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ATP-Binding Cassette Efflux Transporters in Human Placenta

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Abstract

Pregnant women are often complicated with diseases including viral or bacterial infections, epilepsy, hypertension, or pregnancy-induced conditions such as depression and gestational diabetes that require treatment with medication. In addition, substance abuse during pregnancy remains a major public health problem. Many drugs used by pregnant women are off label without the necessary dose, efficacy, and safety data required for rational dosing regimens of these drugs. Thus, a major concern arising from the widespread use of drugs by pregnant women is the transfer of drugs across the placental barrier, leading to potential toxicity to the developing fetus. Knowledge regarding the ATP-binding cassette (ABC) efflux transporters, which play an important role in drug transfer across the placental barrier, is absolutely critical for optimizing the therapeutic strategy to treat the mother while protecting the fetus during pregnancy. Such transporters include P-glycoprotein (P-gp, gene symbol *ABCB1*), the breast cancer resistance protein (BCRP, gene symbol *ABCG2*), and the multidrug resistance proteins (MRPs, gene symbol *ABCCs*). In this review, we summarize the current knowledge with respect to developmental expression and regulation, membrane localization, functional significance, and genetic polymorphisms of these ABC transporters in the placenta and their relevance to fetal drug exposure and toxicity.

Keywords

ABC transporters; BCRP; fetal drug exposure; fetus; P-gp; MRPs; transplacental drug transport

1. INTRODUCTION

The placenta serves as the sole link between the mother and her fetus during pregnancy and forms a physiological barrier that mediates the exchange of nutrients and metabolites between the maternal and fetal circulation. The human placenta is unique in structure and can only be represented by placentas of certain primate species [1]. The human placental barrier consists of the singular epithelium called the syncytiotrophoblast arising from cytotrophoblast, whose apical membrane is in direct contact with the maternal blood and basal membrane is close to the fetal circulation through the umbilical vessels into the placental villi. The syncytiotrophoblast represents the rate-limiting barrier for trans-placental transfer of most substances. As pregnancy advances, the thickness of the human placental barrier diminishes, primarily due to the partial disappearance of the cytotrophoblast [2,3]. The function of the placenta varies during the gestational period. In early pregnancy, the main function of the placenta is to mediate implantation of the embryo into the uterus and produce hormones that prevent the end of the ovarian cycles [4]. After implantation, the placenta is primarily responsible for the transplacental exchange of the bulk of substances

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between the maternal and fetal circulation including oxygen, carbon dioxide, water, and all necessary nutrients required for the developing fetus [5]. The transplacental transfer of substances can be either from the mother to the fetus or from the fetus to the mother [6].

Pregnant women often need to take medication to treat diseases such as viral, fungal, or bacterial infections, epilepsy, hypertension, or pregnancy-induced conditions like depression, nausea, and gestational diabetes [7–9]. Many of these drugs have not been adequately studied with respect to their safety in pregnancy [10–11]. Thus, a major concern arising from the widespread use of drugs by pregnant women is the penetration of drugs across the placental barrier, leading to potential adverse effect and toxicity to the developing fetus. It is now clear that any drug administered to the mother can cross the placental barrier to some extent, unless it is metabolized or altered during transplacental passage, or its physicochemical characteristics limit the transplacental transfer. Transplacental transfer of substances is known to be mediated by passive diffusion, active transport, and facilitated diffusion as well as phagocytosis and pinocytosis [12,13]. Passive diffusion is the predominant form of the transplacental transfer and represents a permeation of substances down the concentration gradient [13]. The extent of transplacental transfer by passive diffusion is affected by drug properties such as molecular weight, pK_a , lipid solubility, and the plasma protein binding of the drug. Only a few drugs have been seen to be transported into the placenta by facilitated diffusion thus far. Phagocytosis and pinocytosis have not been shown to be highly relevant to transplacental transfer due to its slow rate [13].

Increasing attention has now been devoted to active transport because of its recognized role in transplacental transfer of a variety of drugs and xenobiotics [2,10,14–17]. Active transporters expressed in the placenta include ATP-binding cassette (ABC) efflux transporters and ATP-independent transporters in the solute carrier protein (SLC) superfamily such as the organic anion-transporting polypeptides (OATPs), organic cation transporters (OCTs), mono-carboxylate transporters, nucleoside transporters, and others [17,18]. In the placenta, these transporters can be localized on the maternal, the fetal facing sides of the syncytiotrophoblast, and/or the endothelium of fetal capillaries. Active transport across the placental barrier by these transporters requires energy. Almost all active transporters in the placenta can work against concentration gradients, which potentially modulate fetal exposure of the drugs intended to treat the mother, fetus, or both. Among them, ABC efflux transporters are believed to be heavily involved in transporting a wide range of structurally unrelated drugs, xenotoxins, and endogenous metabolites across the placenta. One of the major roles of ABC transporters in the placenta is limiting fetal over-exposure to toxin, drugs or xenobiotics, thus protecting the fetus both pharmacokinetically and pharmacodynamically. ABC transporters expressed in the placenta which have been relatively well defined with respect to their roles in the transport of clinically and toxicologically important drugs/xenobiotics include P-glycoprotein (P-gp or MDR1, gene symbol *ABCB1*), the breast cancer resistance protein (BCRP, gene symbol *ABCG2*), and the multidrug resistance proteins 1–3 and 5 (MRP1–3 and 5, gene symbol *ABCC1-3 and 5*) to a lesser extent [2,10,15,17].

In this review, we will focus on ABC efflux transporters expressed in the placenta with respect to their roles in transplacental transfer of drug/xenobiotics. We will also review developmental expression of these transporters at different gestational ages, the impact of genetic polymorphisms of these transporters on their expression and placental drug transport, and their clinical relevance.

2. P-GLYCOPROTEIN (P-GP OR MDR1, GENE SYMBOL *ABCB1*)

P-gp (also known as MDR1 or ABCB1), a 170 kDa plasma membrane protein, was initially discovered in Chinese hamster ovary cells [19]. The protein is composed of two membrane spanning domains (MSD), each followed by one nucleotide binding domain (NBD). In humans, P-gp is encoded by *MDR1*, while the rodent MDR1 orthologs are encoded by *Mdr1a* and *Mdr1b* [20]. P-gp is widely distributed in many tissues including the large and small intestinal epithelium, brain capillaries, testis, placenta, liver hepatocytes, and renal proximal tubules [21–23]. In particular, in human placenta, P-gp is highly expressed in the maternal-facing apical membrane of the syncytiotrophoblast [24,25] (Table 1). Substrates of P-gp are in an extremely wide range, most of which are cationic or amphipathic in nature and mainly hydrophobic, such as anthracyclines, HIV protease inhibitors, immunosuppressive drugs, and antibiotics [26]. P-gp can be inhibited by its substrates as competitive inhibitors, such as verapamil and cyclosporine, or by inhibitors that are not substrates such as GF120918 [27].

P-gp-mediated transport of drugs in the placenta was first demonstrated in plasma membrane vesicles prepared from the trophoblasts of human placenta [25]. Functional expression of P-gp in the placenta was further confirmed by inhibition of P-gp-mediated transport of calcein-AM and vinblastine by P-gp inhibitors in *in vitro* models of primary human placental trophoblasts and human placental choriocarcinoma derived BeWo cells [28]. In *in vivo* animal studies, a deficiency in the *Mdr1a* gene in the CF-1 mouse strain resulted in an increased teratogenic effect on the fetus due to increased fetal exposure to a teratogenic photoisomer of avermectin, which is known to produce cleft palate in mice [29]. The P-gp substrates digoxin, saquinavir, and paclitaxel accumulated significantly more in the *Mdr1a/1b*($-/-$) fetuses than in the *Mdr1a/1b*($+/+$) fetuses of the same mother [30]. Furthermore, P-gp activity in the placental barrier was completely abrogated by oral administration of the P-gp inhibitors PSC833 or GF120918 to the heterozygous mothers [30]. P-gp in the placental barrier was also shown to limit saquinavir penetration to the fetus in a clinically more realistic setting with [14 C]-saquinavir administered orally to the *Mdr1a/1b*($-/-$) dams [31]. PET imaging, a noninvasive technique, was recently employed to study P-gp activity and its inhibition on a more representative animal model, macaque, using [11 C]-verapamil as the model substrate and cyclosporin A (CsA) as the inhibitor. It was found that CsA significantly increased fetal liver distribution of the radioactivity [32]. All these studies have revealed that P-gp plays an important role in limiting fetal exposure to various potentially harmful drugs and xenobiotics.

A dually perfused human placenta model was employed to directly investigate the influence of P-gp on the pharmacokinetics of transplacental transfer [33–35]. In this human placenta perfusion model, it was first observed that the transplacental clearance of CsA was increased significantly when co-administered with the P-gp inhibitor quinidine or chlorpromazine [33]. Subsequently, it was shown that P-gp could not only limit the entry of rhodamine123 from the maternal circulation to the fetal compartment, but at the same time accelerated the fetomaternal elimination of P-gp substrates [34]. Recently, more evidence emerged, demonstrating direct transfer of P-gp substrates in the perfused human placenta models, such as saquinavir [36,37], methadone [38], indinavir [35,39], as well as rhodamine123 and prazosin [40]. We therefore can conclude that P-gp exerts a protective role for the fetus by restricting the penetration of drugs/xenobiotics from the maternal circulation to the fetal compartment.

Gestational age-dependent expression of P-gp has been reported in the placenta [41–45]. In humans, P-gp expression in pre-term placentas was significantly higher than that in term placentas and gradually decreased with gestational age [41,44,46–47]. The gestational age-

dependent pattern of P-gp expression in guinea pig placenta [48] and mouse placenta [42,45] was similar to that in humans. However, rat P-gp expression could be seen in the rat placental labyrinth from the 12th day of pregnancy [49], with a tendency of increase in mRNA expression of both *Mdr1a* and *Mdr1b* with advancing gestation [43]. Gestational age-dependent changes in placental P-gp expression in mice were also confirmed by the observation of a significant increase in transplacental transfer of digoxin and saquinavir in late gestation as compared with earlier gestational days [50,51]. The physiological relevance of gestational age-dependent expression of placental P-gp in terms of placental function and fetal development is still unclear. With respect to the protective role of placental P-gp for the developing fetus, it is reasonable to assume that the fetus is more sensitive to toxic insult from xenobiotics or endogenous substances in early pregnancy and therefore the expression of P-gp in the placenta would be upregulated in early pregnancy as a mechanism to protect the fetus. As pregnancy advances, this need for protection of the fetus decreases, leading to lower P-gp expression in the placenta later in pregnancy. In cancer cells, in addition to its role in the efflux of drugs, P-gp has been shown to protect the cells through counteracting apoptosis by regulating intracellular concentrations of some extrinsic activators of the intrinsic apoptotic pathway such as sphingomyelin [52]. This is likely also the case for the placenta as formation of the syncytiotrophoblast involves apoptotic processes. Therefore, up-regulation of placental P-gp would be needed to protect the placental cells and hence the fetus, which are particularly vulnerable in early pregnancy.

The exact mechanisms by which placental P-gp expression is regulated at different gestational ages are still unknown. Regulation of the transcriptional activity of the human *MDR1* gene depends on several transacting proteins that bind to the consensus cis-elements of the *MDR1* promoter. For example, Sp nuclear transcriptional factors bind to GC-rich regions in the *MDR1* promoter, which is essential for *MDR1* activation [53]. Expression of Y-boxing binding 1 (YB-1) strongly correlated with activation of *MDR1* gene expression [54,55]. Other transcriptional factors have also been reported to control *MDR1* expression, such as Hypoxia-inducible factor 1 (HIF1) and Pregnane X receptor (PXR) [56]. Thus, it is reasonable to hypothesize that some of these factors may play an important role in regulation of P-gp expression in the placenta. The concentrations of pregnancy-related hormones, such as estrogens and progesterone, drastically increase during pregnancy [57]. Thus, it is also conceivable that P-gp expression in the placenta could be regulated in association with the levels of these hormones, as evidenced by the findings that P-gp expression in *in vivo* or *in vitro* models is regulated by estrogens and progesterone [42,58-63]. However, contradicting data are available in the literature, with some studies showing induction of P-gp by estrogens or progesterone [60-62,64] and other studies showing down-regulation of P-gp by estrogens or no effect from progesterone [63]. Therefore, more research into whether and how these pregnancy-related hormones control gestational age-dependent P-gp expression in the placenta are needed. Synthetic glucocorticoids were recently shown to down-regulate P-gp expression in the guinea pig placental barrier [48].

Substantial variability in P-gp expression and activity in human placenta have been reported. Genetic polymorphisms are believed to be a major cause of such variability in P-gp expression and activity in human placenta [65]. To date, more than 50 single nucleotide polymorphisms (SNPs) have been reported in the *MDR1* gene [66,67]. Among them, of particular interest in the studies using placenta tissues is the identification of three most common SNPs in *MDR1* coding region, C1236T, C3435T, and G2677T/A. The SNPs C1236T and C3435T are synonymous; however, G2677T/A results in an amino acid switch from Ala to Ser/Thr at amino acid position 893 of P-gp. The three SNPs were found in linkage disequilibrium in 31–49% of the Chinese, Malay, and Indian populations, 45–55% of Caucasians, and 5–10% of African Americans [68,69]. At present, consensus on the effect

of these SNPs on P-gp expression and transport activity in the placenta remains obscure. Lower P-gp protein expression was observed in primary human placental trophoblasts carrying the G2677A/T allele, but not the C3435T allele [70]. Interestingly, no significant association was found between the *MDR1* mRNA expression in human placenta and the G2677T or C3435T genotype in the mothers or the infants or for the mothers and infants with the same genotype at position G2677T or C3435T, respectively; however, P-gp protein expression in the placenta was significantly lower when both the mother and the infant were homozygous for the 3435T allele (TT/tt) compared to the 3435C allele (CC/cc) [71]. Moreover, significantly lower P-gp protein expression was observed in placentas from the mothers carrying both the G2677T and C3435T polymorphisms compared to wild-type individuals [71]. In contrast, another study revealed that the C3435T allele was associated with a higher placental P-gp protein level compared with the wild-type alleles, whereas there was no significant association between the G2677T/A polymorphism and placental P-gp expression [37]. In the perfused human placenta, the C3435T allele was associated with significantly higher placental transfer of quetiapine [72]. Neither G2677T nor C3435T polymorphism affected placental transfer of saquinavir, nor was there any correlation between P-gp expression and saquinavir transfer [36,37]. Most recently, Hemauer *et al.* [73] reported that, in human term placenta, the P-gp protein level of the C1236T allele was 11% lower than the wild-type protein, whereas the C3435T and G2677T/A variants each was associated with a 16% reduction in the P-gp protein level. Placental P-gp activity did not seem to correlate with its expression level because the homozygotes for the C1236T and C3435T alleles (TT) were found to have a 42% and 47% increase, respectively, in P-gp-mediated ATP-dependent uptake of the prototypic substrate [³H]-paclitaxel into vesicles prepared from placental brush border membranes. Ten SNPs were identified in the *MDR1* promoter region in the Japanese and Caucasian populations [67]. The haplotypes containing T-1517aC, T-1017aC and T-129C in the promoter region were associated with an increase in transcriptional activity and the *MDR1* mRNA level, but were independent of C3435T. However, in another study, the T-129C allele was found to be associated with a significantly lower placental P-gp protein level [70]. The functional consequence of these SNPs in the *MDR1* promoter region has yet to be determined. Due to the nature of the contrasting data available in the present literature, the clinical relevance of the SNPs of placental P-gp is still unclear. Several factors including rapid degradation of *MDR1* mRNA in placenta tissues after delivery, relatively small sample size, only term placenta used with low P-gp expression, possible contamination of placenta with maternal tissues and blood, and the potential influence of medication on placental P-gp expression may contribute to the inconsistency and large variation in placental P-gp expression associated with each polymorphism. More detailed discussion on these factors can be found in the review article [74]. It is also worth noting that the placenta is of fetal origin and hence the maternal and fetal genotypes may not be the same. Therefore, the correlation of the maternal and fetal genotypes with placental P-gp expression can be different and should be distinguished. Most of the published studies investigated the correlation between the fetal genotype (using placenta tissues or umbilical cord blood) and placental P-gp expression. Thorough and well-designed investigations into the relationship between SNPs of placental P-gp and not only P-gp protein or mRNA expression but, more importantly, P-gp transport activity are needed in future work to address this issue.

3. BREAST CANCER RESISTANCE PROTEIN (BCRP, GENE SYMBOL ABCG2)

Breast Cancer Resistance Protein (BCRP), also known as ABCG2, is the second member of the subfamily G of the large ABC transporter superfamily. BCRP was first discovered in 1998 from a human breast cancer subline (MCF-7/AdrVp) that conferred resistance to

chemotherapeutic agents, such as mitoxantrone and doxorubicin, in the absence of overexpression of other known multidrug transporters P-gp and MRPs [75]. Subsequently, two proteins nearly identical in amino acid sequence to BCRP were identified from the mitoxantrone-resistant human colon carcinoma cells (S1-M1-80) [76] and the expressed sequence tag database [77]. Hence, the two proteins were named mitoxantrone resistance (MXR) and placenta ABC (ABCP) transporters, respectively. A unique feature of the ABCG members is that they comprise only one (NBD) at the N-terminus followed by one MSD at the C-terminus [26,78–80]. Thus, BCRP is a half ABC transporter.

Human BCRP and mouse Bcrp1 confer resistance to a highly diverse range of substrates including hydrophobic chemotherapeutic agents, organic anions, and conjugates of many endogenous and exogenous substances. Typical chemotherapeutic agents that are BCRP/Bcrp1 substrates include anthracyclines (e.g. daunorubicin and doxorubicin) (note that doxorubicin is a substrate of the BCRP/Bcrp1 mutants with substitutions of Arg at amino acid position 482, but not of wild-type BCRP/Bcrp1), camptothecins and its derivatives (e.g. topotecan and SN-38). BCRP/Bcrp1 also transports a large number of non-chemotherapy drugs such as glyburide [81,82] and nitrofurantoin [83]. There is a substantial substrate overlap between BCRP, P-gp, and MRPs, although transport efficiencies can differ widely [75,84–87].

BCRP is highly expressed in stem cells [88,89], in the luminal membrane of the small and large intestines, in the liver canalicular membrane, in the brain microvessel endothelium, and in the apical membrane of placental syncytiotrophoblasts [90–92] (Table 1). BCRP expression in the placenta is the highest among these tissues. Likewise, Bcrp1 is also highly expressed in the apical membrane of syncytiotrophoblasts of mouse placenta [93].

In *in vitro* studies, BCRP has been shown to mediate transport of mitoxantrone [94] and glyburide [95] in membrane vesicles prepared from human term placenta, as well as mitoxantrone and Hoechst 33342 in membrane vesicles isolated from the placental BeWo cells [96] or primary human placental trophoblasts [97]. In *ex vivo* studies, human BCRP and rat Bcrp1 have been shown to significantly limit the maternal-to-fetal transport of PhIP [98], glyburide [99], and cimetidine [100], respectively, in the perfused human and rat placenta models. In pregnant mice deficient in P-gp, co-administration with the Bcrp1 inhibitor GF120918 caused a significant increase in the fetal concentration of topotecan [101]. Increased fetal/maternal plasma concentration ratios in the *Bcrp1*(*-/-*) mice compared with those in wild-type pregnant mice have also been reported for topotecan and phytoestrogens [102,103]. Recently, our laboratory examined the role of Bcrp1 in determining fetal exposure of nitrofurantoin in pregnant mice, clearly suggesting that Bcrp1 significantly limits fetal distribution of nitrofurantoin [104]. We also performed similar studies for glyburide, an antidiabetic drug commonly used to treat gestational diabetes. We found that the fetal AUC of glyburide in the *Bcrp1*(*-/-*) pregnant mice was two times as much as that in the wild-type pregnant mice [81]. These *in vitro* and *in vivo* studies strongly suggest the role of BCRP/Bcrp1 in limiting fetal exposure of exogenous drugs and xenobiotics and in facilitating elimination of drugs and xenobiotics out of the fetal compartment.

Besides the protective role of BCRP against potential toxicity of drugs and xenobiotics for the fetus, other physiological roles of BCRP in the placenta have also been proposed. BCRP may be involved in regulating placental estrogen synthesis by mediating the concentrations of dehydroepiandrosterone sulfate (DHEAS) and estrone 3-sulfate (E_1S) in the placenta [105]. Inhibition of BCRP activity by a BCRP specific inhibitor Ko143 or the knock-down of BCRP expression by siRNA caused cytokine-induced apoptosis in primary human placental trophoblasts and BeWo cells as well as a cytokine-induced increase in the

intracellular ceramide levels [106,107]. The level of BCRP in the placenta from pregnancies with idiopathic fetal growth restriction (IUGR) was also shown to be significantly lower than that from normal pregnancies [106,107]. These results support the hypothesis that the lower BCRP expression in the placenta from IUGR pregnancies may cause a deficit in placental function and survival, thus leading to fetal growth restriction. The mechanism of this phenomenon has yet to be determined. Most recently, BCRP has been shown to actively transport sphingosine-1-phosphate [108], which can inhibit the differentiation of cytotrophoblasts into syncytiotrophoblasts [109]. Therefore, BCRP may also play a role in the control of the differentiation of cytotrophoblasts by mediating the intracellular concentration of sphingosine-1-phosphate. In *Bcrp1*($-/-$) pregnant mice, we did not observe any significant effect of *Bcrp1* knockout on the development of the fetus (e.g. size and weight of the fetus) and the *Bcrp1*($-/-$) offspring. This might be due to the compensation effect through up-regulation of other ABC transporters in *Bcrp1*($-/-$) mice.

BCRP/*Bcrp1* expression is also dependent on gestational age. Our laboratory demonstrated that *Bcrp1* expression (protein and mRNA) in the placenta of pregnant mice peaked at gestation day 15 [93]. Similarly, the *Bcrp* mRNA or protein levels in the placenta of pregnant rats peaked at gestation day 14 or 15 and declines significantly at term [110,111]. In contrast, Kalabis *et al.* showed that the *Bcrp1* mRNA level in the placenta of pregnant mice decreased progressively from gestation day 9.5 toward term; however, the *Bcrp1* protein level did not significantly change during gestation [112]. Current data on BCRP expression in human placenta with advancing gestational age are rather inconsistent [46,113,114]; however, Meyer zu Schwabedissen *et al.* showed with a relatively large number of placenta samples that BCRP protein and mRNA expression in human placenta at preterm (28 ± 1 weeks, 15 placentas) was approximately 2 times greater than that at term (39 ± 2 weeks, 29 placentas) [113]. These human data are consistent with those obtained in mice [93] and rats [110,111].

To date, two *cis* elements in the BCRP gene promoter (the estrogen response element [115] and the peroxisome proliferators-activated response (PPAR) element [116]) and several nuclear receptors (e.g. the peroxisome proliferators-activated receptor gamma (PPAR γ), the hypoxia-inducible factor 1 (HIF-1), and the aryl hydrocarbon receptor (AhR)) have been shown to control BCRP gene transcription [116-119]. Other factors have also been reported to regulate BCRP expression. For example, the aberrant methylation in the predicted CpG island of the BCRP promoter suppresses transcription of BCRP gene [120]. Whether the aforementioned regulation mechanisms are involved in the control of BCRP gene expression in the placenta remains to be determined.

The drastic changes in hormone levels during pregnancy may affect BCRP expression in the placenta. In *in vitro* studies, we have shown that progesterone up-regulates BCRP expression in BeWo cells through progesterone receptor isoform PRB, but not PRA [121]. We have also identified a progesterone response element (PRE) in the BCRP promoter region [121]. In contrast, it has also been reported that progesterone treatment did not affect BCRP expression in primary human placental trophoblasts [64]. It could be reasoned that the trophoblasts secrete a high level of endogenous progesterone, thus saturating the progesterone receptor. Therefore, further addition of exogenous progesterone may not have a strong effect, as we observed in BeWo cells [121,122]. The effect of progesterone on BCRP expression is also dependent on both the levels of PRB and the ratio of PRA/PRB in cell systems used [123,124]. This is likely because we have shown that PRA represses the PRB activity [121]. In future studies, it is worth testing whether BCRP expression in the placenta is regulated by the change in the progesterone level with advancing gestation via PRB, thereby strengthening the protective role of BCRP during pregnancy. Contradicting data are available in the literature with respect to the effect of 17 β -estradiol on BCRP

expression. It has been shown that 17β -estradiol induces BCRP expression in various cancer cell lines including BeWo cells via the estrogen receptor α (ER α) [115,125]. However, Imai *et al.* reported that 17β -estradiol decreased BCRP expression in ER α -positive cancer cell lines through posttranscriptional regulation [126]. We also found that 17β -estradiol down-regulated BCRP expression in BeWo cells [122]. However, combinations of 17β -estradiol and progesterone at certain ratios further induced BCRP expression in BeWo cells compared with the effect of progesterone alone, presumably through induction of PRB by 17β -estradiol [122]. Therefore, it is also important to consider the effect of combinations of the two hormones on BCRP expression.

Estriol, human placental lactogen, and human prolactin could also induce BCRP expression in BeWo cells at physiological concentrations [127]. Testosterone in combination with 17β -estradiol increased BCRP expression, and this induction was abolished by the ER α antagonist ICI-182,780, the testosterone receptor antagonist flutamide, or knockdown of ER α [127]. Tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) were shown to significantly decrease BCRP expression at the mRNA and protein levels, whereas epidermal growth factor (EGF) and insulin-like growth factor II significantly increased BCRP expression [64,113]. Maternal inflammation has been indicated to alter BCRP expression in the placenta [10]. Taken together, gestational age-dependent expression of BCRP in the placenta could be regulated by a complex mechanism, with contributions from multiple hormones and growth factors whose concentrations are drastically changed during the course of pregnancy.

Over 80 naturally occurring SNPs in the *BCRP* gene have been reported thus far [128]. Among those, SNPs G34A and C421A occur at a relatively high frequency in most ethnic populations. The C421A SNP (the Q141K variant) occurs with a low frequency in people of American, European, Hispanic and Middle Eastern descent, but occurs at a much higher level in the Chinese or Japanese population [129]. In the Japanese population, 39–50% are heterozygous and 7% homozygous for C421A [130,131], and 60% are heterozygous in the Chinese population [131]. BCRP SNPs may result in variable expression of the transporter in tissues. Imai *et al.* showed that the C421A polymorphism was associated with decreased expression and activity of BCRP [130]. BCRP SNPs have also been reported in human placenta with lower BCRP protein expression associated with the C421A polymorphism [132]. Therefore, these BCRP SNPs potentially affect expression and activity of the transporter in human placenta, thereby leading to alteration of its protection against fetal drug exposure. Most recently, the apparent K_t and V_{max} values of the Q141K variant for glyburide have been shown to be significantly higher than those of wild-type BCRP, suggesting potential changes in BCRP-mediated transplacental transport of the drug in pregnant women carrying the C421A SNP [133]. It is worth noting that glyburide is being increasingly used to treat gestational diabetes.

4. MULTI-DRUG RESISTANCE PROTEINS (MRPS, GENE SYMBOL ABCCS)

The MRP/ABCC subfamily of the ABC transporter superfamily contains nine members of the multidrug resistance proteins (MRP1-6/ABCC1-6 and MRP7-9/ABCC10-12) that share structural and functional similarities [134,135], the cystic fibrosis transmembrane conductance regulator (CFTR/ABCC7), two sulfonyleurea receptors that are the ATP-sensing subunits of a complex potassium channel (SUR1-2/ABCC8-9), and MRP10/ABCC13 (a pseudogene). MRP10/ABCC13 shares the highest similarity to MRP2/ABCC2, but without transport activity. Of those MRPs, the relatively more extensively studied members in human and rodent placentas are MRP1–3 and 5. However, compared to P-gp and BCRP, much less is known about the functional significance of these MRPs in the placenta. MRPs can be divided into two groups based on the existence of an extra MSD at the N-terminus

(MSD0) which contains five transmembrane α -helices [136–138]. The group that possesses MSD0 includes MRP1, MRP2, MRP3, MRP6, and MRP7, while the group not possessing MSD0 is composed of MRP4, MRP5, MRP8, and MRP9 [139–141]. MRPs are expressed in the placental syncytiotrophoblasts and/or the endothelial cells of fetal capillaries [94,142–144] (Table 1).

MRP1, a 190 kDa plasma membrane protein, was first cloned in 1992 from a lung cancer cell line that was associated with non-P-gp-mediated multidrug resistance [145]. The amino acid sequence identity of MRP1 and P-gp is relatively low (~15%). MRP1 can transport a broad range of substrates. The substrate specificity of P-gp and MRP1 differ markedly, in that P-gp substrates are neutral or mildly positive lipophilic compounds, and MRP1 primarily transports lipophilic anions despite their similar drug resistance profiles to anthracyclines, vinca alkaloids, epipodophyllotoxins, camptothecins, and methotrexate [146–149]. The substrates of MRP1 also include glutathione, sulfate, and glucuronide conjugates such as LTC₄ and GS-prostaglandin A₂, 17 β -estradiol 17 β -D-glucuronide (E₂17 β G), estrone 3-sulfate, and sulfatolithocholate [150–155]. In humans, MRP1 is highly expressed in lung, bladder, spleen, testes, thyroid, and adrenal glands [156]. Particularly, in human placenta, MRP1 is localized in the basolateral membrane of the syncytiotrophoblasts and the endothelium of the fetal vessels [142,157] (Table 1). However, it has also been suggested that MRP1 is present in the apical membrane of the placental syncytiotrophoblasts [144,158] and the BeWo cells [159]. Mouse Mrp1 is highly expressed in tissues such as testes, ovaries, and placenta [160].

Studies using *Mrp1*($-/-$) mice have revealed that Mrp1 functions as an *in vivo* resistance factor against anticancer agents, which is supported by the finding that *Mrp1*($-/-$) mice become hypersensitive to etoposide which is a Mrp1 substrate [161,162]. The involvement of Mrp1 in glutathione homeostasis and in LTC₄-mediated inflammatory processes has also been demonstrated using *Mrp1*($-/-$) mice [161]. Most recently, MRP1 or a MRP1-like transporter located in the apical membrane of the syncytiotrophoblast of human placenta was suggested to play a role in mediating 2,4-dinitrophenyl-S-glutathione (DNP-SG) efflux [158]. A non-steroidal estrogenic mycotoxin, zearalenone, has been shown to induce MRP1 expression in BeWo cells [163]. To date, however, the functional importance and regulation of MRP1 expression in the placenta remains largely unclear.

MRP2/ABCC2, also known as the canalicular multispecific organic anion transporter (cMOAT), was initially discovered as an organic anion transporter in the canalicular membrane of hepatocytes, and its mutations were identified in patients with Dubin-Johnson syndrome [164–165]. MRP2 is mainly expressed in the apical surface of polarized cells in the liver, duodenum, and kidney [166,167]. MRP2 expression has also been found in the apical membrane of the placental syncytiotrophoblasts [144,168]. The substrate specificity of MRP2 is similar to that of MRP1, but the relative affinity of some substrates to MRP2 such as LTC₄ and other glutathione conjugates were significantly lower than that to MRP1 [169]. In contrast, substrates such as methotrexate are more efficiently transported by MRP2 than MRP1 [169]. MRP2 may provide fetal protection against harmful drugs/xenobiotics and endogenous toxic substances as does P-gp [170]. Recently, May *et al.* observed that the maternal-to-fetal transfer of talinolol was increased by the MRP2 inhibitor probenecid, demonstrating the functional significance of MRP2 in human placenta [171]. MRP2 expression in the placenta changes with gestational age. For example, it has been shown that both the mRNA and protein levels of MRP2 in human placenta increase with advancing gestation accompanied by a differentiation of cytotrophoblasts into syncytiotrophoblasts, indicating a developmental control of MRP2 in human placenta [168]. In regard to regulation of MRP2 expression, the synthetic estrogen ethinylestradiol induced Mrp2 mRNA but decreased its protein levels in primary rat hepatocytes [172]. Some steroid

hormones, such as a combination of thyroxine and growth hormone, were found to increase expression of rat Mrp2 in cultured hepatocytes [173]. No information about hormonal regulation of MRP2 expression in the placenta is available.

MRP3/ABCC3 is also an organic anion transporter [174]. MRP3 is localized in the basolateral membranes of polarized cells [175,176]. MRP3 has also been reported to be localized in the fetal vessel endothelial cells and the apical side of the placental syncytiotrophoblasts to a lesser extent [144], although uncertainty remains with respect to the membrane localization of this transporter in the placenta (Table 1). It appears that MRP3 prefers to transport glucuronide conjugates over glutathione and sulfate conjugates, and shares a considerable overlap in substrate specificity with MRP1-2. It transports E₂17βG and LTC₄, but does not transport various GSH conjugates [177]. The functional significance of MRP3 in the placenta has yet to be demonstrated.

MRP5/ABCC5 is widely expressed in mouse and human tissues [136,160,166]. MRP5 is preferentially localized at the basal membrane of syncytiotrophoblasts as well as endothelial cells of fetal vessel [178] (Table 1). MRP5 exhibits a distinct substrate specificity from those of MRP1-3, presumably due to the lack of MSD0 as well as the low amino acid similarity [136]. MRP5 can transport organic anionic compounds, such as glucuronide and sulfate conjugates of steroids including E₂17βG and DHEAS. MRP5 is also an important transporter of cyclic AMP (cAMP) and cyclic GMP (cGMP) [151,179,180]. MRP5 protein expression has been found to decrease towards term with the highest expression level in early pre-term placenta, which implies a specific role in regulating cGMP concentration during pregnancy [178]. The functional role of placental MRP5 in drug transport has not been demonstrated.

SNPs of MRPs have been reported [181–183]. However, the relationship between SNPs and protein expression and activity has yet to be determined. A SNP (G1249A) of MRP2 was found to be associated with decreased expression of the transporter in pre-term human placenta samples [168]. Other MRPs, such as MRP4 [160,184], MRP6 [160], MRP7 [184], and MRP8 [139] have also been detected in the placenta at the mRNA level; however, their membrane localization and functional importance in the placenta remain to be elucidated.

5. CLINICAL SIGNIFICANCE OF ABC TRANSPORTERS IN THE PLACENTA AND FUTURE PERSPECTIVES

It is of great importance to understand the roles of placental ABC transporters in limiting fetal drug exposure as an increasing number of drugs routinely administered to pregnant women are now known to be substrates and/or inhibitors of these ABC transporters. Hence, more research into the effects of these ABC transporters on transplacental pharmacokinetics of drugs is needed to minimize fetal drug toxicity and optimize therapeutic strategies for pregnant women. When two or more drugs are co-administered, drug-drug interactions at the placental barrier could occur if they happen to be substrates and/or inhibitors of the ABC transporters. Altered transporter activity attributable to induction or down-regulation with drug treatment may also result in drug-drug interactions, leading to unpredictable drug concentrations in the fetal compartment, and therefore either potential therapeutic failure or high dose toxicity. Thus, more investigations into transporter-mediated drug-drug interactions at the placental barrier and the underlying mechanisms are required in future studies.

Expression and activity of ABC transporters are dependent on gestational age, and are usually under the tight control of many factors, such as the levels of pregnancy-specific hormones and growth factors produced during pregnancy. Therefore, the maternal-to-fetal

transfer of drugs and fetal drug exposure in early or later gestation may change in association with regulation of the relevant transporters in the placenta. Continued research into the molecular mechanisms by which expression of placental ABC transporters is regulated in the course of pregnancy may help develop rational strategies to control fetal drug exposure and optimize drug therapy for pregnant women. Studies on variability in expression and activity of placental ABC transporters caused by SNPs are also of great importance in deciphering the potentially wide variability in fetal drug exposure after maternal administration and improving personalized medicine. Taken together, a thorough understanding of the behaviors of ABC transporters in the placenta using *in vitro* methods and *in vivo* animal models as well as clinical studies in various aspects reviewed in this article is desperately needed to achieve the goal of improving drug therapy in pregnant women.

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Table 1

Primary ABC Efflux Transporters in Human Placenta

Transporter	Gene Symbol	Localization in Placental Syncytiotrophoblasts	Selected Substrate Drugs
P-gp	<i>ABCB1</i>	Apical	anthracyclines (e.g. daunorubicin, doxorubicin) HIV protease inhibitors (e.g. indinavir, saquinavir) immunosuppressants (e.g. cyclosporine A) phenytoin, quinidine, terfenadine, paclitaxel, ketoconazole, loperamide, atorvastatin, methadone, digoxin, fexofenadine
BCRP	<i>ABCG2</i>	Apical	anthracyclines, mitoxantrone, nitrofurantoin, glyburide, methotrexate, prazosin, zidovudine, topotecan, SN-38, flavopiridol, pantoprazole, cimetidine, imatinib, statins
MRP1	<i>ABCC1</i>	Apical and/or basolateral	etoposide, methotrexate, vinblastine, saquinavir, cisplatin, mitoxantrone, topotecan
MRP2	<i>ABCC2</i>	Apical	etoposide, methotrexate, paclitaxel, vincristine, cisplatin, arsenite, rifampicin, pravastatin
MRP3	<i>ABCC3</i>	Apical and/or basolateral	methotrexate, etoposide, acetaminophen, adefovir
MRP5	<i>ABCC5</i>	Basolateral	methotrexate, nucleoside analogues