER α), estrogen receptor β (ER β), or progesterone receptor and compared them with those of Bcrp1. Bcrp1 mRNA was found to be significantly correlated with transcription of AhR, HIF1 α and ER β in placenta, and that expression of these nuclear receptors was gestationally regulated. Thus, the authors concluded that Bcrp1 expression in mouse placental tissues could be dependent upon gestational age. In a different study, Bcrp1 mRNA levels in the placenta of pregnant mice decreased from gestation day 9.5 towards term; however, protein expression of Bcrp1 did not change significantly during gestation.³¹ Rat Bcrp protein levels in the placenta at gestation day 14 were significantly higher compared to those at gestation day 20 (term in rats is approximately 21 days). Therefore, trends in the placental expression of Bcrp may differ among different laboratories, possibly suggesting other factors involved in Bcrp regulation.

In a study by Meyer zu Schwabedissen et al., ABCC2/MRP2 mRNA and protein amounts were found to significantly increase as a function of gestational age in human placenta.³² Therefore, a fetus in late gestation could have decreased exposure to MRP2 substrates. The authors also studied the effect of single nucleotide polymorphisms (C-24T; G1249A, and C3972T) in the MRP2 gene on placental expression. The polymorphism G1249A was found to result in a significantly reduced expression of MRP2 mRNA in preterms.³²

Evseenko et al. studied the effects of cytokines and growth factors on ABC transporter expression and function in primary human placental trophoblast cells.³³ Treatment of trophoblasts with tumor necrosis factor- α (TNF- α) or interleukin (IL)-1 β was observed to decrease mRNA and protein expression of MDR1 and BCRP transporters by 40–50%. IL-6 was found to increase mRNA and protein expression of MDR3 whereas expression of MRP1

was found to be unaltered. Pretreatment over 48h with TNF- α resulted in significant decrease in BCRP efflux activity with minimal changes in MDR1/3 activity. Epidermal growth factor (EGF) and insulin-like growth factor-2 significantly increased mRNA and protein expression of BCRP; EGF was also found to increase BCRP functional activity. A 48-hour exposure to estradiol (100 nM) increased BCRP, MDR1 and MDR3 mRNA and protein expression by 40–60%, however it had no significant effects on MRP1 expression. It was found to significantly decrease the accumulation of [³H]digoxin, a known MDR1/3 substrate, consistent with the MDR1/3 protein up-regulation observed. Progesterone was found to have modest positive effects on mRNA and protein expression of MRP1. The authors concluded that these changes by cytokines and growth factors in placental transporter expression and function (in some cases) may alter disposition of drugs, may alter fetal susceptibility to xenobiotics, and may also have an impact on placental viability and function.

Effects of placentally-secreted steroid sex hormones on ABCG2 mRNA and protein expression have also been investigated using the BeWo cell line, derived from human placental choriocarcinoma.³⁴ Estrone, 17 β -estradiol and estriol were found to induce the expression of ABCG2 mRNA in a concentration- dependent manner where as only 17 β -estradiol was found to induce the expression of ABCG2 protein. In addition, progesterone was found to suppress the induction of ABCG2 expression by 17 β -estradiol. The authors concluded that placental expression of ABCG2 was regulated by sex hormones like estrogen and progesterone which are secreted by the placenta during gestation. In a different study with BeWo cells, ABCG2 protein and mRNA expression were significantly increased approximately two- to three-fold by estriol, human placental lactogen, and human prolactin at physiological concentrations.³⁵ The estrogen receptor antagonist ICI-182,780 abolished the induction of ABCG2 by estriol. In the same study, testosterone did not affect BCRP expression by itself at physiological concentrations; however, testosterone and 17 β -estradiol dosed together increased BCRP mRNA and protein expression. This induction was abolished by ICI-182,780 or the testosterone receptor antagonist flutamide. Human chorionic gonadotropin did not affect BCRP expression at physiological concentrations. In addition, Wang et al showed that progesterone isoform PRB but not PRA was responsible for induced BCRP expression in BeWo cells and also identified a progesterone response element (PRE) in the promoter region of BCRP.³⁶ Meanwhile, Evseenko et al. did not observe any changes in BCRP protein expression in primary trophoblasts incubated with progesterone,³³ possibly due to high previous exposure to progesterone.²⁸ These data elucidate the hormonal regulation patterns of BCRP/Bcrp.

INTERINDIVIDUAL VARIATIONS IN ABC TRANSPORTER EXPRESSION

Interindividual variations observed in drug distribution may be attributed to factors like gender, race, genetics, diet, diseased state and concurrent medications. Interindividual differences have also been reported in ABC transporter activity and expression which may have an effect on pharmacokinetics of drugs.^{37,38} The impact of such differences on pharmacokinetics would depend upon the overall contribution of the affected pathway. Although the placenta may not contribute substantially to maternal pharmacokinetics, it does influence fetal exposure. Thus, variability in placental ABC transporter expression could result in variable fetal exposure.

Tanabe et al. evaluated if mutations in the human MDR1 gene correlate with placental P-gp expression.³⁸ They sequenced the MDR1 cDNA and measured P-gp expression by western blotting in 100 placentae obtained from Japanese women. They reported nine MDR1 single nucleotide polymorphisms (SNPs) in the human placenta. T-129C and G2677 (A, T) were found to be correlated with P-gp expression levels. T-129C (T/C) was associated with significantly lower levels of P-gp than T/T (wild type). Although the difference was not significant, G2677 (A, T) was also associated with lower levels of P-gp in placentae. In a different study, placental P-gp expression was lower when both mother and child were carriers

of 3435T compared to levels obtained for pairs of 3435C.³⁹ Thus, SNPs may influence the pharmacokinetic and pharmacodynamic properties of clinically useful drugs that are P-gp substrates.

Kobayashi et al. studied the contribution of BCRP gene polymorphisms to placental BCRP expression.⁴⁰ The authors carried out RT-PCR and western blotting studies to determine mRNA and protein levels. Of the polymorphisms detected, G34A (Val12Met) and C421A (Gln141Lys) were found to appear commonly in Japanese subjects. C421A variant was found to be widespread both in Japanese as well as Caucasian subjects. The authors observed lower levels of placental BCRP protein in homozygotes for the A421 allele compared to those for the C421 allele, and Japanese subjects had significantly higher frequencies of G34A and C421A than the Caucasian and African American subjects. C376T polymorphism was detected only in Japanese; however its frequency was quite low. The authors also identified various other SNPs that occurred at low frequencies in placenta of the Japanese population, like G1322A and T1465C. In other studies, BCRP SNPs including A616C and A1768T have been reported that are comparatively less frequent.²⁸ Thus, there has been an interindividual variability observed with activity and expression of certain placental transporters and the data suggests that C421A polymorphism could decrease BCRP expression and activity in human placenta, leading to increased fetal drug exposure.²⁸

Although polymorphisms of the ABCC1 gene have been reported, their effect on protein expression and function is a bit uncertain.⁴¹ The G2012T SNP that results in Gly-Val substitution has been reported but any changes in the protein expression or function has not been observed.⁴² The G1299T SNP, on the other hand, is reported to result in an Arg-Ser substitution resulting in a decrease in transport of several organic anions but an increase in doxorubicin resistance.⁴³ The G128C SNP results in a Cys-Ser substitution resulting in a decreased resistance to sodium arsenite and vincristine.⁴⁴ None of the reported SNPs have resulted in a complete loss of expression or function of the protein. They do moderate its substrate affinity though.⁴⁵

Patients with Dubin-Johnson syndrome have mutations in the ABCC2 gene which causes a mild conjugated hyperbilirubinemia. Decreased excretion of these conjugates has been observed in patients with loss of ABCC2 function.⁴¹ In addition, the G1249A polymorphism is reported to cause a significant decrease in placental ABCC2 mRNA expression in preterm placentae.³²

CLINICAL SIGNIFICANCE OF PLACENTAL DRUG TRANSPORTERS

A pregnant woman may take several drugs between conception and birth. The risk-benefit ratio needs to be critically considered before administering a drug to the pregnant woman and complete knowledge of feto-placental disposition should be available. The majority of drugs used in pregnancy are administered to treat the mother; however, the fetus may be the target for some therapies. Maternally directed efflux of these drugs would limit fetal exposure and should be considered with regards to therapeutic goals.

Anticancer drugs are known to be substrates for ABC transporters and have been used to treat pregnant women. *In-vitro* transport studies of doxorubicin, which is a P-gp and MRP2 substrate, have shown no maternal-to-fetal passage of the drug even at high doses.⁴⁶ Roboz et al. reported absence of doxorubicin in amniotic fluid at 20 weeks of gestation, suggesting minimal fetal transfer.⁴⁷

P-gp also effluxes many cardiovascular drugs. Using a placental perfusion model, digoxin has a low fetal transfer ratio of 0.36 ± 0.04 .⁴⁸ Ushigome et al. have shown the involvement of P-gp in the active transport of digoxin using the BeWo cell culture model.⁹ In a different study,

however, P-gp inhibitors, quinidine and verapamil, did not affect the transplacental transfer of digoxin *in vitro* in normal human placentae using the isolated perfused placenta technique.⁴⁹ In spite of these conflicting results published in the literature, it has been suggested that pharmacological inhibition of P-gp may prove beneficial to enhance digoxin availability to the fetus for the treatment of fetal tachycardia.⁵⁰ Transplacental transfer in case of amiodarone is extremely low, with a fetal-to-maternal ratio found to be around 0.01–0.03.⁵¹ This low transplacental transfer may also be due to the drug's high plasma protein binding (96%).

If seizures in pregnancy are left untreated, they may lead to maternal trauma, placental abruption, fetal hypoxia and intracerebral hemorrhage and increased risk of congenital malformations.⁵ Because of such risks, anticonvulsants like valproic acid, phenytoin, phenobarbital and carbamazepine are used during pregnancy to treat epilepsy despite their known teratogenicities. These anticonvulsants are weak substrates of ABC transporters, however, *in-vitro* and *in-vivo* studies have shown that their fetal concentrations are equivalent to maternal levels,⁵² likely due to high permeabilities.

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY DURING PREGNANCY

To treat HIV infected patients, multiple antiretroviral drugs are used in what is known as Highly Active Antiretroviral Therapy (HAART). The aim of the therapy is not only the treatment of maternal HIV infection but also the prevention of viral transmission to the fetus. The use of multiple drugs has led to a significant reduction in occurrence of perinatal transmission to less than 2%, but at the same time increased the chances of short-term toxicity and long-term impact on the mother and the child.⁵³ Because of toxicities observed after use of these antiretroviral drugs in pregnant women, guidelines have been published for use of these drugs in pregnant HIV-infected women (<http://aidsinfo.nih.gov/ContentFiles/PerinatalGL.pdf>).

As mentioned earlier in this review, different physiological changes that occur during pregnancy may also alter the pharmacokinetics of different drugs. In pregnancy, the concentrations of nucleoside and non-nucleoside reverse transcriptase inhibitors may not to change significantly but concentrations of protease inhibitors (PI) are significantly reduced.⁵⁴ As protease inhibitors are an integral part of HAART, lower concentrations may result in treatment failure and poor therapeutic outcomes. Thus, the observed physiological changes during pregnancy may necessitate dose adjustments to achieve therapeutic concentrations in pregnant women. Most of the HIV protease inhibitors are administered with zidovudine, which acts to inhibit excretion mediated by P-gp and metabolism mediated by CYP3A4, thus causing an increase in concentrations of the HIV protease inhibitors.⁵⁵

HIV protease inhibitors, as substrates of ABC transporters, have been the targets of many studies that have been carried out to see the effect of pharmacokinetic changes during pregnancy, on the placental transfer of this category of drugs. The literature shows several cases in which efflux transporters are responsible for low drug concentrations reaching the fetus. For example, a deficiency in mouse placental P-gp has been shown to enhance fetal susceptibility to chemically induced birth defects by avermectins.⁵⁶ Similarly, Smit et al. showed that 2.4-, 7-, or 16-fold more [³H] digoxin, [¹⁴C] saquinavir, or paclitaxel, respectively, entered the P-gp deficient Mdr1a^{-/-}/1b^{-/-} fetuses than entered wild type fetuses in the study carried out in mice.⁵⁷ They also used the P-gp inhibitors PSC833 or GG918 to show that blocking P-gp using these inhibitors resulted in increased transplacental passage of these drugs into the fetus. Molsa et al. observed similar results in studies carried out with human placentae, in which preperfusion with PSC833 increased the maternal-to-fetal transfer of saquinavir by 7.9-fold (0.18% ± 0.09% vs 1.4% ± 0.67%), and preperfusion with GG918 increased it by 6.2-fold (0.18% ± 0.09% vs 1.1% ± 0.39%).⁵⁸ The authors also observed 108-fold higher saquinavir transfer in the fetal-to-maternal direction than from maternal to fetal direction (0.18% ± 0.09%

vs $19.5\% \pm 14.5\%$). PSC833 did not affect saquinavir transfer in the fetal-to-maternal direction ($16.6\% \pm 14.2\%$ vs $19.5\% \pm 14.5\%$), possibly because it was already relatively high. The authors also found that P-glycoprotein expression was correlated with PSC833-induced change in saquinavir transfer, but did not observe ABCB1 polymorphism to affect the PSC833- or GG918-induced change in saquinavir transfer in a small number of samples.⁵⁸

The interaction of HIV protease inhibitors with P-gp has been reported.⁴ At a concentration of $5\text{ }\mu\text{M}$, ritonavir, nelfinavir, and indinavir significantly increased calcein fluorescence in CEM/VBL100 cells in the calcein-AM assay, indicating P-gp was inhibited. However, at a higher concentration of $50\text{ }\mu\text{M}$, saquinavir was also observed to have an effect. This observed increase in calcein fluorescence was probably due to a decreased P-gp activity caused by the HIV protease inhibitors used in the study. The drugs caused a decrease in calcein-AM efflux, which was seen as an increased fluorescence. Thus, all four protease inhibitors studied were found to interact with P-gp with affinities in the order ritonavir > nelfinavir > indinavir > saquinavir.⁴ In the same study, all four drugs were also found to increase the calcein fluorescence in MRP1⁺ CEM/VM-1–5 cells suggesting inhibition of MRP1 by HIV protease inhibitors.⁴

In several different studies, ritonavir, saquinavir, indinavir and nelfinavir were observed to inhibit P-gp.^{4,55,59–63} Ritonavir was the most potent of them. In a study by Drewe et al., ritonavir ($\text{IC}_{50} = 0.2\text{ }\mu\text{M}$) was found to be a more potent P-gp inhibitor than the MDR-reversing agent SDZ PSC833 ($\text{IC}_{50} = 1.13\text{ }\mu\text{M}$). The authors used porcine primary brain capillary endothelial cell monolayers as an *in vitro* system to study the effect of P-gp inhibition on the uptake of saquinavir into the cells. A 5.65-fold higher concentration of SDZ PSC833 was required to inhibit the P-gp mediated extrusion of saquinavir. The authors concluded that administering ritonavir with saquinavir may facilitate the brain uptake of the drug.⁶⁴

Sudhakaran et al. investigated the effect of PSC833, a known P-gp inhibitor, and ritonavir, on the clearance index of indinavir.⁶⁵ They carried out the dual *in-vitro* perfusion of the isolated human placenta and found an increase in clearance index of indinavir between control (0.25 ± 0.03) and PSC833 treatment (0.37 ± 0.14). In contrast, clearance index of indinavir was observed to be unchanged between control (0.34 ± 0.14) and ritonavir treatment (0.39 ± 0.13).⁶⁵ The authors suggested the use of P-gp inhibitors to achieve higher transfer across the placenta for drugs which are substrates of P-gp. However, since indinavir also interacts with MRP1⁴ and MRP2,⁶⁶ it is possible that the above results may have been due to the contribution of other transporters.

Some protease inhibitors have also been reported to be inducers of P-gp expression. Vishnuvardhan et al. reported that acute treatment with lopinavir inhibits P-gp activity; however, on extended exposure lopinavir not only increased the efflux of rhodamine 123 but also the expression of P-gp protein and mRNA.⁶⁷ The authors demonstrated that lopinavir potently inhibited P-gp mediated rhodamine 123 efflux in Caco-2 monolayer cells ($\text{IC}_{50} = 1.7\text{ }\mu\text{M}$). Upon chronic lopinavir exposure (72 hours) in LS180V cells, the content of intracellular rhodamine 123 was reduced by approximately 50%, which indicated increased efflux activity. In these cells, lopinavir induced P-gp mRNA and protein levels up to three-fold in a concentration dependent manner.⁶⁷

The influence of important anti-HIV drugs on BCRP activity *in vitro* has also been determined.⁶⁸ The authors assessed the BCRP inhibition by an increase in pheophorbide A accumulation in MDCKII-BCRP cells and compared it with the corresponding parental cell line MDCKII lacking human BCRP. The authors observed the following IC_{50} values regarding BCRP inhibition by the following anti-HIV drugs: lopinavir ($7.66\text{ }\mu\text{M}$) < nelfinavir ($13.5\text{ }\mu\text{M}$) < delavirdine ($18.7\text{ }\mu\text{M}$) ≤ efavirenz ($20.6\text{ }\mu\text{M}$) < saquinavir ($27.4\text{ }\mu\text{M}$) < atazanavir ($69.1\text{ }\mu\text{M}$) < amprenavir ($181\text{ }\mu\text{M}$) < abacavir ($385\text{ }\mu\text{M}$). Nevirapine and zidovudine were found to have

a weak inhibitory effect. They could not estimate the inhibitory effects of ritonavir and tipranavir because of their low solubility. Other drugs in the study like indinavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zalcitabine, were found not to have an inhibitory BCRP effect.⁶⁸ Gupta et al. investigated the effect of HIV protease inhibitors on BCRP efflux activity by measuring intracellular mitoxantrone fluorescence in human embryonic kidney (HEK) cells stably expressing wild-type BCRP (482R) and its two mutants (482T and 482G).⁶⁹ Ritonavir, saquinavir, and nelfinavir were found to inhibit wild-type BCRP (482R) with IC₅₀ values of $19.5 \pm 0.8 \mu\text{M}$, $19.5 \pm 7.6 \mu\text{M}$, and $12.5 \pm 4.1 \mu\text{M}$, respectively. All the three drugs were observed to inhibit 482T and 482G with IC₅₀ that was found to be twice that observed with 482R. Indinavir and amprenavir were found not to inhibit BCRP activity. In the same study, direct efflux of above mentioned radiolabelled drugs in HEK cells was studied. None of them were found to be substrates of BCRP.⁶⁹

MRP inhibition by indinavir, amprenavir, ritonavir, lamivudine or zidovudine has been investigated.⁷⁰ Of the drugs studied, only ritonavir was found to inhibit the functional activity of MRP-1. The inhibitory effects were studied in UMCC-1/VP cells which over-express MRP-1 and the inhibitory activity was demonstrated by re-sensitization of MRP-1 over-expressing cells to cytotoxic effects of etoposide, a cytotoxic compound.⁷⁰

HIV protease inhibitors transfer poorly across the placenta. Cord/maternal blood concentration ratios of protease inhibitors have been observed to range from 0.01 in case of indinavir to 0.27 in case of amprenavir.^{71,72} In contrast, nucleoside and non-nucleoside reverse transcriptase inhibitors, which are much lesser plasma protein bound compared to protease inhibitors, transfer to a much greater extent. Cord/maternal ratios range from 0.38 for didanosine to 1.32 for stavudine.^{71–76} Similar results were observed in a study by Mirochnick et al.⁷⁷ The authors in the above studies mention the lower placental transfer of protease inhibitors to be due to their efflux by P-gp. However, the drugs are known to be substrates of other ABC transporters as well. Therefore, other transporters in addition to P-gp may also contribute to the lower placental transfer observed with protease inhibitors. Also, the drugs' high plasma protein binding may also contribute, as discussed later in this review.

van Heeswijk et al. studied the pharmacokinetics of nelfinavir and its active metabolite hydroxy-t-butylamidenelfinavir (M8) during pregnancy and post partum in a group of patients.⁷⁸ A 24% reduction in AUC_{0–12} of nelfinavir was observed during third trimester of pregnancy compared with post partum, though this reduction was not found to be statistically significant. The trough concentration (C₁₂) was also reduced by 57% during pregnancy. In addition, AUC of M8 was reduced by 70% in pregnant women. The authors found just 3 out of 11 patients with trough concentrations above the therapeutic nelfinavir concentration during third trimester of pregnancy compared to 6 patients post partum. The authors recommended the adjustment of nelfinavir doses during late pregnancy to maintain therapeutic plasma concentrations. A decrease in exposure with other protease inhibitors in pregnant women has also been observed. For saquinavir⁷⁹ and indinavir,⁸⁰ values for AUC_{0–8h} were 77 % and 68 % lower (respectively), antepartum compared to postpartum. Thus, dosage adjustments in pregnancy may be necessary for other protease inhibitors as well.

Mathias et al. evaluated whether systemic clearance, bioavailability, or plasma protein binding of nelfinavir was altered during pregnancy in mice.⁸¹ The mice replicated the pregnancy related changes observed in the oral nelfinavir disposition in pregnant women. The authors observed a significant decrease in nelfinavir C_{max} and AUC in pregnancy and a significant increase in oral plasma clearance of the drug, irrespective of whether it was normalized to body weight or not. The authors also determined if enhanced activity and/or expression of mouse CYP3A or P-gp or both were responsible for the reduced nelfinavir exposure observed during pregnancy. In contrast to oral administration, AUC and CL after intravenous administration, were not

significantly different in pregnant mice compared to those observed in non-pregnant mice. Bioavailability was significantly reduced in pregnant mice due to increased expression and activity of hepatic CYP3A. On the other hand, intestinal CYP3A as well as hepatic and intestinal P-gp expression were not found to be significantly different among pregnant and non-pregnant mice. Also, the percentage of nelfinavir unbound in plasma was found to be lower in pregnant mice compared to that in non-pregnant mice. This means that the increase in systemic clearance observed in pregnant mice was not due to a decrease in plasma protein binding. The authors concluded that the effect of pregnancy on nelfinavir disposition was due to a change in bioavailability and not due to change in plasma protein binding or systemic clearance of the drug.⁸¹

PLASMA PROTEIN BINDING

Unlike other epithelia separating two fluid compartments (i.e. blood vs intestinal chyme), the placenta separates maternal and fetal blood circulations. The concentrations of drug binding proteins are unequal and dynamic. Although at equilibrium (in the absence of other factors) unbound concentrations should be equivalent, this protein concentration gradient may favor partitioning of total drug on the maternal side for highly plasma protein bound drugs. Studies of drug transfer across the placenta need to differentiate the effects of plasma protein binding from the activity of placental ABC transporters. Specifically, maternal serum albumin concentrations have been found to range from 25 to 35 g/L between 12 and 41 weeks gestation.⁸² Fetal serum albumin ranges from 7.5 to 16 g/L at 12–15 weeks, approaching maternal concentrations by 30 weeks and then exceeding maternal concentrations by 20% after 35 weeks. Maternal serum AAG concentration, on the other hand, ranges from 0.38 to 1.05 g/L. These authors were unable to determine the fetal AAG concentrations before 16 weeks with the technique they used; however, they found the concentration to increase at a constant rate after that but the levels never reached the maternal levels.⁸² Thus, HIV protease inhibitors, being highly plasma protein bound (most >98%), may be affected by the plasma protein concentration changes that occur during the gestation period. Several studies have been carried out taking this into account.

Using an isolated perfused placental cotyledon model in which they perfused a protein-free buffer, Sudhakaran et al. observed a significantly higher transplacental clearance index for indinavir in the fetal-to-maternal direction (0.97 ± 0.12) compared to maternal-to-fetal direction (0.39 ± 0.09).⁸³ Since, the perfusate in the study did not contain human serum albumin, differential protein binding in maternal and fetal perfusate may not account for the differential clearance observed.⁸³ This difference in clearance index may be attributed to P-gp or other ABC transporters. The transplacental clearance index for indinavir determined in this study was similar to that for amprenavir (0.38 ± 0.09 and 0.14 ± 0.08 at peak and trough concentrations, respectively) previously determined.⁸⁴ However, both these determinations were done in the absence of proteins in the perfusate. The study carried out by Forestier et al. involved saquinavir in the presence of human serum albumin at a concentration of 2g/L in the perfusate.⁸⁵ The mean ($\pm$ SD) clearance index was found to be substantially lower (0.05 ± 0.05) in the maternal-to-fetal direction, compared to the freely diffusible marker antipyrine. Because clearance index in the fetal-to-maternal direction was not determined in the studies by Bawdon⁸⁴ and Forestier et al.,⁸⁵ it was difficult to assess whether the drugs' lower clearance index in the maternal-to-fetal direction was due to their binding to the proteins in the perfusate or their efflux by any transporter.

Gavard et al. investigated the placental transfer of lopinavir and ritonavir in an ex vivo human perfused cotyledon model.⁸⁶ The authors found a positive correlation between maternal lopinavir concentration and the clearance index. Placental transfer of lopinavir was found to be higher than other protease inhibitors studied under similar conditions.^{83–85,87} The albumin

concentration had a strong impact on placental transfer of the drugs under study. A reduction in placental transfer for lopinavir as well as ritonavir was observed when the albumin concentration was increased from 2g/L to a physiologic concentration of 40g/L.⁸⁶ Thus, plasma protein binding is an important factor to be considered for placental transfer of lopinavir and ritonavir.

Sudhakaran et al. examined the role of differential protein binding in the transplacental disposition of indinavir and saquinavir.⁸⁸ They measured the extent of drug binding to human serum albumin (HSA) and α_1 -acid glycoprotein (AAG). The concentration ranges they used were 20 to 40 g/L for HSA and 0.20 to 2.00 g/L for AAG. They also estimated the drug binding with varying AAG concentration keeping HSA concentration constant in a protein mixture. Unbound fraction (f_u) of saquinavir decreased with increasing HSA concentration, however, f_u of indinavir changed minimally. With an increase in AAG concentration, f_u for both the drugs decreased. Similar results were obtained with an increase in AAG concentration in the protein mixture. Unbound drug fractions in matched maternal and umbilical cord plasma were also determined to assess if HSA and AAG concentration gradients influenced the extent of binding in matched samples. Unbound fractions of both the drugs in umbilical cord plasma significantly differed from that in maternal plasma (0.36 ± 0.11 in maternal plasma vs 0.53 ± 0.12 in cord plasma for indinavir; 0.0066 ± 0.0039 in maternal plasma vs 0.0090 ± 0.0046 in cord plasma for saquinavir). Thus protein binding of both the drugs was dependent upon protein concentration. The observed difference in plasma protein binding may be due to transplacental AAG concentration gradient, which could contribute to the low cord: maternal total plasma concentration ratios.⁸⁸ We recommend determining unbound concentrations in cord and maternal plasma samples to gain better insight into the processes involved in transplacental drug transfer.

VARIABILITY IN ANTIRETROVIRAL DRUG DISPOSITION

As discussed earlier in the review, there is inter-individual variability reported in the expression of various placental ABC transporters. This section of the review covers various aspects of antiretroviral drugs-ABC transporter interaction and the genotypic differences reported in the literature.

Fellay et al. carried out a pharmacogenetics study to analyse the association between response to antiretroviral treatment and allelic variants of the gene for MDR1, which codes for P-gp, genes coding for CYP3A4, CYP3A5, CYP2D6, and CYP2C19, and of the gene for chemokine receptor CCR5.⁸⁹ The authors determined if the above mentioned genes contributed to drug concentrations and treatment effectiveness of nelfinavir and efavirenz *in-vivo*. In this study, the MDR1 3435 TT genotype was associated with low expression of the MDR1 transcript and P-gp in peripheral blood mononuclear cells and with low plasma drug concentrations. The authors also observed differences in MDR1 3435 genotype distribution in different human populations. According to the study, in caucasians as well as in participants of this study, MDR1 3435 polymorphism showed a 25% TT, 50% CT, and 25% CC distribution. In a different study, frequency of CC genotype was found to be 67–83% in African American people and that of TT genotype was around 2–5% compared to about 20–25% in Caucasians.⁹⁰ As a result, the clinical response and disposition of HIV protease inhibitors are expected to vary in differing human populations.

Effects of protease inhibitors (saquinavir, ritonavir, lopinavir, indinavir, nelfinavir, amprenavir) and non-nucleoside reverse transcriptase inhibitors (efavirenz and nevirapine) on P-gp expression were investigated using peripheral blood mononuclear cells (PBMCs).⁹¹ The authors also compared the effects of each compound between individuals with differing genotypes at position 3435 of exon 26 of MDR1. Nelfinavir and efavirenz, both at 10 μ M,

caused a significant increase in the P-gp expression. The authors did not observe any significant differences in induction between genotypes (CC, CT, and TT). A higher concentration (100 μ M) of the protease inhibitors, except for amprenavir, caused a significant up-regulation of MDR1.⁹¹

Genotypic analysis at two MDR1 loci, C3435T and G2677T, were performed in a multicenter study using DNA from 103 patients on atazanavir or lopinavir as the primary antiretrovirals.⁹² The authors compared median trough concentrations for lopinavir and atazanavir among the groups (CC, CT and TT for C3435T; GG, GT and TT for G2677T) to investigate the influence of MDR1 genotype on the drugs' pharmacokinetics. The C/T and G/T alleles at the MDR1 C3435T and G2677T loci were equally frequent in the Caucasian population, but the wild-type alleles were more prevalent in the African-American population. Additionally, trough plasma concentrations of lopinavir or atazanavir did not correlate with the variant T allele.⁹²

The impact of human MDR1 G1199A polymorphism on P-gp dependent trans-epithelial permeability of HIV protease inhibitors (amprenavir, indinavir, lopinavir, ritonavir, and saquinavir) has been evaluated.⁹³ Recombinant epithelial cells expressing wild-type MDR1 (MDR1_{wt}) or the G1199A variant (MDR1_{1199A}) were used in this study. The authors observed a significantly greater trans-epithelial permeability ratio (basolateral-to-apical transport divided by apical-to-basolateral transport) in MDR1_{1199A} cells compared to MDR1_{wt} cells. For individual drugs, this greater ratio was observed to be 1.7-fold, 1.8-fold, 1.5-fold, 2.8-fold, and 2.1-fold in case of amprenavir, indinavir, lopinavir, ritonavir, and saquinavir, respectively.⁹³ This shows that G1199A polymorphism may affect oral bioavailability of HIV protease inhibitors because of alterations in their trans-epithelial permeability.

The effect of several MDR1 and CYP3A5 polymorphisms on the pharmacokinetic parameters of indinavir in HIV-infected patients has been investigated.⁹⁴ Indinavir pharmacokinetics were studied over a 12-hour interval in patients receiving indinavir alone or together with ritonavir. The authors assessed the genetic polymorphisms by real-time PCR assays and direct sequencing for MDR1 and by PCR-SSCP analysis for CYP3A5. The authors found that the MDR1 C3435T genotype affected the absorption constant of indinavir and suggested the role of P-gp in the drug's pharmacokinetic variability. Ritonavir, being a CYP3A and P-gp inhibitor, may attenuate the pharmacokinetic variability linked to the genetic differences and thus the inter-individual variability of indinavir.⁹⁴

Peripheral blood mononuclear cells from individuals receiving nelfinavir have been used for a comprehensive evaluation of 39 SNPs in MDR1, 7 in ABCC1, 27 in ABCC2, and 16 in ABCG2.⁹⁵ No significant association between cellular nelfinavir AUC and SNPs or haplotypes at ABCC1, ABCC2, ABCG2 was found. Additionally, association with cellular exposure for two loci in strong linkage disequilibrium: MDR1 3435C>T; AUC_{TT}>AUC_{CT}>AUC_{CC} was observed.

Another study by Anderson et al. showed that genetically determined CYP3A5 expressors had 44% faster indinavir oral clearance versus non-expressors.⁹⁶ In the same study, MRP2-24C/T variant carriers were found to have 24% faster indinavir oral clearance. However, no correlation was observed between MRP2 G1249A variant carrier status and pharmacokinetics or pharmacodynamics of indinavir. Lamivudine-triphosphate concentrations were 20% elevated in MRP4 T4131G variant carriers. A trend for elevated zidovudine-triphosphates was also observed in MRP4 G3724A variant carriers. The authors however, did not find any relationship with BCRP.⁹⁶ In a different study of 34 HIV-infected patients treated with HIV protease inhibitors (saquinavir and lopinavir/ritonavir) has described three-fold higher saquinavir concentrations in patients with MRP2 G1249A GG genotype compared with variant carriers.⁹⁷

Acosta et al. observed high variability in indinavir plasma concentrations among patients receiving the same dose.⁹⁸ They found that the patients with undetectable plasma HIV RNA levels had higher indinavir concentrations and lower oral clearance than the patients with detectable plasma HIV RNA. This was attributed to the intersubject differences in CYP metabolic activity that may have been present. Variability has also been observed between children receiving nelfinavir.⁹⁹ High interpatient variability has also been observed with combination antiretroviral therapy. For example, high variation in saquinavir plasma levels was seen in patients receiving saquinavir/ritonavir (1600 mg/100 mg) once daily.¹⁰⁰ In another study, high variation in saquinavir plasma levels was observed when administered alone as well as with ritonavir or nelfinavir.¹⁰¹

Table 2 summarizes the variability in antiretroviral drug disposition discussed in this section. Thus, the observed differences in concentrations achieved in different individuals taking the same dose of an antiretroviral drug may be attributed (in part) to the inter-individual variations observed in the expression of various ABC transporters, as mentioned in the review earlier.

CONCLUSIONS

Based on the interactions of antiretroviral drugs with the placental ABC transporters, we can conclude that amongst the antiretroviral drugs administered during pregnancy, HIV protease inhibitors more than other antiretrovirals interact with placental ABC transporters. Interactions between HIV protease inhibitors and P-gp and BCRP are most established in the literature, though MRPs may also contribute. Plasma protein binding effects must be considered before attributing the differences in cord/maternal plasma total drug concentrations to the activity of placental ABC transporters. Additionally, pharmacogenetic differences may result in interpatient variability in transplacental transfer of antiretroviral drugs. Based on the above factors and considering changes in maternal pharmacokinetics in pregnancy, doses of HIV protease inhibitors may need to be altered during pregnancy in order to achieve optimal maternal and fetal antiretroviral activity and therapeutic outcomes.

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References

1. Syme MR, Paxton JW, Keelan JA. Drug transfer and metabolism by the human placenta. *Clinical pharmacokinetics* 2004;43(8):487–514. [PubMed: 15170365]
2. Anderson GD. Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clinical pharmacokinetics* 2005;44(10):989–1008. [PubMed: 16176115]
3. Loebstein R, Lalkin A, Koren G. Pharmacokinetic changes during pregnancy and their clinical relevance. *Clinical pharmacokinetics* 1997;33(5):328–343. [PubMed: 9391746]
4. Srinivas RV, Middlemas D, Flynn P, Fridland A. Human immunodeficiency virus protease inhibitors serve as substrates for multidrug transporter proteins MDR1 and MRP1 but retain antiviral efficacy in cell lines expressing these transporters. *Antimicrobial agents and chemotherapy* 1998;42(12):3157–3162. [PubMed: 9835508]
5. Evseenko D, Paxton JW, Keelan JA. Active transport across the human placenta: impact on drug efficacy and toxicity. *Expert Opin Drug Metab Toxicol* 2006;2(1):51–69. [PubMed: 16863468]
6. Ceckova-Novotna M, Pavek P, Staud F. P-glycoprotein in the placenta: expression, localization, regulation and function. *Reproductive toxicology* (Elmsford, NY 2006;22(3):400–410.
7. Pavek P, Staud F, Fendrich Z, Sklenarova H, Libra A, Novotna M, Kopecky M, Nobilis M, Semecky V. Examination of the functional activity of P-glycoprotein in the rat placental barrier using rhodamine

123. The Journal of pharmacology and experimental therapeutics 2003;305(3):1239–1250. [PubMed: 12626638]
8. Atkinson DE, Greenwood SL, Sibley CP, Glazier JD, Fairbairn LJ. Role of MDR1 and MRP1 in trophoblast cells, elucidated using retroviral gene transfer. *Am J Physiol Cell Physiol* 2003;285(3):C584–591. [PubMed: 12724138]
9. Ushigome F, Takanaga H, Matsuo H, Yanai S, Tsukimori K, Nakano H, Uchiumi T, Nakamura T, Kuwano M, Ohtani H, Sawada Y. Human placental transport of vinblastine, vincristine, digoxin and progesterone: contribution of P-glycoprotein. *European journal of pharmacology* 2000;408(1):1–10. [PubMed: 11070177]
10. Utoguchi N, Chandorkar GA, Avery M, Audus KL. Functional expression of P-glycoprotein in primary cultures of human cytotrophoblasts and BeWo cells. *Reproductive toxicology* (Elmsford, NY 2000;14(3):217–224.
11. Nagashige M, Ushigome F, Koyabu N, Hirata K, Kawabuchi M, Hirakawa T, Satoh S, Tsukimori K, Nakano H, Uchiumi T, Kuwano M, Ohtani H, Sawada Y. Basal membrane localization of MRP1 in human placental trophoblast. *Placenta* 2003;24(10):951–958. [PubMed: 14580377]
12. Patel P, Weerasekera N, Hitchins M, Boyd CA, Johnston DG, Williamson C. Semi quantitative expression analysis of MDR3, FIC1, BSEP, OATP-A, OATP-C, OATP-D, OATP-E and NTCP gene transcripts in 1st and 3rd trimester human placenta. *Placenta* 2003;24(1):39–44. [PubMed: 12495658]
13. Evseenko DA, Paxton JW, Keelan JA. ABC drug transporter expression and functional activity in trophoblast-like cell lines and differentiating primary trophoblast. *American journal of physiology* 2006;290(5):R1357–1365. [PubMed: 16322349]
14. Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, Gibb W. Expression of the multidrug resistance P-glycoprotein, (ABCB1 glycoprotein) in the human placenta decreases with advancing gestation. *Placenta* 2006;27(6–7):602–609. [PubMed: 16143395]
15. Brangi M, Litman T, Ciotti M, Nishiyama K, Kohlhagen G, Takimoto C, Robey R, Pommier Y, Fojo T, Bates SE. Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. *Cancer research* 1999;59(23):5938–5946. [PubMed: 10606239]
16. Mao Q, Unadkat JD. Role of the breast cancer resistance protein (ABCG2) in drug transport. *The AAPS journal* 2005;7(1):E118–133. [PubMed: 16146333]
17. Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, Schellens JH, Schinkel AH. Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *Journal of the National Cancer Institute* 2000;92(20):1651–1656. [PubMed: 11036110]
18. St-Pierre MV, Serrano MA, Macias RI, Dubs U, Hoechli M, Lauper U, Meier PJ, Marin JJ. Expression of members of the multidrug resistance protein family in human term placenta. *American journal of physiology* 2000;279(4):R1495–1503. [PubMed: 11004020]
19. Langmann T, Maurer R, Zahn A, Moehle C, Probst M, Stremmel W, Schmitz G. Real-time reverse transcription-PCR expression profiling of the complete human ATP-binding cassette transporter superfamily in various tissues. *Clinical chemistry* 2003;49(2):230–238. [PubMed: 12560344]
20. Schuetz JD, Connelly MC, Sun D, Paibir SG, Flynn PM, Srinivas RV, Kumar A, Fridland A. MRP4: A previously unidentified factor in resistance to nucleoside-based antiviral drugs. *Nature medicine* 1999;5(9):1048–1051.
21. Meyer Zu Schwabedissen HE, Grube M, Heydrich B, Linnemann K, Fusch C, Kroemer HK, Jedlitschky G. Expression, localization, and function of MRP5 (ABCC5), a transporter for cyclic nucleotides, in human placenta and cultured human trophoblasts: effects of gestational age and cellular differentiation. *The American journal of pathology* 2005;166(1):39–48. [PubMed: 15631998]
22. Novotna M, Libra A, Kopecky M, Pavek P, Fendrich Z, Semecky V, Staud F. P-glycoprotein expression and distribution in the rat placenta during pregnancy. *Reproductive toxicology* (Elmsford, NY 2004;18(6):785–792.
23. Kalabis GM, Kostaki A, Andrews MH, Petropoulos S, Gibb W, Matthews SG. Multidrug resistance phosphoglycoprotein (ABCB1) in the mouse placenta: fetal protection. *Biology of reproduction* 2005;73(4):591–597. [PubMed: 15917342]

24. Gil S, Saura R, Forestier F, Farinotti R. P-glycoprotein expression of the human placenta during pregnancy. *Placenta* 2005;26(2–3):268–270. [PubMed: 15708129]
25. Mathias AA, Hitti J, Unadkat JD. P-glycoprotein and breast cancer resistance protein expression in human placentae of various gestational ages. *American journal of physiology* 2005;289(4):R963–969. [PubMed: 15961534]
26. Yeboah D, Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, Gibb W. Expression of breast cancer resistance protein (BCRP/ABCG2) in human placenta throughout gestation and at term before and after labor. *Canadian journal of physiology and pharmacology* 2006;84(12):1251–1258. [PubMed: 17487233]
27. Meyer zu Schwabedissen HE, Grube M, Dreisbach A, Jedlitschky G, Meissner K, Linnemann K, Fusch C, Ritter CA, Volker U, Kroemer HK. Epidermal growth factor-mediated activation of the map kinase cascade results in altered expression and function of ABCG2 (BCRP). *Drug metabolism and disposition: the biological fate of chemicals* 2006;34(4):524–533. [PubMed: 16415123]
28. Mao Q. BCRP/ABCG2 in the placenta: expression, function and regulation. *Pharmaceutical research* 2008;25(6):1244–1255. [PubMed: 18202831]
29. Yasuda S, Itagaki S, Hirano T, Iseki K. Expression level of ABCG2 in the placenta decreases from the mid stage to the end of gestation. *Bioscience, biotechnology, and biochemistry* 2005;69(10):1871–1876.
30. Wang H, Wu X, Hudkins K, Mikheev A, Zhang H, Gupta A, Unadkat JD, Mao Q. Expression of the breast cancer resistance protein (Bcrp1/Abcg2) in tissues from pregnant mice: effects of pregnancy and correlations with nuclear receptors. *Am J Physiol Endocrinol Metab* 2006;291(6):E1295–1304. [PubMed: 17082346]
31. Kalabis GM, Petropoulos S, Gibb W, Matthews SG. Breast Cancer Resistance Protein (Bcrp1/Abcg2) in Mouse Placenta and Yolk Sac: Ontogeny and its Regulation by Progesterone. *Placenta*. 2007
32. Meyer zu Schwabedissen HE, Jedlitschky G, Gratz M, Haenisch S, Linnemann K, Fusch C, Cascorbi I, Kroemer HK. Variable expression of MRP2 (ABCC2) in human placenta: influence of gestational age and cellular differentiation. *Drug metabolism and disposition: the biological fate of chemicals* 2005;33(7):896–904. [PubMed: 15821043]
33. 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(4):595–601. [PubMed: 17237156]
34. Yasuda S, Itagaki S, Hirano T, Iseki K. Effects of sex hormones on regulation of ABCG2 expression in the placental cell line BeWo. *J Pharm Pharm Sci* 2006;9(1):133–139. [PubMed: 16849015]
35. Wang H, Unadkat JD, Mao Q. Hormonal regulation of BCRP expression in human placental BeWo cells. *Pharmaceutical research* 2008;25(2):444–452. [PubMed: 17823853]
36. Wang H, Lee EW, Zhou L, Leung PC, Ross DD, Unadkat JD, Mao Q. Progesterone receptor (PR) isoforms PRA and PRB differentially regulate expression of the breast cancer resistance protein in human placental choriocarcinoma BeWo cells. *Molecular pharmacology* 2008;73(3):845–854. [PubMed: 18042733]
37. Beringer PM, Slaughter RL. Transporters and their impact on drug disposition. *The Annals of pharmacotherapy* 2005;39(6):1097–1108. [PubMed: 15886292]
38. Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, Takahashi M, Kurata Y, Kigawa J, Higuchi S, Terakawa N, Otsubo K. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *The Journal of pharmacology and experimental therapeutics* 2001;297(3):1137–1143. [PubMed: 11356939]
39. Hitzl M, Schaeffeler E, Hoche B, Slowinski T, Halle H, Eichelbaum M, Kaufmann P, Fritz P, Fromm MF, Schwab M. Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). *Pharmacogenetics* 2004;14(5):309–318. [PubMed: 15115917]
40. Kobayashi D, Ieiri I, Hirota T, Takane H, Maegawa S, Kigawa J, Suzuki H, Nanba E, Oshimura M, Terakawa N, Otsubo K, Mine K, Sugiyama Y. Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug metabolism and disposition: the biological fate of chemicals* 2005;33(1):94–101. [PubMed: 15475413]

41. Conseil G, Deeley RG, Cole SP. Polymorphisms of MRP1 (ABCC1) and related ATP-dependent drug transporters. *Pharmacogenetics and genomics* 2005;15(8):523–533. [PubMed: 16006996]
42. Conrad S, Kauffmann HM, Ito K, Deeley RG, Cole SP, Schrenk D. Identification of human multidrug resistance protein 1 (MRP1) mutations and characterization of a G671V substitution. *Journal of human genetics* 2001;46(11):656–663. [PubMed: 11721885]
43. Conrad S, Kauffmann HM, Ito K, Leslie EM, Deeley RG, Schrenk D, Cole SP. A naturally occurring mutation in MRP1 results in a selective decrease in organic anion transport and in increased doxorubicin resistance. *Pharmacogenetics* 2002;12(4):321–330. [PubMed: 12042670]
44. Leslie EM, Letourneau JJ, Deeley RG, Cole SP. Functional and structural consequences of cysteine substitutions in the NH2 proximal region of the human multidrug resistance protein 1 (MRP1/ABCC1). *Biochemistry* 2003;42(18):5214–5224. [PubMed: 12731862]
45. Atkinson DE, Brice-Bennett S, D'Souza SW. Antiepileptic medication during pregnancy: does fetal genotype affect outcome? *Pediatric research* 2007;62(2):120–127. [PubMed: 17597651]
46. Grohard P, Akbaraly JP, Saux MC, Gimenez S, Robert J, Brachet-Liermain A, Leng JJ. Transplacental passage of doxorubicin. *Journal de gynécologie, obstétrique et biologie de la reproduction* 1989;18(5):595–600.
47. Roboz J, Gleicher N, Wu K, Chanihian P, Kerenyi T, Holland J. Does doxorubicin cross the placenta? *Lancet* 1979;2(8156–8157):1382–1383. [PubMed: 92735]
48. Derewlany LO, Leeder JS, Kumar R, Radde IC, Knie B, Koren G. The transport of digoxin across the perfused human placental lobule. *The Journal of pharmacology and experimental therapeutics* 1991;256(3):1107–1111. [PubMed: 2005575]
49. Holcberg G, Sapir O, Tsadkin M, Huleihel M, Lazer S, Katz M, Mazor M, Ben-Zvi Z. Lack of interaction of digoxin and P-glycoprotein inhibitors, quinidine and verapamil in human placenta in vitro. *European journal of obstetrics, gynecology, and reproductive biology* 2003;109(2):133–137.
50. Ito S. Transplacental treatment of fetal tachycardia: implications of drug transporting proteins in placenta. *Seminars in perinatology* 2001;25(3):196–201. [PubMed: 11453617]
51. Schmolling J, Renke K, Richter O, Pfeiffer K, Schlebusch H, Holler T. Digoxin, flecainide, and amiodarone transfer across the placenta and the effects of an elevated umbilical venous pressure on the transfer rate. *Therapeutic drug monitoring* 2000;22(5):582–588. [PubMed: 11034264]
52. Myllynen P, Pienimäki P, Vahakangas K. Human placental perfusion method in the assessment of transplacental passage of antiepileptic drugs. *Toxicology and applied pharmacology* 2005;207(2 Suppl):489–494. [PubMed: 16023691]
53. Watts DH. Treating HIV during pregnancy: an update on safety issues. *Drug Saf* 2006;29(6):467–490. [PubMed: 16752931]
54. Marzolini C, Kim RB. Placental transfer of antiretroviral drugs. *Clinical pharmacology and therapeutics* 2005;78(2):118–122. [PubMed: 16084846]
55. Huisman MT, Smit JW, Wiltshire HR, Hoetelmans RM, Beijnen JH, Schinkel AH. P-glycoprotein limits oral availability, brain, and fetal penetration of saquinavir even with high doses of ritonavir. *Molecular pharmacology* 2001;59(4):806–813. [PubMed: 11259625]
56. Lankas GR, Wise LD, Cartwright ME, Pippert T, Umbenhauer DR. Placental P-glycoprotein deficiency enhances susceptibility to chemically induced birth defects in mice. *Reproductive toxicology* (Elmsford, NY) 1998;12(4):457–463.
57. Smit JW, Huisman MT, van Tellingen O, Wiltshire HR, Schinkel AH. Absence or pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. *The Journal of clinical investigation* 1999;104(10):1441–1447. [PubMed: 10562306]
58. Molsa M, Heikkinen T, Hakola J, Hakala K, Wallerman O, Wadelius M, Wadelius C, Laine K. Functional role of P-glycoprotein in the human blood-placental barrier. *Clinical pharmacology and therapeutics* 2005;78(2):123–131. [PubMed: 16084847]
59. Choo EF, Leake B, Wandel C, Imamura H, Wood AJ, Wilkinson GR, Kim RB. Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. *Drug metabolism and disposition: the biological fate of chemicals* 2000;28(6):655–660. [PubMed: 10820137]

60. Gutmann H, Fricker G, Drewe J, Toeroek M, Miller DS. Interactions of HIV protease inhibitors with ATP-dependent drug export proteins. *Molecular pharmacology* 1999;56(2):383–389. [PubMed: 10419558]
61. Miller DS, Nobmann SN, Gutmann H, Toeroek M, Drewe J, Fricker G. Xenobiotic transport across isolated brain microvessels studied by confocal microscopy. *Molecular pharmacology* 2000;58(6):1357–1367. [PubMed: 11093774]
62. Profit L, Eagling VA, Back DJ. Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors. *AIDS (London, England)* 1999;13(13):1623–1627.
63. Washington CB, Wiltshire HR, Man M, Moy T, Harris SR, Worth E, Weigl P, Liang Z, Hall D, Marriott L, Blaschke TF. The disposition of saquinavir in normal and P-glycoprotein deficient mice, rats, and in cultured cells. *Drug metabolism and disposition: the biological fate of chemicals* 2000;28(9):1058–1062. [PubMed: 10950849]
64. Drewe J, Gutmann H, Fricker G, Torok M, Beglinger C, Huwyler J. HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833. *Biochemical pharmacology* 1999;57(10):1147–1152. [PubMed: 11230802]
65. Sudhakaran S, Rayner CR, Li J, Kong DC, Gude NM, Nation RL. Inhibition of placental P-glycoprotein: impact on indinavir transfer to the foetus. *British journal of clinical pharmacology* 2008;65(5):667–673. [PubMed: 18093255]
66. Huisman MT, Smit JW, Crommentuyn KM, Zelcer N, Wiltshire HR, Beijnen JH, Schinkel AH. Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. *Aids* 2002;16(17):2295–2301. [PubMed: 12441801]
67. Vishnuvardhan D, Moltke LL, Richert C, Greenblatt DJ. Lopinavir: acute exposure inhibits P-glycoprotein; extended exposure induces P-glycoprotein. *AIDS (London, England)* 2003;17(7):1092–1094.
68. Weiss J, Rose J, Storch CH, Ketabi-Kiyanvash N, Sauer A, Haefeli WE, Efferth T. Modulation of human BCRP (ABCG2) activity by anti-HIV drugs. *The Journal of antimicrobial chemotherapy* 2007;59(2):238–245. [PubMed: 17202245]
69. Gupta A, Zhang Y, Unadkat JD, Mao Q. HIV protease inhibitors are inhibitors but not substrates of the human breast cancer resistance protein (BCRP/ABCG2). *The Journal of pharmacology and experimental therapeutics* 2004;310(1):334–341. [PubMed: 15007102]
70. Olson DP, Scadden DT, D'Aquila RT, De Pasquale MP. The protease inhibitor ritonavir inhibits the functional activity of the multidrug resistance related-protein 1 (MRP-1). *AIDS (London, England)* 2002;16(13):1743–1747.
71. Chappuy H, Treluyer JM, Rey E, Dimet J, Fouche M, Firtion G, Pons G, Mandelbrot L. Maternal-fetal transfer and amniotic fluid accumulation of protease inhibitors in pregnant women who are infected with human immunodeficiency virus. *American journal of obstetrics and gynecology* 2004;191(2):558–562. [PubMed: 15343237]
72. Marzolini C, Rudin C, Decosterd LA, Telenti A, Schreyer A, Biollaz J, Buclin T. Transplacental passage of protease inhibitors at delivery. *AIDS (London, England)* 2002;16(6):889–893.
73. Chappuy H, Treluyer JM, Jullien V, Dimet J, Rey E, Fouche M, Firtion G, Pons G, Mandelbrot L. Maternal-fetal transfer and amniotic fluid accumulation of nucleoside analogue reverse transcriptase inhibitors in human immunodeficiency virus-infected pregnant women. *Antimicrobial agents and chemotherapy* 2004;48(11):4332–4336. [PubMed: 15504861]
74. Mandelbrot L, Peytavin G, Firtion G, Farinotti R. Maternal-fetal transfer and amniotic fluid accumulation of lamivudine in human immunodeficiency virus-infected pregnant women. *American journal of obstetrics and gynecology* 2001;184(2):153–158. [PubMed: 11174495]
75. Moodley J, Moodley D, Pillay K, Coovadia H, Saba J, van Leeuwen R, Goodwin C, Harrigan PR, Moore KH, Stone C, Plumb R, Johnson MA. Pharmacokinetics and antiretroviral activity of lamivudine alone or when coadministered with zidovudine in human immunodeficiency virus type 1-infected pregnant women and their offspring. *The Journal of infectious diseases* 1998;178(5):1327–1333. [PubMed: 9780252]

76. Watts DH, Brown ZA, Tartaglione T, Burchett SK, Opheim K, Coombs R, Corey L. Pharmacokinetic disposition of zidovudine during pregnancy. *The Journal of infectious diseases* 1991;163(2):226–232. [PubMed: 1988507]
77. Mirochnick M, Dorenbaum A, Holland D, Cunningham-Schrader B, Cunningham C, Gelber R, Mofenson L, Culnane M, Connor J, Sullivan JL. Concentrations of protease inhibitors in cord blood after in utero exposure. *The Pediatric infectious disease journal* 2002;21(9):835–838. [PubMed: 12352805]
78. van Heeswijk RP, Khaliq Y, Gallicano KD, Bourbeau M, Seguin I, Phillips EJ, Cameron DW. The pharmacokinetics of nelfinavir and M8 during pregnancy and post partum. *Clinical pharmacology and therapeutics* 2004;76(6):588–597. [PubMed: 15592330]
79. Acosta EP, Zorrilla C, Van Dyke R, Bardeguez A, Smith E, Hughes M, Huang S, Pitt J, Watts H, Mofenson L. Pharmacokinetics of saquinavir-SGC in HIV-infected pregnant women. *HIV clinical trials* 2001;2(6):460–465. [PubMed: 11742433]
80. Unadkat JD, Wara DW, Hughes MD, Mathias AA, Holland DT, Paul ME, Connor J, Huang S, Nguyen BY, Watts DH, Mofenson LM, Smith E, Deutsch P, Kaiser KA, Tuomala RE. Pharmacokinetics and safety of indinavir in human immunodeficiency virus-infected pregnant women. *Antimicrobial agents and chemotherapy* 2007;51(2):783–786. [PubMed: 17158945]
81. Mathias AA, Maggio-Price L, Lai Y, Gupta A, Unadkat JD. Changes in pharmacokinetics of anti-HIV protease inhibitors during pregnancy: the role of CYP3A and P-glycoprotein. *The Journal of pharmacology and experimental therapeutics* 2006;316(3):1202–1209. [PubMed: 16293714]
82. Krauer B, Dayer P, Anner R. Changes in serum albumin and alpha 1-acid glycoprotein concentrations during pregnancy: an analysis of fetal-maternal pairs. *British journal of obstetrics and gynaecology* 1984;91(9):875–881. [PubMed: 6477846]
83. Sudhakaran S, Ghabrial H, Nation RL, Kong DC, Gude NM, Angus PW, Rayner CR. Differential bidirectional transfer of indinavir in the isolated perfused human placenta. *Antimicrobial agents and chemotherapy* 2005;49(3):1023–1028. [PubMed: 15728898]
84. Bawdon RE. The ex vivo human placental transfer of the anti-HIV nucleoside inhibitor abacavir and the protease inhibitor amprenavir. *Infectious diseases in obstetrics and gynecology* 1998;6(6):244–246. [PubMed: 9972485]
85. Forestier F, de Renty P, Peytavin G, Dohin E, Farinotti R, Mandelbrot L. Maternal-fetal transfer of saquinavir studied in the ex vivo placental perfusion model. *American journal of obstetrics and gynecology* 2001;185(1):178–181. [PubMed: 11483925]
86. Gavard L, Gil S, Peytavin G, Ceccaldi PF, Ferreira C, Farinotti R, Mandelbrot L. Placental transfer of lopinavir/ritonavir in the ex vivo human cotyledon perfusion model. *American journal of obstetrics and gynecology* 2006;195(1):296–301. [PubMed: 16678781]
87. Casey BM, Bawdon RE. Placental transfer of ritonavir with zidovudine in the ex vivo placental perfusion model. *American journal of obstetrics and gynecology* 1998;179(3 Pt 1):758–761. [PubMed: 9757985]
88. Sudhakaran S, Rayner CR, Li J, Kong DC, Gude NM, Nation RL. Differential protein binding of indinavir and saquinavir in matched maternal and umbilical cord plasma. *British journal of clinical pharmacology* 2006;63(3):315–321. [PubMed: 16981898]
89. Fellay J, Marzolini C, Meaden ER, Back DJ, Buclin T, Chave JP, Decosterd LA, Furrer H, Opravil M, Pantaleo G, Retelska D, Ruiz L, Schinkel AH, Vernazza P, Eap CB, Telenti A. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002;359(9300):30–36. [PubMed: 11809184]
90. Schaeffeler E, Eichelbaum M, Brinkmann U, Penger A, Asante-Poku S, Zanger UM, Schwab M. Frequency of C3435T polymorphism of MDR1 gene in African people. *Lancet* 2001;358(9279):383–384. [PubMed: 11502320]
91. Chandler B, Almond L, Ford J, Owen A, Hoggard P, Khoo S, Back D. The effects of protease inhibitors and nonnucleoside reverse transcriptase inhibitors on p-glycoprotein expression in peripheral blood mononuclear cells in vitro. *Journal of acquired immune deficiency syndromes (1999)* 2003;33(5):551–556. [PubMed: 12902797]
92. Ma Q, Brazeau D, Zingman BS, Reichman RC, Fischl MA, Gripshover BM, Venuto CS, Slish JC, DiFrancesco R, Forrest A, Morse GD. Multidrug resistance 1 polymorphisms and trough

- concentrations of atazanavir and lopinavir in patients with HIV. *Pharmacogenomics* 2007;8(3):227–235. [PubMed: 17324111]
93. Woodahl EL, Yang Z, Bui T, Shen DD, Ho RJ. MDR1 G1199A polymorphism alters permeability of HIV protease inhibitors across P-glycoprotein-expressing epithelial cells. *AIDS (London, England)* 2005;19(15):1617–1625.
94. Solas C, Simon N, Drogoul MP, Quaranta S, Frixon-Marin V, Bourgarel-Rey V, Brunet C, Gastaut JA, Durand A, Lacarelle B, Poizot-Martin I. Minimal effect of MDR1 and CYP3A5 genetic polymorphisms on the pharmacokinetics of indinavir in HIV-infected patients. *British journal of clinical pharmacology* 2007;64(3):353–362. [PubMed: 17517050]
95. Colombo S, Soranzo N, Rotger M, Sprenger R, Bleiber G, Furrer H, Buclin T, Goldstein D, Decosterd L, Telenti A. Influence of ABCB1, ABCC1, ABCC2, and ABCG2 haplotypes on the cellular exposure of nelfinavir in vivo. *Pharmacogenetics and genomics* 2005;15(9):599–608. [PubMed: 16041239]
96. Anderson PL, Lamba J, Aquilante CL, Schuetz E, Fletcher CV. Pharmacogenetic characteristics of indinavir, zidovudine, and lamivudine therapy in HIV-infected adults: a pilot study. *Journal of acquired immune deficiency syndromes (1999)* 2006;42(4):441–449. [PubMed: 16791115]
97. Kruse, G.; Staszewski, S.; Cascorbi, I.; Breske, A.; Moecklinghoff, C.; Stocker, H. Mutations in the drug transporter genes MDR1 and MRP2 and pharmacokinetics in patients treated with saquinavir/lopinavir. 4th International Workshop on Clinical Pharmacology of HIV Therapy; Cannes, France. 2003.
98. Acosta EP, Henry K, Baken L, Page LM, Fletcher CV. Indinavir concentrations and antiviral effect. *Pharmacotherapy* 1999;19(6):708–712. [PubMed: 10391416]
99. Acosta, EP.; Nachman, S.; Wiznia, A.; Turner, M.; Sommadossi, JP.; Yogev, R. Pharmacokinetic (PK) Evaluation of Nelfinavir (NFV) in Combination with Nevirapine (NVP) or Ritonavir (RTV) in HIV-Infected Children - PACTG 403. *Intersci Conf Antimicrob Agents Chemother ed., Univ. of Alabama at Birmingham; Birmingham, AL.* 2000.
100. Montaner JS, Schutz M, Schwartz R, Jayaweera DT, Burnside AF, Walmsley S, Saag MS. Efficacy, safety and pharmacokinetics of once-daily saquinavir soft-gelatin capsule/ritonavir in antiretroviral-naïve, HIV-infected patients. *MedGenMed* 2006;8(2):36. [PubMed: 16926775]
101. Langmann P, Zilly M, Weissbrich B, Schlor C, Vath T, Richter E, Klinker H. Therapeutic drug monitoring of saquinavir in patients during protease inhibitor therapy with saquinavir alone or in combination with ritonavir or nelfinavir. *European journal of medical research* 2000;5(2):59–62. [PubMed: 10720564]

Table 1

Effect of physiological changes observed during pregnancy on pharmacokinetics, in general.^{2–4}

PHARMACOKINETIC CHANGES OBSERVED DURING PREGNANCY	
ABSORPTION	<ul style="list-style-type: none">• Reduction in gastric acid secretion causes an increase in gastric pH• Decrease in intestinal motility causes an increase in gastric emptying time and intestinal transit time• Nausea and vomiting, which are pronounced during the earlier stages of pregnancy, may also affect drug absorption
DISTRIBUTION	<ul style="list-style-type: none">• Total body water, plasma volume and body fat stores also increase and may affect drug distribution• Protein concentrations also change during pregnancy. Albumin concentration is reported to decrease whereas change in concentration of α-acid glycoprotein is equivocal.• Volume of distribution increases which causes a decrease in peak drug concentrations
METABOLISM	<ul style="list-style-type: none">• Estrogen and progesterone levels increase, which may affect the activity and expression of many metabolizing enzymes. Activity of CYP3A4, 2D6, 2C9 and 2A6 increases whereas that of CYP1A2 and 2C19 decreases during pregnancy
EXCRETION	<ul style="list-style-type: none">• Renal plasma flow and glomerular filtration rate increase, which increases the renal clearance of a drug• Activity and expression of different transporters are also altered

Table 2

Variability in Antiretroviral Drug Disposition⁸⁹⁻⁹⁷

Drug	Gene (Protein)	Single nucleotide polymorphism (SNP) studied	Frequency of genotype	Effect observed	Experimental model used
Lopinavir	MDR1 (P-gp) MDR1 (P-gp) MDR1 (P-gp)	C3435T C3435T, G2677T G1199A	Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT). ⁹⁴ C/T, G/T alleles at the MDR1 C3435T and G2677T loci - equally frequent in Caucasians; wild-type alleles - more prevalent in African-Americans. ⁹⁵	Increased P-gp expression. ⁹⁴ Trough drug plasma concentrations did not correlate with the variant T allele. ⁹⁵ Significantly lower trans-epithelial permeability ratio in cells expressing wild-type MDR1 cells compared to G1199A variant. ⁹⁶	Humans, Peripheral Blood Mononuclear Cells Humans Recombinant epithelial cells expressing wild-type MDR1 or G1199A variant
Ritonavir	MDR1 (P-gp) MDR1 (P-gp)	C3435T G1199A	Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT). ⁹⁴	Increased P-gp expression. ⁹⁴ Significantly lower trans-epithelial permeability ratio in cells expressing wild-type MDR1 cells compared to G1199A variant. ⁹⁶	Humans, Peripheral Blood Mononuclear Cells Recombinant epithelial cells expressing wild-type MDR1 or G1199A variant
Nelfinavir	MDR1 (P-gp) MDR1 (P-gp) MDR1 (P-gp), ABCC1 (MRP1), ABCC2 (MRP2), ABCG2 (BCRP)	C3435T C3435T A comprehensive evaluation of 39 SNPs in MDR1, 7 in ABCC1, 27 in ABCC2, and 16 in ABCG2	25% TT, 50% CT, 25% CC in Caucasians. ⁹² 67-83% CC, 2-5% TT in African-Americans. ⁹³ Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT). ⁹⁴	MDR1 3435 TT genotype associated with low P-gp expression and low plasma drug concentrations. ⁹² Increased P-gp expression. ⁹⁴ No significant association between cellular nelfinavir AUC and SNPs or haplotypes at ABCC1, ABCG2, ABCG2. Association with cellular exposure for two loci in strong linkage disequilibrium: MDR1 3435C>T; AUC _{TT} >AUC _{CT} >AUC _{CC} . ⁹⁸	Humans, Peripheral Blood Mononuclear Cells Humans, Peripheral Blood Mononuclear Cells peripheral blood mononuclear cells from individuals receiving nelfinavir
Indinavir	MDR1 (P-gp) MDR1 (P-gp) MDR1 (P-gp) ABCC2 (MRP2) ABCC2 (MRP2)	C3435T G1199A C3435T -24C/T G1249A	Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT). ⁹⁴	Increased P-gp expression. ⁹⁴ Significantly lower trans-epithelial permeability ratio in cells expressing wild-type MDR1 cells compared to G1199A variant. ⁹⁶ The genotype affected the absorption constant of indinavir. ⁹⁷ MRP2-24C/T variant carriers had 24% faster indinavir oral clearance. ⁹⁹ No correlation observed between G1249A variant carrier status and pharmacokinetics or pharmacodynamics of indinavir. ⁹⁹	Humans, Peripheral Blood Mononuclear Cells Recombinant epithelial cells expressing wild-type MDR1 or G1199A variant. Humans (HIV-infected patients) Humans
Saquinavir	MDR1 (P-gp) MDR1 (P-gp) ABCC2 (MRP2)	C3435T G1199A G1249A	Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT). ⁹⁴	Increased P-gp expression. ⁹⁴ Significantly lower trans-epithelial permeability ratio in cells expressing wild-type MDR1 cells compared to G1199A variant. ⁹⁶ Three-fold higher drug concentrations in patients with MRP2 G1249A GG genotype compared to variant carriers. ¹⁰⁰	Humans, Peripheral Blood Mononuclear Cells Recombinant epithelial cells expressing wild-type MDR1 or G1199A variant. Humans (HIV-infected patients)

Drug	Gene (Protein)	Single nucleotide polymorphism (SNP) studied	Frequency of genotype	Effect observed	Experimental model used
Ampranavir	MDR1 (P-gp) MDR1 (P-gp)	C3435T G1199A	Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT). ⁹⁴	Increased P-gp expression not observed even at a higher concentration of 100μM. ⁹⁴ Significantly lower trans-epithelial permeability ratio in cells expressing wild-type MDR1 compared to G1199A variant. ⁹⁶	Humans, Peripheral Blood Mononuclear Cells Recombinant epithelial cells expressing wild-type MDR1 or G1199A variant
Atazanavir	MDR1 (P-gp)	C3435T, G2677T	C/T, G/T alleles at the MDR1 C3435T and G2677T loci - equally frequent in Caucasians; wild-type alleles - more prevalent in African-Americans. ⁹⁵	Trough drug plasma concentrations did not correlate with the variant T allele. ⁹⁵	Humans
Lamivudine	ABCC4 (MRP4) ABCG2 (BCRP)	T4131G C421A, G34A		Drug concentrations-20% elevated in MRP4 T4131G variant carriers. ⁹⁹ None of the BCRP variants associated with drug concentrations. ⁹⁹	Humans Humans
Zidovudine	ABCC4 (MRP4) ABCG2 (BCRP)	G3724A C421A, G34A		Trend for elevated zidovudine concentrations in MRP4 G3724A variant carriers; relationship not statistically significant. ⁹⁹ None of the BCRP variants associated with drug concentrations. ⁹⁹	Humans Humans
Efavirenz	MDR1 (P-gp) MDR1 (P-gp)	C3435T C3435T	25% TT, 50% CT, 25% CC in Caucasians. 92 67-83% CC, 2-5% TT in African-Americans. ⁹³ Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT). ⁹⁴	MDR1 3435 TT genotype associated with low P-gp expression and low plasma drug concentrations. ⁹² Increased P-gp expression. ⁹⁴	Humans, Peripheral Blood Mononuclear Cells Humans, Peripheral Blood Mononuclear Cells
Nevirapine	MDR1 (P-gp)	C3435T	Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT). ⁹⁴	Increased P-gp expression. ⁹⁴	Humans, Peripheral Blood Mononuclear Cells