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MINIREVIEW

Regulation of Placental Efflux Transporters During Pregnancy Complications

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Abbreviations

ATP-binding cassette (ABC), breast cancer resistance protein (BCRP), chorioamnionitis (CA), extravillous trophoblast cells (EVTs), fetal growth restriction (FGR), FMS-like tyrosine kinase 1 (sFlt-1), gestational diabetes mellitus (GDM), hemolysis, elevated liver enzymes, low platelet count (HELLP), human immunodeficiency virus (HIV), large for their gestational age (LGA), lipopolysaccharide (endotoxin, LPS), multidrug resistance protein 1 (MDR1), multidrug resistance-associated proteins (MRPs), organic anion transporters (OATs), organic anion transporting polypeptide (OATPs), organic cation transporters (OCTs), P-glycoprotein (P-gp), polyinosinic/polycytidlic acid [poly(I:C)], small for their gestational age (SGA), syncytiotrophoblasts (STBs)

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Abstract

The placenta is essential for regulating the exchange of solutes between the maternal and fetal circulations. As a result, the placenta offers support and protection to the developing fetus by delivering crucial nutrients and removing waste and xenobiotics. ATP-binding cassette (ABC) transporters, including multidrug resistance protein 1 (MDR1), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP), remove chemicals through active efflux and are considered the primary transporters within the placental barrier. Altered transporter expression at the barrier could result in fetal exposure to chemicals and/or accumulation of xenobiotics within trophoblasts. Emerging data demonstrate that expression of these transporters is changed in women with pregnancy complications, suggesting potentially compromised integrity of placental barrier function. The purpose of this review is to summarize the regulation of placental efflux transporters during medical complications of pregnancy including: (1) placental inflammation/infection and chorioamnionitis, (2) hypertensive disorders of pregnancy, (3) metabolic disorders including gestational diabetes and obesity, and (4) fetal growth restriction/altered fetal size for gestational age. For each disorder, we review the basic pathophysiology and consider impacts on the expression and function of placental efflux transporters. Mechanisms of transporter dysregulation and implications for fetal drug and toxicant exposure are discussed. Understanding how transporters are up- or down-regulated during pathology is important in assessing possible exposures of the fetus to potentially harmful chemicals in the environment as well as the disposition of novel therapeutics intended to treat placental and fetal diseases.

Significance Statement:

Diseases of pregnancy are associated with reduced expression of placental barrier transporters that may impact fetal pharmacotherapy and exposure to dietary and environmental toxicants.

Keywords: P-glycoprotein, MDR1, MRP, BCRP, chorioamnionitis, pre-eclampsia, gestational diabetes mellitus, fetal growth restriction

Introduction

Following implantation of the fertilized egg in the uterus, trophoblast stem cells within the embryo serve as precursor cells that give rise to the placenta. Directed by specific transcription factors and signaling pathways (Hu and Cross, 2010; Latos and Hemberger, 2014), trophoblast stem cells differentiate into a variety of placenta cell lineages (Maltepe and Fisher, 2015). These cells exert distinct functions and are involved in the attachment of the placenta to the maternal decidua, invasion of maternal blood vessels, transport of solutes and gases, removal of waste products, and the secretion of hormones.

Implantation and Oxygenation. A successful pregnancy requires the secure anchoring of the placenta to the uterine wall. In order to achieve this tight interaction, placenta cells need to remodel the maternal uterine vasculature. Following differentiation from trophoblast stem cells, extravillous trophoblast cells (EVTs) begin the invasion of endometrial spiral arteries (Maltepe et al., 2010; Soares et al., 2012). As the EVT's accumulate in the arterial lumen, a plug is formed that limits blood flow. Around gestational weeks 10 to 11, the plug begins to dissolve and allow maternal blood cells to enter the intervillous space (Pijnenborg et al., 1981). Further intrusion of endovascular trophoblasts into uterine arteries occurs throughout pregnancy (Brosens et al., 2011; Pijnenborg et al., 2011). As arterial smooth muscle cells are depleted and trophoblasts replace portions of the endothelium, the spiral arteries dilate and reduce the velocity of maternal blood entering the placenta. This establishes proper maternal-fetal perfusion and delivery of solutes and gases (Red-Horse et al., 2004) (**Figure 1**). Notably, the placentas of both humans and rodents are considered hemochorial as maternal blood is in direct contact with the chorion, the outermost fetal membrane surrounding the embryo (Rai and Cross, 2014).

Tight control of invasion by EVT's into the maternal uterus during early gestation limits the delivery of oxygen to the placenta. As a result, early pregnancy is considered a state of a physiological hypoxia (Patel et al., 2010; Schneider, 2011). Low oxygen concentrations enable extensive remodeling of spiral arteries, a deeper invasion of trophoblasts into the uterus, (Ho-Chen et al., 2007; Rosario et al., 2008; Founds et al., 2009), and proliferation of trophoblast cell populations (Genbacev et al., 1996;

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Caniggia et al., 2000). As plugs clear from the maternal spiral arteries, more blood flows to the placenta and oxygen concentrations rise (Schneider, 2011) thereby promoting further organ development.

Passive Transfer. The large surface area of the placenta and its direct contact with maternal blood allows for efficient exchange of nutrients and gases (Dilworth and Sibley, 2013). Within the intervillous space of the human placenta, the maternal and fetal blood compartments are largely separated by an epithelial lining comprised of syncytiotrophoblasts (STBs). STBs are formed through the fusion of mononuclear cytotrophoblasts that have developed from trophoblast stem cells. The multinucleated syncytium regulates the transfer of oxygen (Benirschke et al., 1998) and the maternal-fetal exchange of nutrients, electrolytes, water, and waste products (Shennan et al., 1986). For small, hydrophobic, and uncharged molecules, the lipid-rich plasma membrane of STBs facilitates transfer using simple diffusion. For larger and hydrophilic compounds, such as glucose (Illsley, 2000), amino acids (Desforges and Sibley, 2010), and calcium (Belkacemi et al., 2005), facilitated transport using carrier proteins is needed to transfer chemicals down their concentration gradient to the fetal circulation. Beginning in the second trimester and throughout the remainder of gestation, maternal immunoglobulins are transferred across the placenta to the fetus using Fc receptor-mediated transcytosis (Simister, 2003; Schneider and Miller, 2010). While the syncytium is largely continuous at the surface of the placenta villi, there are regions where fibrin deposition disrupts this barrier and thereby allows passage of small molecules (Edwards et al., 1993; Brownbill et al., 2000). These regions are infrequent and as a result there is limited paracellular transfer of chemicals in the placenta.

Active Transport. Within the placenta, there are number of solute carriers responsible for the active uptake of nutrients and xenobiotics. These transporters include organic anion transporters (OATs) (Cha et al., 2000; Dallmann et al., 2019), organic cation transporters (OCTs), and organic anion transporting polypeptide (OATPs) (Briz et al., 2003; Chatuphonprasert et al., 2018; Dallmann et al., 2019). Depending upon the isoform, these carriers are localized to either the apical (maternal-facing) or basal (fetal-facing)

side of STBs. Of these transporters, the primary isoforms detected and quantified by proteomics include OAT4, OATP2B1, and OCT3 (Anoshchenko et al., 2020). The removal of chemicals from STBs is accomplished by ABC transporters (Wilkins, 2015) (**Figure 2**). Upon substrate binding, ABC transporters generate energy from the hydrolysis of ATP which allows substrate translocation across the plasma membrane (Pantham et al., 2012; Scian et al., 2014). It has been posited that active removal of xenobiotics from STBs is a mechanism to protect the fetus from direct exposure to potentially harmful chemicals. As a result, efflux transporters particularly in the apical membrane are thought to confer protection of the fetus and serve as the primary regulators of the ‘placental barrier’. ABC efflux transporters that are highly expressed in the human placenta include: (1) multidrug resistance protein or P-glycoprotein (MDR1 or P-gp, *ABCB1* gene), (2) multidrug resistance-associated proteins (MRP1-3,5, *ABCC1-3*, 5 genes), and (3) breast cancer resistance protein (BCRP, *ABCG2* gene).

MDR1 Transporter. In the placenta, MDR1 localizes to the apical microvillous surface of STBs (Hodges et al., 2011; Gormley et al., 2017) where it restricts fetal exposure to drugs and xenobiotics that enter from the maternal blood (Nakamura et al., 1997; Mölsä et al., 2005). The *ABCB1* and *Abcb1a/b* genes in humans and rodents, respectively, encode MDR1/Mdr1 proteins (van der Bliek et al., 1988; Booth-Genthe et al., 2006). The murine *Abcb1a* and *Abcb1b* genes share over 80% homology with the human *ABCB1* gene (Booth-Genthe et al., 2006). Substrates of MDR1 range from small organic cations to large lipophilic and amphipathic compounds (Romsicki and Sharom, 1998; Gormley et al., 2017). These include endogenous and exogenous chemicals, most notably chemotherapeutic drugs, anti-infective drugs, cardiovascular drugs, immunomodulators, and steroids (Raggers et al., 1999; Lee et al., 2005; Gormley et al., 2017). Notably, in mice lacking Mdr1 in the placenta, there is greater fetal accumulation of digoxin, saquinavir, and paclitaxel (Smit et al., 1999). Placental MDR1 expression is dynamic and changes as gestation advances. During early pregnancies, human placentas express high levels of MDR1 mRNA and protein, however, protein levels decline by more than half as pregnancy advances to term (Gil et al., 2005; Mathias et al., 2005; Sun et al., 2006; Anoshchenko et al., 2020).

MRP Transporters. Within the placenta, the most highly expressed MRPs are MRP1, 2, 3 and 5. MRPs translocate both endogenous and exogenous substances including many organic lipophilic anions and glutathione-conjugated chemicals (Yoon et al., 1996; Chang, 2007). MRP1 is highly expressed in the basal membrane of STBs whereas MRP3 is localized to the fetal endothelium (Nagashige et al., 2003). There is also some indication that MRP3 may also be present on the apical microvillous membrane of STBs, the primary site of MRP2 expression (St-Pierre et al., 2000). MRP5 is expressed on the basal membrane of STBs. In the human placenta, MRP1, 2 and 3 actively export drugs and toxicants. *In vitro* MRP1 protects against methyl mercury-induced cellular stress through the apical-to-basal transfer of the toxic metal, and by subsequently maintaining placenta glutathione levels (Straka et al., 2016; Granitzer et al., 2020). By comparison, MRP5 has been hypothesized to play a major role in signal transduction by reducing the intracellular concentrations of cyclic AMP and GMP (Jedlitschky et al., 2000; Wielinga et al., 2003). Trophoblast differentiation is enhanced by cAMP; thus, MRP5 protein expression may influence cytotrophoblast fusion and formation of the syncytium. MRP5 expression decreases over the course of healthy gestation (Meyer Zu Schwabedissen et al., 2005a), whereas the expression of MRP1 and MRP2 tends to increase (Meyer zu Schwabedissen et al., 2005b).

BCRP Transporter. Bcrp is extensively expressed on placental STBs, EVT, and fetal endothelial cells (Allikmets et al., 1998; Zenclussen et al., 2003). Endogenous substrates of BCRP include estrogens, porphyrins, bile acids, and riboflavin (Robey et al., 2009). Other substrates of this transporter include a wide array of drugs such as glyburide for gestational diabetes (Gedeon et al., 2008), and various anticancer drugs (i.e., mitoxantrone) (Kolwankar et al., 2005; Mao and Unadkat, 2015). BCRP protects the fetus by lowering concentrations of xenobiotics (Pollex et al., 2008; Zhou et al., 2008; Szilagyi et al., 2019b). Moreover, BCRP aids in early placental development by inhibiting excessive EVT invasion of spiral arteries (Lye et al., 2019). Regulation of placenta BCRP expression across gestation is unclear. Using quantitative proteomics, it was shown that the protein expression of BCRP declines by

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approximately 50% from early to late gestation (Anoshchenko et al., 2020). However, some earlier studies report no change (Mathias et al., 2005) or suggest increased (Yeboah et al., 2006) expression through gestation. These inconsistent findings may also reflect the high inter-individual expression of BCRP in the placenta (Bircsak et al., 2018).

About 40 to 80% of pregnant women report prescription drug use during pregnancy, including about 50% reporting use in the first trimester (Scaffidi et al., 2017). Many commonly prescribed medications as well as toxicants in our environment, diet, and workplace are substrates of ABC transporters. These pumps work to actively remove potentially harmful chemicals and xenobiotics that enter the placenta. However, emerging data suggest that the enrichment of ABC transporters in the placenta is altered by disease. This review summarizes the differential expression of ABC efflux transporters in rodent and human placentas associated with pregnancy complications. Understanding the mechanisms by which transporters are up- or down-regulated provides critical insight into the potential disposition of endobiotics and xenobiotics at the placental barrier during disease.

Key Recent Advances: Placental Transporter Expression During Pregnancy Complications.

There are a variety of complications that occur during pregnancy. Using placenta biospecimens, placental explants, cultured cell lines, and animal models, researchers have begun to characterize the expression of ABC transporters in diseased placentas. These complications include (1) placental inflammation/infection and chorioamnionitis, (2) hypertensive disorders of pregnancy, (3) metabolic disorders including gestational diabetes and obesity, and (4) fetal growth restriction/altered fetal size for gestational age.

Inflammation/Infection/Chorioamnionitis (CA). The immune system is critical for the establishment and maintenance of pregnancy ensuring proper immunologic tolerance of the fetus and maturation of the placenta (Robertson et al., 2018; Schumacher and Zenclussen, 2019; Fujiwara et al., 2020). It is also important for responding to infection. A local infection of the intra-amniotic tissue around the fetus, or inflammation of the placental fetal membranes (chorion and amnion), may induce chorioamnionitis (CA).

CA is frequently observed in patients presenting with spontaneous or preterm labor, often accompanied by a premature rupture of the membranes (Seong et al., 2008). As a result, histological CA typically occurs in less than 5% of term-delivered placentas and almost all placentas that are delivered between 21-24 weeks of gestation (Hecht et al., 2008; Kim et al., 2015b). Between 27 and 34 weeks, cases of CA steadily decrease with advancing gestational age.

Inflammation of the fetal membranes during CA can be evidence of either (1) a maternal-host neutrophilic response in the absence of detectable bacteria (Hillier et al., 1988; McNamara et al., 1997; Redline et al., 2003; Kim et al., 2015a), or (2) an invading organism that leads to further complications including funisitis and inflammation of the umbilical cord (Tsiartas et al., 2013). Regardless of the etiology, the consequences of CA typically include preterm birth (Hillier et al., 1988; Marconi et al., 2011; Erdemir et al., 2013), a predisposition to neonatal infections (Dexter et al., 1999; Hornik et al., 2012), and neurodevelopmental delays (Yoon et al., 1996; Grether and Nelson, 1997; Stoll et al., 2004).

Pathogenesis. Bacterial infections that ascend through the maternal genital tract are the primary cause of CA (Sherman et al., 1997). Other exposure routes include (1) maternal systemic bacterial infection and transfer of the organism (Cunningham et al., 1973; Craig et al., 1996) into the fallopian tubes and intrauterine space (Benirschke, 1960), and (2) accidental exposure during medical sampling of amniotic fluid (Rode et al., 2000). Infections tend to weaken fetal membranes predisposing them to rupture (Lannon et al., 2014), which in turn, may disrupt the placental protective barrier and can allow the migration of invading organisms into fetal tissues (Plakkal et al., 2013).

Inflammation that ensues during CA is a response to the release of chemokines in the placenta either due to cellular stress and injury or from the presence of microbes (Romero et al., 2014; Romero et al., 2015). Chemokines stimulate a robust inflammatory response including release of pro-inflammatory cytokines and chemotaxis of neutrophils (Scapini et al., 2000) into the chorioamniotic membranes or umbilical cord (McNamara et al., 1997; Steel et al., 2005). An excess of leukocytes can also contribute to hyperinflammatory response in the placenta.

ABC Transporter Alterations. To date, placental biospecimens evaluated from pregnancies afflicted with CA have yielded conflicting results with regard to ABC transporter expression (**Table 1**). In one study, women with CA exhibited a 50% reduction in placental BCRP expression in the third trimester (Petrovic et al., 2015). Other studies have shown differential BCRP and MDR1 expression in preterm CA placentas (Mason et al., 2011; do Imperio et al., 2018) and no change in MDR1 in term CA placentas (Petrovic et al., 2015). Notably, all three of these studies had fairly small sample sizes.

Therefore, in order to further explore the relationships between infection, inflammation, and ABC transporters, researchers have used rodent models of inflammation and infection. Administration of polyinosinic/polycytidlic acid [poly(I:C)] recapitulates features of a viral infection whereas treatment with lipopolysaccharide (endotoxin, LPS) simulates infection with gram-negative bacteria and the resulting inflammatory responses. Notably, exposure of pregnant mice to LPS (Chen et al., 2005; Wang et al., 2005; Petrovic et al., 2008) as well as poly(I:C) reduced placental expression of Bcrp and Mdr1 mRNA and protein (Petrovic and Piquette-Miller, 2010) as well as Bcrp expression in the yolk sac (Martinelli et al., 2020b). Down-regulation of Bcrp expression in LPS-treated pregnant mice correlated with greater accumulation of the antidiabetic drug and Bcrp substrate, glyburide, in the placenta and fetus compared to vehicle-treated mice (Petrovic et al., 2008). Acute exposure to LPS for 4 hours increased placental accumulation of ³H-digoxin suggesting that there is reduced Mdr1 activity compared to vehicle-treated mice although there were no change in mRNA levels (Bloise et al., 2013). Similarly, fetuses of pregnant rats treated with LPS and administered the Mdr1 substrate, (99m)Tc-sestamibi, showed 3.5-fold greater accumulation compared to vehicle treated control mice which corresponded with down-regulation of Mdr1a and 1b mRNAs (Wang et al., 2005). Likewise, exposure of pregnant rats to polyI:C for 24 hours similarly reduced placental expression of Mdr1a and 1b mRNAs and increased fetal concentrations of the antiviral drug and Mdr1 substrate, lopinavir (Petrovic and Piquette-Miller, 2015). Together, these data suggest that inflammation and/or infection can significantly reduce ABC transporter expression in rodent placentas and enable transfer of xenobiotics.

A more recent paper has evaluated zonal differences in transporter expression in placentas from mice treated with LPS (Reginatto et al., 2021). Immunostaining for Mdr1 and Bcrp proteins was reduced in the labyrinth zone and enhanced in the junctional zone of LPS-treated mice at gestation day 18.5 (Reginatto et al., 2021). This increased staining was largely on spongiotrophoblasts that provide structural support for growth of the labyrinth (Reginatto et al., 2021). These data necessitate an in-depth analysis of cell-specific or regional differences in transporter regulation during disease.

While transporters are typically considered as important players in the placental barrier, they also regulate other placental functions. Notably, BCRP and MDR1 are expressed on EVT cells (in addition to STBs) (Dunk et al., 2018; Lye et al., 2019). BCRP inhibits migration of EVT cells without altering cell proliferation whereas MDR1 stimulates EVT migration. Interestingly, exposure of an immortalized EVT cell line to LPS or single stranded RNAs containing viral antigens reduced BCRP expression and enhanced cell migration (Lye et al., 2019). Regulation of MDR1 in EVTs was not evaluated in this study. These findings provide interesting insight into novel mechanisms by which transporters impact placental health and disease beyond regulation of barrier function.

Researchers often culture small portions of first or third trimester human placentas in the laboratory for up to 1 week after delivery. During this period, the explants will shed and then regenerate the syncytium. This model allows for *ex vivo* exposure to chemicals and environmental conditions and assessment of trophoblast responses. Interestingly, treatment of first-trimester placenta explants with LPS decreased the mRNA and protein expression of BCRP and MDR1. By comparison, however, treatment of first-trimester explants with poly(I:C) elicited no effect on transporter expression. Rather, exposure of third-trimester explants to poly(I:C) reduced MDR1 mRNA expression (Lye et al., 2015). These findings suggest that bacterial and viral infections, or their resulting inflammation, may impair ABC transporter expression and function and potentially render the developing fetus susceptible to xenobiotic exposure.

Chronic maternal infections including malaria or human immunodeficiency virus (HIV) can also induce a persistent inflammatory state in the placenta. The presence of the *Plasmodium* parasite (malaria) during pregnancy primes the placenta to become a hyperactive pro-inflammatory microenvironment that

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can alter transporter expression. Rodents infected with malarial parasites exhibit reduced mRNA and protein expression of Bcrp, Mdr1a, and Mrp2 in the placenta (Cressman et al., 2014; Fontes et al., 2019). Similarly, mRNA expression of Abcb1b is reduced in the yolk sac of mice infected with malarial parasites (Martinelli et al., 2020a). However, placentas from women who were either currently infected, or had prior infection with malaria (with or without inflammation), demonstrated almost no alterations in the same transporters compared to uninflamed and uninfected placentas (Muehlenbachs et al., 2007). By comparison, placentas from HIV-infected women had reduced BCRP and MRP1 and 2 expression and increased levels of MDR1 compared to healthy women (Camus et al., 2006; Kojovic et al., 2020a). Notably though these observations were not recapitulated in HIV-Tg rats, a rodent model that mirrors chronic AIDS-like phenotypes. Exposure of HIV-Tg rats to a low dose of LPS reduced placental Mdr1a mRNA and protein expression in contrast to the up-regulation observed in infected human placentas (Ghoneim et al., 2017). The disparity in findings across species may suggest that there are other factors that play a role in HIV-infected women but are not observed in the HIV-Tg rat model. Collectively, infections and inflammatory states induced in the placenta appear to impact the expression of key ABC transporter; however, the timing and directional change in transporter levels (either up- or down-regulation) is not entirely clear and requires further evaluation particularly in larger birth cohorts.

Hypertensive Disorders of Pregnancy. Pre-eclampsia is a hypertensive disorder of pregnancy that is characterized by late-onset systemic vasoconstriction ($\geq 20^{\text{th}}$ week of gestation) leading to hypertension and proteinuria in women who are typically normotensive prior to conception (Brown et al., 2000; Lenfant, 2001; Sibai, 2003). Placental vasoconstriction as a result of pre-eclampsia greatly reduces blood flow to the fetus and can result in developmental complications. Pre-eclampsia affects 2 to 7% of healthy, nulliparous women with increased frequency and severity noted in multifetal pregnancies or in women with pre-existing complications including chronic hypertension and pre-gestational diabetes (Caritis et al., 1998; Sibai et al., 2000; Wen et al., 2004). A severe state of pre-eclampsia can advance into Hemolysis, Elevated Liver enzymes, Low platelet count (HELLP) syndrome (Dusse et al., 2015) which affects 0.5 to

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0.9% of all pregnancies and manifests late in gestation. Although the etiology is poorly understood, a diagnosis of HELLP includes the presence of increased liver enzymes (Knapen et al., 1998), thrombocytopenia, and hemolysis (Magann and Martin, 1999) or more severe symptoms such as edema (Weinstein, 1985; Sibai et al., 2000). Both pre-eclampsia and HELLP can cause significant maternal and fetal morbidity as well as mortality.

Pathogenesis. Pre-eclampsia is thought to involve a deficiency in the extent to which EVT's invade and remodel uterine spiral arteries and is accompanied by an inflammatory response (Dekker and Sibai, 2001; Dekker and Robillard, 2003). Premature loss of the EVT plugs within the spiral arteries can result in a reduced placenta size and an early-onset of pre-eclampsia (Burton and Jauniaux, 2004). Failed remodeling and invasion by EVT's can further lead to irregular placental perfusion and uncontrolled hypoxia (Burton et al., 2009; Blazquez et al., 2014) resulting in the presence of reactive oxygen species, oxidative stress, and apoptosis in the syncytium (Huppertz, 2008). An exacerbated stress state can in turn stimulate the production of inflammatory cytokines and result in systemic hyperinflammation.

ABC Transporter Alterations. Human placenta explants and biospecimens from patients diagnosed with pre-eclampsia or HELLP can be used as experimental tools to study the impact of disease on ABC transporter expression (**Table 2**). In general, placentas from pre-eclamptic pregnancies tend to exhibit reduced transporter expression when evaluated by microarray (Herse et al., 2007; Nishizawa et al., 2007; Founds et al., 2009; Pantham et al., 2012; Vaiman et al., 2013; Gormley et al., 2017). Additional studies where mRNA and protein expression were quantified confirm that pre-eclampsia is often associated with reduced expression of placental MDR1, MRP1, 2, and BCRP transporters though some transporters are unchanged or slightly increased (Afrouzian et al., 2018; Kojovic et al., 2020b). When pre-eclampsia is severe and early in its onset, a prominent reduction in placental MDR1 protein expression has been observed (Dunk et al., 2018). HELLP further reduces expression of BCRP (Ruebner et al., 2012; Jebbink

et al., 2015). These findings collectively suggest that there may be a compromise of the placental barrier in women with hypertensive disorders of pregnancy.

Metabolic Disorders. Gestational diabetes mellitus (GDM) is a state of glucose intolerance that is typically diagnosed during the second or third trimester in pregnant women who often have no prior history of diabetes (Coustan, 2013; Association, 2015). In 2017, the nationwide prevalence of GDM was 13% with greater incidence observed in women of older age (Coustan, 2013; Law and Zhang, 2017; Melchior et al., 2017). Although GDM typically ceases with parturition, GDM patients are at a heightened risk of experiencing pregnancy and childbirth complications (Crowther et al., 2005; Metzger et al., 2008), as well as developing chronic type II diabetes (Kim et al., 2002; Bellamy et al., 2009; Rayanagoudar et al., 2016). Additionally, the offspring from GDM mothers are vulnerable to becoming obese and developing diabetes over their lifetime (Dabelea, 2007; Damm, 2009; Law and Zhang, 2017).

Pathogenesis. Risk factors for developing GDM include obesity, weight gain during pregnancy, high systolic blood pressure, and a family history of diabetes (Christian and Porter, 2014; Leng et al., 2015; Law et al., 2017). Although the complete pathogenesis of GDM is poorly understood, weight gain and obesity during pregnancy can induce maternal hyperglycemia, leading to dysregulation in glucose homeostasis and glucose intolerance (Herring et al., 2009). Further, histopathologic changes of GDM placentas include villous immaturity and edema, syncytial nodes, fibrinoid necrosis, and fibrin thrombus (Rudge et al., 2011; Edu et al., 2016). Glucose is an essential nutrient that transfers from the maternal circulation for optimal fetal development; therefore, proper transfer is crucial (Baumann et al., 2002; Augustin, 2010; Illsley and Baumann, 2020). GDM has been associated with a limited fetal nutrient transfer due to altered placenta functioning (Daskalakis et al., 2008; Dashe et al., 2009), which can adversely impact fetal growth and development.

ABC Transporter Alterations. Maternal obesity and GDM can impact the expression of placental ABC transporters (**Table 3**). A common approach to recapitulate these diseases in rodents is to feed with a high-fat diet prior to conception. Notably, in both obese pregnant women and mice, expression of MDR1 mRNA and protein in the placenta is reduced (Wang et al., 2015). Although no functional studies have been performed, it can be inferred that placentas from obese women may accumulate greater burden of MDR1 substrates and potentially pass these chemicals to the fetus.

A second experimental model has been pursued to evaluate ABC transporter expression in the placentas of rodents with GDM and compare these results to biospecimens from pregnant women with GDM. This is accomplished by administering streptozotocin to rodents during gestation which destroys pancreatic insulin-producing beta-cells and induces hyperglycemia (Anger and Piquette-Miller, 2011). GDM, if untreated, is associated with increased placental MDR1 expression in rodents and humans. When the disease is managed with insulin treatment, transporter expression returned to levels similar to a healthy pregnancy (Anger and Piquette-Miller, 2011; Anger et al., 2012). Interestingly, plasma levels of HbA1c (glycated hemoglobin) positively correlated with placenta BCRP mRNA and protein expression (Anger et al., 2012). Likewise, rats with GDM also exhibited elevated expression of Mdr1b, Bcrp and Mrp2 mRNA (Anger and Piquette-Miller, 2011). In fact, the up-regulation of Mdr1b in placenta correlated with a reduction in fetal exposure to the Mdr1 substrate and antiviral drug, lopinavir, though changes in maternal hepatic metabolism may have also reduced fetal concentrations (Anger and Piquette-Miller, 2011).

Glibenclamide (glyburide), a substrate of BCRP (Pollex et al., 2008; Zhou et al., 2008), MDR1, MRP1 (Hemauer et al., 2010), and MRP3 (Gedeon et al., 2006) has been used as a treatment for GDM (Camelo Castillo et al., 2014). Metformin and rosiglitazone are other drugs that can be used either in monotherapy or in combination with glyburide to treat GDM, both of which are substrates of MDR1 (Hemauer et al., 2010). Notably, metformin has been the more recent preferred treatment for GDM and is also a substrate of BCRP (Hemauer et al., 2010). These therapies work to stimulate systemic insulin secretion or peripheral tissue sensitivity with an overall goal of lowering blood glucose levels.

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Consequently, proper placental ABC transporter expression and function is imperative to limit fetal drug exposure.

Altered Fetal Growth. Prenatal growth is highly dependent upon the gestational age of the fetus and nutrition received via the placenta. Abnormal *in utero* growth can lead to low or high birth weights that can impact growth trajectories during the postnatal period (Godfrey et al., 2011). Measurements from ultrasounds conducted during the first trimester are often used to assess fetal growth. Babies that are born less than two standard deviations compared to a proportionate population are classified as being small for their gestational age (SGA) (Alexander et al., 1996). Fetal growth restriction (FGR) is a more extreme condition and describes the slow growth of a fetus compared to the expected rate (Mandrizzato et al., 2008). Impairment of fetal growth is seen in approximately 10% of pregnancies (Unterscheider et al., 2013); the majority are attributed to SGA and to a lesser extent to FGR. Furthermore, babies born SGA are at an increased risk of being short stature and developing cardio-metabolic diseases early in life (Castagno et al., 2019; Muñoz Fontán et al., 2019).

Pathogenesis. SGA and FGR can result from maternal, placental, fetal, and genetic etiologies. The healthy progression of a pregnancy requires appropriate placental blood flow for optimal fetal growth and development (Sutton et al., 1990). Improper placentation can alter blood perfusion to the fetus and delivery of oxygen and nutrients (Gatford et al., 2010) leading to pathologic hypoxia, poor nutrition, improper placental vascular development and growth (Burton and Jauniaux, 2004; Hendrix and Berghella, 2008; Herrera et al., 2014).

ABC Transporter Alterations. Studies evaluating ABC transporter expression changes in placentas from SGA or FGR pregnancies have been limited (**Table 4**). Placentas from FGR-complicated pregnancies exhibit reduced BCRP and MDR1 mRNA expression compared to healthy pregnancies (Evseenko et al., 2007a). A recent paper has begun to draw potential ties between transporter expression during disease and

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placental accumulation of xenobiotics. Cao et al. demonstrated that placentas from FGR pregnancies had reduced BCRP expression which this correlated with over 2-fold greater accumulation of the endocrine disrupting chemical, bisphenol A (an established BCRP substrate) (Dankers et al., 2013; Cao et al., 2022). Placental BCRP mRNA expression is also inversely related to babies being born SGA (Deyssenroth et al., 2017). Currently, there are no pharmacological interventions to treat either SGA or FGR and restore transporter expression (Lausman and Kingdom, 2013; Bendix et al., 2020).

A number of studies have evaluated relationships between FGR caused by toxicants and regulation of Mdr1 and yielded mixed results. The primary toxicants that have been investigated include tobacco smoke (or its nicotine component) and ethanol which cause FGR in rodents. In mice, treatment with nicotine caused FGR and reduced Mdr1 protein expression (Wang et al., 2009) which is similar to findings in rats exposed to both tobacco and ethanol (Li et al., 2011). However, the opposite results have been observed in two studies where exposure tobacco smoke *in utero* enhanced expression of Mdr1 (Yan et al., 2005; Yan et al., 2006).

Pathways Regulating ABC Transporter Expression During Pregnancy Complications

Emerging data provide insights into the molecular signals regulating aberrant transporter expression during pathogenic pregnancies. One mechanism explored has been the dysregulation of the maternal endocannabinoid system which normally functions to regulate the actions of trophoblast cells (Costa, 2016). Endocannabinoid dysfunction, as seen in pre-eclampsia for example, can lead to an aberrant state of hypoxia and decreased BCRP expression (Abán et al., 2013; Szilagyi et al., 2019a). Likewise, activation of hypoxia signaling through the hypoxia inducible factor -1 alpha transcription factor can reduce BCRP expression both *in vitro* and in placentas from healthy women living at high altitudes (Francois et al., 2017).

Exposure of the placenta to cytokines and growth factors as observed in some placental pathologies can also alter ABC transporter expression. Up-regulation of interleukin-6, tumor necrosis factor- α , and interleukin-1 β signaling is seen in CA, pre-eclampsia, and GDM; consequently, these

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cytokines have been shown to reduce expression of MDR1 and BCRP by almost half (Hartmann et al., 2001; Sukhai et al., 2001; Evseenko et al., 2007b). The activity of BCRP can also be reduced by TNF- α . Other pro-inflammatory cytokines, sex steroids, and growth factors have opposing regulatory effects on ABC transporter levels. For instance, in isolated cytotrophoblast cells from term placentas, insulin-like growth factor II and EGF increase BCRP expression and function, respectively (Evseenko et al., 2007b). *In vitro* exposure to progesterone had a modest effect on MRP1 and MDR1 expression on the cell culture (Evseenko et al., 2007b). In addition to MRP1 and MDR1, BCRP also has been shown to be strongly induced by progesterone in trophoblast (Wang et al., 2008), whereas 17 β -estradiol decreases BCRP expression (Wang et al., 2006). In HIV-infected pregnant women, there is a positive correlation between maternal 17 β -estradiol concentrations and placental expression of MDR1 and MRP1 (Kojovic et al., 2020a).

Circulating concentrations of the angiogenic factor FMS-like tyrosine kinase 1 (sFlt-1) are elevated in pregnant women with pre-eclampsia. Interestingly, exposure of human BeWo trophoblast cells to sFlt-1 reduces BCRP expression by 85 to 90% which can be rescued by vascular endothelial growth factor. These findings may be important in understanding transporter regulation during pre-eclampsia (Kojovic et al., 2020b). Epigenetic factors may also play a role in transporter regulation during disease. In placentas from women with chorioamnionitis, there was an increase in miR-331-5p and a reduction of Mdr1 immunostaining compared to controls (do Imperio et al., 2018). Previous studies have demonstrated that elevated miR-331-5p can suppress Mdr1 expression (Feng et al., 2011). Most likely, it is a combination of these various hormonal, immune, endocrine, and epigenetic pathways that are responsible for the dysregulation of ABC transporter expression in diseased placentas.

Perspectives on Future Directions

Taken together, pathologic pregnancies appear to largely be associated with reduced placental barrier transporter expression although exceptions have been noted. Future studies should apply more quantitative proteomic and genetic approaches to more robustly characterize changes. For example,

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genetic variation in BCRP is an important determinant of protein expression in healthy human placentas (Bircsak et al., 2018); though its importance in pregnancies with gestational complications is unknown. Animal models should be further refined to mirror observations in human disease cohorts and their use should be expanded to evaluate xenobiotic disposition as performed for maternal-fetal disposition of known substrates. In conclusion, ABC transporters are key players in regulating the placental disposition of drugs and environmental chemicals and their down-regulation has the potential to increase the placental accumulation and fetal exposure xenobiotics.

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Footnotes

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Figure Legends

Figure 1. Human Placenta Structure and Invasion of the Uterus. Diagrams of two placental villous structures from the maternal surface of the placenta have been magnified. The villous on the *left* represents the early placenta (early first trimester) where extravillous trophoblasts have formed a plug in the uterine spiral artery preventing maternal blood flow and creating a physiological hypoxic state. The villous on the *right* represents the dissociation of the plug as extravillous trophoblasts have invaded deeper into the uterus during the late first trimester and endovascular trophoblasts have remodeled the spiral artery allowing for dilatation. This process allows for maternal oxygenated blood to enter the intervillous space and reach the syncytiotrophoblasts that express uptake and efflux transporters and secrete hormones. Created with BioRender.com.

Figure 2. Enrichment of ABC Transporters in the Human Placental Barrier. ATP-binding cassette transporters are expressed on syncytiotrophoblasts and fetal endothelial cells in the human placenta. The apical surface of the syncytium faces the maternal blood whereas the basal membrane faces the interstitium containing cytotrophoblasts (not shown) and fetal capillaries. ABC transporters expressed include the multidrug resistance protein or P-glycoprotein (MDR1 or P-gp, *ABCB1* gene), multidrug resistance-associated proteins (MRP1-3,5, *ABCC1-3*, 5 genes) and breast cancer resistance protein (BCRP, *ABCG2* gene). Arrows denote the direction of substrate efflux towards the maternal or fetal compartments. The exact orientation and localization of MRP transporters on the fetal endothelium is not well-characterized. Created with BioRender.com.

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Table 1. Placental Expression of ABC Transporters in Response to Inflammation, Infection, and Chorioamnionitis.

Diseases	Species	Study Size	MDR1 / ABCB1	MRPs / ABCCs	BCRP / ABCG2	References
Inflammation (LPS)	Rats	N = 3 – 7 Control N = 3 – 7 LPS (0.1, 0.5, 1.0 mg/kg)	↓ mRNA <i>Abcb1a</i> , <i>Abcb1b</i>	↓ mRNA <i>Abcc1</i> , <i>Abcc2</i> , <i>Abcc3</i>	↓ mRNA and protein	(Petrovic et al., 2008)
	Rats	N = 4 – 6 Control N = 4 – 6 LPS (0.5, 1.0, mg/kg)	↓ mRNA <i>Abcb1a</i> , <i>Abcb1b</i>	NE	NE	(Wang et al., 2005)
	Mice	N = 3 – 6 Control N = 3 – 6 LPS (0.1, 0.2, 0.5 mg/kg)	↓ mRNA <i>Abcb1a</i>	NE	NE	(Chen et al., 2005)
	Human 1 st T Placenta Explants	N = 6 Control N = 6 LPS (0.1-10 µg/mL)	↓ mRNA and protein	NE	↓ mRNA and protein	(Lye et al., 2015)
Inflammation [Poly(I:C)]	Human 1 st T Placenta Explants	N = 6 Control N = 6 Poly(I:C) (1-50 µg/mL)	↓ mRNA ↔ protein	NE	↔ mRNA or protein	
	Rats	N = 4 – 6 Control N = 4 – 6 Poly(I:C) (2.5, 5.0 mg/kg)	↓ mRNA <i>Abcb1a/b</i> ↔ protein	↓ mRNA <i>Abcc1,3</i> ; ↔ mRNA <i>Abcc2</i>	↓ mRNA ↔ protein	(Petrovic and Piquette-Miller, 2010)
	Rats	N = 7 – 10 Control N = 7 – 10 Poly(I:C) (5.0 mg/kg)	↓ mRNA <i>Abcb1a/b</i>	↓ mRNA <i>Abcc2</i>	NE	(Petrovic and Piquette-Miller, 2015)
Infection (Malaria)	Mice	N = 5 Control N = 5 Infected	↓ mRNA <i>Abcb1a/b</i> ; ↓ protein	↓ mRNA <i>Abcc1-3</i>	↓ mRNA	(Cressman et al., 2014)
	Mice	N = 12 Control N = 20 Infected	↓ mRNA <i>Abcb1a/b</i> ; ↓ protein	↔ mRNA <i>Abcc2, 5</i>	↓ mRNA and protein	(Fontes et al., 2019)
Infection (HIV-1+)	Human Subjects	N = 23 Uninfected N = 25 Infected	↑ mRNA; ↔ protein	↓ mRNA <i>ABCC1, 2, 4</i>	↓ mRNA; ↔ protein	(Kojovic et al., 2020a)
	Human Subjects	N = 35 Uninfected N = 24 Infected	↑ mRNA and protein	NE	NE	(Camus et al., 2006)
	Rats	N = 4 – 7 HIV-Tg and vehicle N = 4 – 7 HIV-Tg and LPS (0.1, 0.25 mg/kg)	↓ mRNA <i>Abcb1a</i> ; ↓ protein	NE	NE	(Ghoneim et al., 2017)
Infection (Hepatitis C)	Human Subjects	N = 7 Uninfected N = 7 Infected	↑ mRNA and protein	NE	↔ mRNA; ↑ protein	(Pfeifer et al., 2018)
Infection (Zika)	Mice	N = 6 Control N = 6 Zika (immunocompetent) N = 6 Zika (immunocompromised)	↓ protein	↓ protein	NE	(Andrade et al., 2021)
Chorioamnionitis (CA)	Human Subjects	N = 8 – 14 Control, Preterm N = 8 – 14 Preterm CA	↔ mRNA or protein	NE	↓ mRNA and protein	(Petrovic et al., 2015)
	Human Subjects	N = 6 Control, Preterm N = 6 Preterm with CA	↑ mRNA, ↓ protein	↑ mRNA <i>ABCC2</i>	↑ mRNA and protein	(do Imperio et al., 2018)
	Human Subjects	N = 8 – 10 Control, Preterm N = 8 – 10 Preterm with inflammation and CA	↑ mRNA and protein	↔ mRNA <i>ABCC1, 2</i>	↑ mRNA and protein	(Mason et al., 2011)

Abbreviations: MDR1/*ABCB1*: multidrug resistance protein 1, P-glycoprotein 1 (P-gp); MRPs/*ABCCs*: multidrug resistance-associated proteins; BCRP/*ABCG2*: breast cancer resistance protein; LPS: lipopolysaccharide; [poly(I:C)]: polyinosinic:polycytidylic acid; HIV: human immunodeficiency virus; CA: chorioamnionitis; T: trimester; NE: not examined

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Table 2. Placental Expression of ABC Transporters in Response to Hypertensive Disorders of Pregnancy.

Diseases	Species	Study Size	MDR1 / <i>ABCB1</i>	MRPs / <i>ABCCs</i>	BCRP / <i>ABCG2</i>	References
Pre-eclampsia (PE)	Human Subjects	N = 26 Control N = 20 PE	NE	↑ mRNA and protein <i>ABCC1</i>	↔ mRNA; ↑ protein	(Afrouzian et al., 2018)
	Human Subjects	N = 24 Control N = 34 PE	↔ mRNA or protein	↓ mRNA <i>ABCC1</i> and <i>ABCC2</i> ; ↔ mRNA <i>ABCC4</i>	↓ mRNA and protein	(Kojovic et al., 2020b)
Severe Early Onset Pre-eclampsia	Human Subjects	N = 9 Control N = 5 – 9 PE	↓ mRNA and protein	NE	NE	(Dunk et al., 2018)
Hemolysis, Elevated Liver enzymes, Low platelet count (HELLP)	Human Subjects	N = 10 Control N = 10 PE N = 10 HELLP	NE	NE	↓ mRNA	(Ruebner et al., 2012)
	Human Subjects	N = 12 Control N = 6 PE/HELLP	NE	NE	↓ protein	(Jebbink et al., 2015)

Abbreviations: MDR1/*ABCB1*: multidrug resistance protein 1; MRPs/*ABCCs*: multidrug resistance-associated proteins; BCRP/*ABCG2*: breast cancer resistance protein; PE: pre-eclampsia; HELLP: hemolysis, elevated liver enzymes, low platelet count; NE: not examined

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Table 3. Placental Expression of ABC Transporters in Response to Metabolic Disorders.

Disease	Species	Study Size	MDR1 / ABCB1	MRPs / ABCCs	BCRP / ABCG2	References
Obesity	Mice	N = 6 – 8 Control N = 6 – 8 Obese	↓ mRNA <i>Abcb1a</i> and protein	NE	NE	(Wang et al., 2015)
	Human Subjects	N = 20 Control/lean N = 10 Obese	↓ mRNA and protein	NE	NE	
Gestational Diabetes Mellitus (GDM)	Rats	N = 4 – 5 Control N = 4 – 5 Insulin-managed GDM N = 4 – 5 Non-insulin managed GDM	↑ mRNA <i>Abcb1b</i> ; ↔ mRNA <i>Abcb1a</i> (non-insulin managed)	↑ mRNA <i>Abcc2</i> (non-insulin managed)	↑ mRNA (non-insulin managed)	(Anger and Piquette- Miller, 2011)
	Human Subjects	N = 14 Control N = 13 GDM	↑ mRNA	↔ mRNA <i>ABCC2</i>	↔ mRNA	(Anger et al., 2012)

Abbreviations: MDR1/*ABCB1*: multidrug resistance protein 1; MRPs/*ABCCs*: multidrug resistance-associated proteins; BCRP/*ABCG2*: breast cancer resistance protein; GDM: gestational diabetes mellitus; NE: not examined

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Table 4. Placental Expression of ABC Transporters in Response to Small or Large for Gestational Age or Fetal Growth Restriction.

Disease	Species	Study Size	MDR1 / ABCB1	MRPs / ABCCs	BCRP / ABCG2	References
Fetal Growth Restriction (FGR)	Human Subjects	N = 25 control N = 25 FGR	↓ mRNA	↔ mRNA <i>ABCC1, 2</i>	↓ mRNA	(Evseenko et al., 2007a)
	Human Subjects	N = 18 control N = 18 FGR	NE	NE	↓ mRNA and protein	(Cao et al., 2022)
	Mice	N = 4 control N = 4 nicotine-induced FGR	↓ mRNA <i>Abcb1a</i> ; ↔ mRNA <i>Abcb1b</i> ; ↓ protein	NE	NE	(Wang et al., 2009)
	Rats	N = 5 control N = 5 tobacco/ethanol-induced FGR	↓ mRNA <i>Abcb1a</i> ; ↔ mRNA <i>Abcb1b</i>	NE	NE	(Li et al., 2011)
	Rats	N = 4 control N = 4 tobacco smoke-induced FGR	↑ mRNA <i>Abcb1a</i> ; ↔ mRNA <i>Abcb1b</i> ; ↑ protein	NE	NE	(Yan et al., 2005)
	Rats	N = 4 control N = 4 tobacco-induced FGR	↑ mRNA <i>Abcb1a</i>	NE	NE	(Yan et al., 2006)
Small and Large for Gestational Age (SGA/LGA)	Human Subjects	N = 387 control N = 130 SGA N = 160 LGA	NE	NE	↓ mRNA	(Deyssenroth et al., 2017)

Abbreviations: MDR1/*ABCB1*: multidrug resistance protein 1; MRPs/*ABCCs*: multidrug resistance-associated proteins; BCRP/*ABCG2*: breast cancer resistance protein; SGA: small for gestational age; LGA: large for gestational age; FGR: fetal growth restriction; NE: not examined

FIGURE 1