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REVIEW

Drug transporters in the human blood-placental barrier

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Studies on the increasing number of transporters found in the placental barrier are gaining momentum, because of their tissue-specific expression, significance in physiology and disease, and the possible utilization of the emerging knowledge in pharmacology. In the placenta, both syncytiotrophoblast and fetal capillary endothelium express transporters. Fetal exposure is determined by the net effect of combination of transporters, their nature and localization in relation to placental cells and their substrate specificity. Although the significance of placental transporters on human fetal drug exposure is almost an unstudied field so far, their potential use to design drugs that do not cross the placenta is already being pursued. It is thus of interest to review the existing knowledge of human placental transporters. Transporters in all groups which take part in drug transport are found in human placenta. Especially, ATP-binding cassette transporters ABCG2/breast cancer resistance protein, ABCB1/P-glycoprotein and ABCC2/MRP2 are all expressed at the apical surface of syncytiotrophoblast facing maternal blood and are putatively important protective proteins both for placental tissue and the fetus, because they are efflux transporters and their substrates include many drugs and also environmental chemicals. Such protective effect has been shown in animals, but these results cannot be directly extrapolated to humans due to interspecies differences in placental structure and function. Experimental models utilizing human placental tissue, especially human placental perfusion, offer valuable possibilities, which have been insufficiently studied so far.
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Keywords: syncytiotrophoblast; ABC transporters; breast cancer resistance protein; P-glycoprotein; organic anion transporters; organic cation transporters; monoamine transporters

Abbreviations: ABC, ATP-binding cassette; ABCP, placental ABC protein; BCRP, breast cancer resistance protein; CAR, constitutive androstane receptor; FXR, farnesoid X receptor; MCT, monocarboxylate transporter family; MDR1, multidrug resistance protein 1; MRP1-3, multidrug resistance-associated protein 1-3; MXR, mitoxantrone resistance protein; NET, noradrenaline transporter; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCTN2, organic cation transporter; P-gp, P-glycoprotein; PXR, pregnane X receptor; SERT, 5-HT (serotonin) transporter; SLC, solute carrier protein; SXR, steroid and xenobiotic receptor

Introduction

Important paths for endogenous and exogenous compounds through biological membranes, including membranes in the blood-placental barrier, are provided by specialized transporter proteins (Klaassen and Lu, 2008). Transporters in several groups take part in drug transport: In the large group of ATP-binding cassette (ABC) transporters with 52 members, five proteins are heavily involved in drug transport: ABCB1/P-glycoprotein (P-gp)/MDR1, ABCC1-3/MRP1-3 and ABCG2/breast cancer resistance protein (BCRP) (see Leslie *et al.*, 2005; Sarkadi *et al.*, 2006; Behravan and Piquette-Miller, 2007; Wang *et al.*, 2007; Zhou, 2008). In addition, ATP-independent transporters in solute carrier protein (SLC) families as well as monoamine, monocarboxylate and nucleoside transporters also transport drugs (Unadkat *et al.*, 2004; Koepsell *et al.*, 2007). Transporters in all of these groups are found in human placenta (Table 1).

Due to their active role in pharmacokinetics, drug interactions involving transporters are of clinical importance (Mizuno *et al.*, 2003; Henrich *et al.*, 2007; Dutta *et al.*, 2008; Zhou *et al.*, 2008b). In addition, environmental toxic agents may be substrates of the same transporters and affect their expression. For example, aflatoxin B1, a compound toxic and carcinogenic to liver, is a substrate of ABCG2/BCRP (van Herwaarden *et al.*, 2006), but not of ABCB1/P-gp, which it,

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Table 1 Characteristics of drug transporters found in human placenta

Name	*Alternative names*	*Chromosome*	*Cellular localization in placenta*	*Substrates*	*Inhibitors*	*Reference*
ABCB1	P-gp MDR1	7q21.1	ST (apical)	Anticancer drugs, protease inhibitors, drugs of abuse, steroids, verapamil, cyclosporin	Verapamil, cyclosporin, PSC833 (valspodar), GF120918, simvastatin, lovastatin, atorvastatin	1, 2, 3, 4, 5
ABCC1	MRP1	16p13.1	ST (basol), ET	Organic anions, glutathione conjugates, nucleotide analogues, anticancer drugs	Probenecid, cyclosporin, PSC833, MK571	1, 3, 4, 6
ABCC2	MRP2	10q24	ST (apical)	Organic anions, glutathione conjugates, nucleotide analogues, anticancer drugs	Probenecid, cyclosporin, PSC833, MK571	1, 3, 4
ABCC3	MRP3	17q22	ST (apical), ET	Anticancer drugs	MK571, benzbromarone, probenecid	1, 3, 4
ABCC5	MRP5		ST (basol), ET	cAMP, cGMP	Probenecid, sildenafil	4, 7, 8
ABCG2	BCRP	4q22	ST (apical), ET	Anticancer drugs, oestrone sulphates, cimetidine, glyburide, nitrofurantoin	GF120918, KO143, fumitremorgin C, glucocorticoids, digoxin, novobiocin, nicardipine	1, 9, 10, 11, 12, 13
SLC6A2	NET	16q12.2	ST (basol)	MPP+, amphetamines	Amphetamines, tricyclic antidepressants	1, 3, 14, 15, 16
SLC6A4	SERT	17q11.1–17q12	ST (apical)	MPP+, amphetamines	Tricyclic antidepressants, SSRI, cocaine	1, 3, 14, 16
SLC10A6	SOAT	4q21.3	T	DHEAS, oestrone sulphate, pregnenolone sulphate, methylpyrene sulphates	Methylpyrene-sulphates (2-SMP, 4-SMP), BSP	17
SLC21A3	OATP-A, SLCO1A2	12p12	T	17-β-D glucuronyl oestradiol	Anti-HIV drugs, dexamethasone, erythromycin, verapamil	1, 18
SLC21A6	OATP-C, SLCO1B1	12p12	T	17-β-D glucuronyl oestradiol	Glycyrrhizin, cyclosporin A, FK-506	1, 18
SLC21A8	OATP-8, SLCO1B3	12p12	T	Digoxin, fexofenadine	Glycyrrhizin	1, 18, 19, 20
SLC21A9	OATP-B OATP2B1, SLCO2B1	11q13	ST (basol), CT (basol)	oestrone sulphate, 17-β-D glucuronyl oestradiol		1, 19, 21, 22
SLC21A11	OATP-D, SLCO3A1	15q26	Unknown			1
SLC21A12	OATP-E, SLCO4A1	20q13.33	ST (apical)		BSP	1
SLC22A3	OCT3 EMT	6q26–q27	Unknown, ST (basol)	antidepressants, amphetamines, MPP+	Corticosterone, β-oestradiol, MPTP, imipramine, desipramine	1, 3, 16
SLC22A4	OCTN1	5q31.1	Unknown	verapamil, quinidine	D-carnitine, nicotine, cimetidine	1, 3
SLC22A5	OCTN2	5q31	ST (apical)	L-carnitine, verapamil, quinidine, beta-lactam antibiotics	Corticosterone, MPTP, nicotine	1, 3, 23
SLC22A9 SLC22A11	OAT4	11q12.3	ST (basol), CT (basol)	DHEAS, oestrone sulphate, tetracycline, zidovudine, methotrexate, salicylate, ketoprofen	Taurocholate, probenecid, BSP, corticosterone	1, 21, 24

Substrates and inhibitors found in any experimental system are included.

Data from (1) Wang *et al.* (2007), (2) Holtzman *et al.* (2006), (3) Unadkat *et al.* (2004), (4) Schinkel & Jonker (2003), (5) McDevitt & Callaghan (2007), (6) Atkinson *et al.* (2003), (7) Meyer zu Schwabedissen *et al.* (2005a), (8) McAleer *et al.* (1999), (9) Ceckova *et al.* (2006), (10) Pavek, *et al.* (2005), (11) Gedeon *et al.* (2008), (12) Pollex *et al.* (2008), (13) Merino *et al.* (2005), (14) Ramamoorthy *et al.* (1995), (15) Bruss *et al.* (1993), (16) Martel and Keating (2003), (17) Geyer *et al.* (2007), (18) Briz *et al.* (2003), (19) Kullak-Ublick *et al.* (2001), (20) Shimizu *et al.* (2005), (21) Ugele *et al.* (2003), (22) Ugele *et al.* (2008), (23) Grube *et al.* (2005), (24) Rizwan & Burckhardt (2007).

ABC, ATP-binding cassette; basol, basolateral; BCRP, breast cancer resistance protein; BSP, bromosulphophthalein; cAMP, cyclic adenosine monophosphate; cGMP, guanosine triphosphate; CT, cytotrophoblast; DHEAS, dehydroepiandrosterone sulfate; ET, endothelium of fetal blood vessels; MPP+, 1-methyl-4-phenyl pyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRP, multidrug resistance-associated protein; P-gp, P-glycoprotein; OATP, organic anion transporter; OCT, organic cation transporter; SERT, 5-HT (serotonin) transporter; SLC, solute carrier protein; SOAT, sodium-dependent organic anion transporter; SSRI, serotonin specific reuptake inhibitors; ST, syncytiotrophoblast; T, trophoblasts.

however, induces (Santoni-Rugiu and Silverman, 1997). The glutathione conjugate of aflatoxin B1 has been shown to be a substrate of ABCC/MRP transporters (Cole and Deeley, 1998; Deeley and Cole, 2006), and ochratoxin A is a substrate of hOATs (Babu *et al.*, 2002, see also Rizwan and Burckhardt, 2007). Studies on the increasing number of transporters found in the placental barrier are gaining momentum, because of their tissue-specific expression, significance in physiology and disease, and the possible utilization of the emerging knowledge in pharmacology (Bottalico *et al.*, 2004; Ganapathy and Prasad, 2005; Evseenko *et al.*, 2006b; Gedeon and Koren, 2006; Behravan and Piquette-Miller, 2007; Cleal and Lewis, 2008). Polymorphisms of many transporters, including ABCB1/MDR1, ABCC1/MRP1, ABCC2/MRP2 and SLC22A5/OCTN2, have been detected (see Mizuno *et al.*, 2003). The few studies existing on ABCB1/P-gp polymorphisms in human placenta show that transporter polymorphisms in the placenta may have significance in fetal exposure (Rahi *et al.*, 2008 and references within).

Although much important information on the possible substrate specificity of transporters has been gained through *in vitro* studies, the ultimate proof of functional significance requires *in vivo* studies (Klaassen and Lu, 2008). As there are interspecies differences in placental structure (Benirschke *et al.*, 2006) and the transfer to the fetus (see e.g. Carney *et al.*, 2004; Myllynen *et al.*, 2005), models utilizing human placental tissue or *in vivo* human studies are essential for final conclusions applicable to clinical practice (Vähäkangas and Myllynen, 2006). In particular, human placental perfusion has appeared useful (see Myllynen *et al.*, 2003; 2008; Nanovskaya *et al.*, 2005; May *et al.*, 2008; Rahi *et al.*, 2008) because it retains the tissue structure and the polarized nature of the syncytiotrophoblast better than tissue explants (Di Santo *et al.*, 2003). However, it still has its restrictions and the results have to be compared with those from *in vivo* human studies (Myllynen and Vähäkangas, 2002; Myllynen *et al.*, 2003). Naturally, only drugs necessary during pregnancy can be studied *in vivo*.

The importance of drug transporters in protection from fetotoxic effects of chemicals has been confirmed in transporter knockout animals (for recent reviews see Behravan and Piquette-Miller, 2007; Klaassen and Lu, 2008). For instance, lack of P-gp in the placenta increases susceptibility of mouse fetuses to the teratogenic effect by a pesticide, ivermectin, and increases drug concentration in fetuses of dams treated with digoxin, saquinavir and paclitaxel (Smit *et al.*, 1999 and references therein). Accordingly, inhibitors of ABCG2/BCRP and ABCB1/P-gp/MDR1, like GF120918, increase fetal exposure to topotecan (Jonker *et al.*, 2000) and cimetidine (Staud *et al.*, 2006). The important role of ABCG2/BCRP is supported by the fact that the BCRP genotype multiplies fetal nitrofurantoin concentration compared with wild type mice (Zhang *et al.*, 2007). In this review, we concentrate on what is known so far about the existence and role of drug transporters in human placenta.

Drug transporters in human placenta

Macrostructure and microstructure of human placenta is complex, and there are significant differences in placental

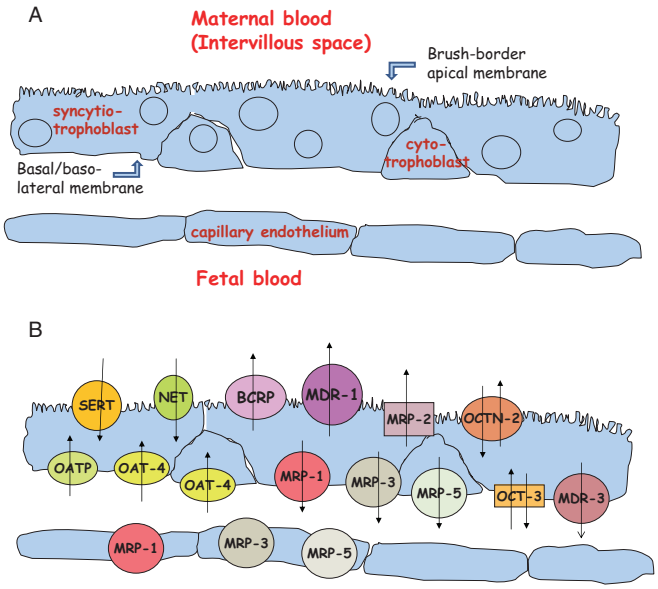

Figure 1 Human placental barrier at term. (A) The microstructure of placental barrier. (B) Main transporter proteins expressed in human placental barrier and their localization. BCRP, breast cancer resistance protein; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; NET, noradrenalin transporter; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCTN, organic cation transporter; SER, serotonin transporter.

structure and function between different species (Nau, 1986; Carney *et al.*, 2004; Enders and Carter, 2004; Carter, 2007). In the maternal side of human placenta, spiral arteries bring blood from uterine wall to the blood space around placental villi. A villus is formed by fetal vessels that branch from cord vessels, and are covered by endothelium inside and the trophoblasts outside facing maternal blood. The placental barrier or materno-fetal diffusion distance thins out during pregnancy so that the distance of over 50 μm at the late second month has reduced to less than 5 μm by the 37th week of pregnancy, consisting of an endothelial cell layer, thin layer of connective tissue and a continuous syncytiotrophoblast with some individual trophoblasts underneath (Figure 1A; Benirschke *et al.*, 2006; Castellucci and Kaufmann, 2006).

In the placenta, both syncytiotrophoblast and fetal capillary endothelium express transporters. In syncytiotrophoblast, transporters are found both in the brush border (apical membrane) facing maternal blood and basolateral membrane close to fetal capillaries where they transfer compounds in and out of syncytiotrophoblast (Syme *et al.*, 2004; Evseenko *et al.*, 2006b; Myllynen *et al.*, 2007). Apical and basal membranes of syncytiotrophoblast express different transporters, leading to polarized transport of the trophoblastic layer (Figure 1B). Transporters can either increase (uptake transporters) or decrease (efflux transporters) the concentration of compounds in the cell (see Lankas *et al.*, 1998; Smit *et al.*, 1999; Klaassen and Lu, 2008). Consequently, an efflux transporter at the apical surface of syncytiotrophoblast can protect the fetus from exposure while an efflux transporter on the basolateral surface can have a totally opposite effect. However, transporters localized in different membranes probably function in a synergistic manner. Grube and coworkers

(2007) have recently gained data to support such a functional interaction between ABCG2/BCRP and SLCO2B1/organic anion-transporting polypeptide (OATP)2B1 using a transfected cell model. The net effect, which is determined by the nature and localization of transporters in relation to placental cells and their substrate specificity, thus affects fetal exposure.

There is still a restricted amount of data about drug transporters and their role in human placenta, and even the localization of many transporters is still unknown (Table 1). A large number of drug transporters are expressed in human placental tissue: ABC transporters (ABCB1/MDR1/P-gp, ABCC1-3/MRP1-3, ABCG2/BCRP), organic anion transporters (OAT) (SLC21A8/OATP-8, SLC21A12/OATPA-E, SLC22A11/OAT4), organic cation transporters (SLC22A3/OCT3, SLC22A4/OCTN1, SLC22A5/OCTN2) and 5-HT (serotonin) transporter (SLC6A4/SERT), as well as a noradrenaline transporter (SLC6A2/NET) (Table 1; Wang *et al.*, 2007). Most of the ABC efflux transporters identified in the placenta are expressed on the apical membrane of syncytiotrophoblast facing maternal blood, and thus putatively displaying protection for the fetus against chemical insults (Behravan and Piquette-Miller, 2007). The most abundant transporters in the apical surface of syncytiotrophoblast are ABCB1/P-gp and ABCG2/BCRP. Although the significance of placental transporters on human fetal drug exposure is almost an unstudied field so far (Ganapathy and Prasad, 2005; Hodge and Tracy, 2007; Weier *et al.*, 2008), its potential use to design drugs that do not cross the placenta is already being pursued (Gedeon and Koren, 2006). Apart from transport, transporter proteins have been shown to have important physiological roles in murine development (Tachikawa *et al.*, 2005; Sawicki *et al.*, 2006) and in tissue defence (see Leslie *et al.*, 2005). Such functions in human placenta are almost an unexplored area. Both ABCB1/P-gp and ABCG2/BCRP are, however, putatively important in cellular protection in the placenta (Shiverick *et al.*, 2007).

Nishimura and Naito (2005; 2008) have studied the expression of a wide variety of human transporters at the mRNA level (Table 2). They found large differences in the expression level between tissues, and many of the transporters were expressed at the highest level in the placenta. The mRNA of SLC22A11/hOAT4 was expressed only in kidney and in placenta at about the same level. Among the studied tissues, the expression of ABCG2/BCRP mRNA was highest in placenta. Interpretation of mRNA results has to be cautious, however, because they do not necessarily correlate with the expression of proteins or functional activity (Table 3). It is interesting that in many cases, the same transporters are highly expressed both in placenta and testis (Table 2; Nishimura and Naito, 2005; Geyer *et al.*, 2007).

As expected from the function of the placenta in nutrient transport from mother to fetus, a number of amino acid transporters have been shown in human placenta (SLC1A, SLC6A, SLC7A, SLC16A, SLC36A, SLC38A, SLC43A) (Jansson, 2001; Cleal and Lewis, 2008). Although probably not important in drug transfer, amino acid transport through them can be inhibited by drugs and other chemicals. Pastrakuljic and coworkers (2000) reported various levels of inhibition by nicotine, cocaine or their combination depending on the amino acid, representing different types of amino acid transporters. L-type amino acid transporters are also involved, in addition to OATP and monocarboxylate transporter family, in the transport of thyroid hormones (James *et al.*, 2007). All of these are expressed in human placenta.

Table 2 Transporters with the highest mRNA expression in the placenta among 23 tissues, including spinal cord, but no other neuronal tissues (Nishimura and Naito, 2005)

Name	*Alternative names*	*Notes*
ABCB8	MACB1	Testis almost as high
ABCC10	MRP7	
ABCG2	BCRP	
SLC2A1	GLUT1	About 20 times higher than in any other tissue
SLC3A2	CD98	
SLC4A2	AE2, HKB3	10 times higher expression than in other tissues
SLC5A6	SMVT	Five times more than in testis; difference to other tissues very large
SLC6A2	NET, NAT1	About 10 times higher than in testis and adrenal gland; minute amounts or non-existent in other tissues
SLC7A1	CAT-1, HCAT1	Testis almost as high
SLC7A4	CAT4, HCAT3	Testis almost as high
SLC10A3	P3, DXS253E	
SLC15A3	hPTR3	
SLC21A12	OATP1, OATP-E	As high (or higher) in lung
SLC29A3	ENT3	

Forty-six human ABC transporters and 108 SLC transporters were analysed, including all the transporters mentioned in Table 1, except SLC21A8.
ABC, ATP-binding cassette; AE2, anion exchange protein 2; BCRP, breast cancer resistance protein; CAT-1/4, cationic amino acid transporter 1/4; ENT3, equilibrative nucleoside transporter 3; GLUT1, glucose transporter 1; HCAT1/3, human cationic amino acid transporter 1/3; HKB3, erythrocyte membrane protein band 3-like 1; MACB1, mitochondrial ATP-binding cassette 1; MRP7, multidrug resistance-associated protein 7; NAT1, noradenaline transporter 1; NET, noradrenaline transporter; OATP, organic anion-transporting polypeptide; SLC, soute carrier protein; SMVT, sodium-dependent multivitamin transporter.

Table 3 Drug transporters identified in human placenta and human choriocarcinoma cell lines

Placenta, primary trophoblasts	*JAR*	*Jeg-3*	*BeWo*
P-gp (1, 4)	mRNA (2), P (4)	mRNA (11)	mRNA (2), NP (4, 10)
BCRP (1, 4)	mRNA (2), P (4)	mRNA (2)	mRNA (2, 10), P (4, 10)
MRP1 (1, 4)	mRNA (2), P (4)	mRNA (2)	mRNA (2, 10), P (4)
MRP2 (1, 4)	mRNA (2), NP (4)		mRNA (2, 10), NP (4)
SERT (1)	F (3)		
NET (1)	NmRNA, NF (8)		
OCT2	NF (3)		
OCT3, EMT (1, 9)	NF (3)		
OCTN2 (5)			F (5,6)
hOAT4 (7)			NP (7)

Data from (1) Nishimura and Naito (2005), (2) Serrano *et al.* (2007), (3) Martel and Keating, 2003, (4) Evseenko *et al.* (2006a), (5) Hirano *et al.* (2008), (6) Rytting and Audus (2008), (7) Zhou *et al.* (2006), (8) Ramamoorthy *et al.* (1993d), (9) Wessler *et al.* (2001), (10) Myllynen *et al.* (2008), (11) Pavek *et al.* (2007).
BCRP, breast cancer resistance protein; EMT, extraneuronal monoamine transporter; F, functional; hOAT, human organic anion transporter; mRNA, mRNA found; MRP, multidrug resistance-associated protein; NET, noradrenaline transporter; NF, not functional; NmRNA, no mRNA; NP, minimal or no protein; OCT, organic cation transporter; P, protein found; P-gp, P-glycoprotein; SERT, 5-HT (serotonin) transporter.

Table 4 Significance of drug transporter polymorphisms in human placenta

Protein	*Gene polymorphisms*	*Type of study*	*Significance in placenta*	*Reference*
P-gp/MDR1	G2677A/T (Ala toThr/Ser) T-129C	100 human placentas	Less P-gp protein Less P-gp protein	1
P-gp/MDR1	G2677T/A C3435T	73 human placentas from Caucasians	No effect on MDR1 mRNA Homozygosity of 3435T and 2677T lead to lower protein levels	2
P-gp/MDR1	C3435T	44 human placentas	T allele associated with a higher expression	3
P-gp/MDR1	C3435T and G2677A/T	Human placental perfusion	No effect on saquinavir transfer	3, 4
P-gp/MDR1	C3435T	Human placental perfusion	3435T associated with increased transfer of quetiapine	5
MRP2	G1249A	58 human placentas	Reduced expression of MRP2 mRNA in preterm placentas only	6
BCRP	G34A (Val12Met) C421A (Gln141Lys)	99 human placentas	No effect on protein level Protein decreased	7

References: (1) Tanabe *et al.* (2001), (2) Hitzl *et al.* (2004), (3) Rahi *et al.* (2008), (4) Mölsä *et al.* (2005), (5) Rahi *et al.* (2007), (6) Meyer zu Schwabedissen *et al.* (2005b), (7) Kobayashi *et al.* (2005).
BCRP, breast cancer resistance protein; MDR, multidrug resistance protein; MRP, multidrug associated protein; OAT, organic anion transporter; P-gp, P-glycoprotein.

Regulation of transporters is complex. In pharmacology, their significance was first found in chemotherapy of cancer, where induction of the ABC transporters is one of the main mechanisms of chemoresistance to cytostatic medication (see Lage, 2008). Transporter proteins are induced in response to many xenobiotics and endobiotics as well as in response to inflammatory diseases, and this induction is mediated through xenobiotic-activated nuclear hormone receptors like the steroid and xenobiotic receptor (human orthologue of rodent pregnane X receptor), constitutive androstane receptor (CAR) and the farnesoid X receptor (see Teng and Piquette-Miller, 2008). Expression of mRNA of these nuclear receptors has been demonstrated both in primary trophoblastic cells isolated from term placenta as well as in human choriocarcinoma cell lines BeWo, JAR and Jeg-3 (Serrano *et al.*, 2007). Because nuclear receptors take part in the regulation of transporters, it is feasible to assume that transporters in placenta are also amenable to environmental induction and inhibition. However, because the regulation of transporters by nuclear receptors is tissue specific (Teng and Piquette-Miller, 2008), the role of such regulation in the responses of placental transporters needs to be studied separately. It is known that the major cytochrome P450, CYP1A1, in the placenta is induced by cigarette smoke (Vähäkangas *et al.*, 1989; Kolwankar *et al.*, 2005) as in other tissues and that this induction is mediated through a nuclear Ah-receptor (Hakkola *et al.*, 1997). However, when comparing 10 placentas from smokers with 10 placentas from non-smokers, Kolwankar *et al.* (2005) found no statistically significant differences in ABCB1/P-gp/MDR1 or ABCG2/BCRP protein level.

Transporters are under hormonal regulation in the placenta. ABCG2/BCRP expression is regulated by oestradiol and progesterone in BeWo cells, a human trophoblastic cell line (Vore and Leggas, 2008 and references within; Wang *et al.*, 2008a,b). Glucocorticoids regulate P-gp in human primary trophoblasts (Hahn *et al.*, 1999) and in trophoblastic cell lines BeWo and Jeg-3, where Pavek and coworkers (2007) showed glucocorticoid receptor α-mediated transcriptional regulation of P-gp. Diseases like infection, inflammation and cancer may change the expression of transporters (Petrovic *et al.*, 2007; Morgan *et al.*, 2008). Interestingly, Evseenko and coworkers (2007a), using human primary term trophoblasts, showed that the apical ABC transporters are differentially regulated from basal ABC transporters by a number of cellular factors like growth hormone and cytokines. Down-regulation of apical transporters and concurrent up-regulation of basal transporters by such factors may lead to decreased protection of the fetus from toxic effects of xenobiotics.

Recently found proteins containing the PDZ domain (the most common protein-protein interaction domain in humans; Jemth & Gianni, 2007) bind to the C-terminus of many transporters (Sugiura *et al.*, 2006). PDZK1 increases *in vitro* the transport activity of SLC22A11/OAT4 (cell surface expression increases) and SLC22A5/OCTN2 (cell surface expression does not increase), which are expressed in human placenta (Wang *et al.*, 2007). In addition, ABCC2/MRP2, also expressed in human placenta, interacts directly with PDZK1. It remains to be seen whether this regulation pathway has significance in human placenta. Genetic polymorphisms (Kobayashi *et al.*, 2005; Mourad *et al.*, 2008) and ethnic differences in the frequency of polymorphic variants (Tanabe *et al.*, 2001) probably cause the ethnic variation (see Cropp *et al.*, 2008) and individual variation in the level and activity of transporters (Table 4). The significance for the function of placenta of such variation is incompletely understood currently.

ABCB1 and ABCB4 (P-gps) in placenta

ABC transporter proteins can be classified into two classes, full and half transporters (Rocchi *et al.*, 2000). The majority of functional ABC transporters including ABCB1 and ABCB4 (P-gp) belongs to the family of full transporters. These transporters consist of two ATP-binding domains for the energy to transport substrates across the membrane and two sets of transmembrane domains providing a passageway for the transported molecule (Rocchi *et al.*, 2000; Doyle and Ross, 2003). The organization of transmembrane domains differs between the ABC transporters and is essential for the transport itself (Borst and Oude Elferink, 2002; Leslie *et al.*, 2005).

The ABCB1 gene encodes the MDR1 P-gp and ABCB4 gene MDR3 P-gp, both of which are drug transporters (Smith *et al.*, 2000; Schinkel and Jonker, 2003; Evseenko *et al.*, 2007a). ABCB1/MDR1 is a phosphorylated 170 kDa-sized glycoprotein (Schinkel and Jonker, 2003; Evseenko *et al.*, 2007a) and probably the most widely studied drug efflux transporter. It is expressed mostly in the barriers of human body, like in the blood–brain, blood–testis, blood–nerve and fetal–maternal barrier. The first to describe P-gp expression in normal human full-term placenta using immunohistochemistry were Sugawara and coworkers (1988), a finding later confirmed by Cordon-Cardo *et al.* (1990). Currently, P-gp is among the best studied transporters in human placenta (for reviews, see Ceckova-Novotna *et al.*, 2006; Behravan and Piquette-Miller, 2007; Wang *et al.*, 2007). In human placenta, ABCB1/MDR1 is localized in the maternal-facing apical membrane of placental syncytiotrophoblast where it putatively protects the developing fetus from xenobiotics and drugs present in maternal circulation by active back-transport from early gestation until term (Schinkel and Jonker, 2003; Sun *et al.*, 2006).

The ABCB4/MDR3 protein is 170 kDa in size and a close homologue to ABCB1/MDR1. ABCB4/MDR3 is a transmembrane protein, which functions as a lipid translocator, and is mainly located in the liver (Oude Elferink and Paulusma, 2007), but it is also expressed in placenta in the basolateral membrane of syncytiotrophoblast (Evseenko *et al.*, 2007a).

ABCB1/P-gp has been detected in human placental trophoblasts from the first trimester to term, but its expression does not stay constant. The expression of both the ABCB1 gene and P-gp decreases in human placenta by gestation (Mathias *et al.*, 2005; Sun *et al.*, 2006), in contrast to rat placenta where it has been reported to increase with advancing gestation (Novotna *et al.*, 2004). However, in mouse, P-gp expression decreases with advancing gestation, as in humans. Implying that this decrease in mouse has functional consequences, Petropoulos *et al.* (2007) recently demonstrated a significant increase in transplacental transfer of ^{3}H-digoxin in late gestation when compared with earlier embryonic days.

ABCB1/MDR1/P-gp transports a large variety of substrates, mainly hydrophobic compounds, including a wide variety of therapeutic drugs (Schinkel & Jonker, 2003; Zhou, 2008). Drugs from the same group may behave differently, in terms of drug interactions, due to different metabolism and interaction with transporters. Among the clinically important group of statins, simvastatin, lovastatin and atorvastatin inhibit P-gp *in vitro* and may also be substrates, while pravastatin and fluvastatin do not significantly inhibit P-gp (Holtzman *et al.*, 2006). It would be important to take such differences into account in studies of placental transport and inhibition of transport, in pharmacotherapy during pregnancy. However, so far, a limited number of studies exist. In human placental perfusion, functional inhibition of placental P-gp leads to increased transfer of saquinavir (Mölsä *et al.*, 2005), but does not affect the transfer of quetiapine, an atypical antipsychotic (Rahi *et al.*, 2007).

Many polymorphisms of ABCB1/MDR1/P-gp have been characterized with clinical significance to drug resistance and pharmacokinetics of its substrates (Wang *et al.*, 2007; Zhou, 2008). ABCB1/MDR1 gene mutations, and the SNPs G2677T and C3435T, are associated with variations in the expression level of placental P-gp (Tanabe *et al.*, 2001; Hitzl *et al.*, 2004; Rahi *et al.*, 2008). In human placental perfusion, ABCB1/P-gp genotypes C3435T and G2677A/T had no effect on saquinavir transfer (Mölsä *et al.*, 2005; Rahi *et al.*, 2008), while the 3435T allele was associated with an increase in the placental transfer of quetiapine (Rahi *et al.*, 2007). Interestingly, the haplotype, not only non-synonymous SNPs seem to determine the function of a transporter. In the case of P-gp, two synonymous SNPs, C1236T and C3435T, in combination with a non-synonymous SNP, G2677T, alter the interaction of P-gp with its substrates, while G2677T alone has no effect (Sauna *et al.*, 2007). Such alterations may well affect the exposure of human fetus to drugs used in pregnancy with a possibility of harmful effects.

ABCG2/BCRP in placenta

ABCG2 is also known as BCRP, mitoxantrone resistance protein (Miyake *et al.*, 1999) or placental ABC protein (Allikmets *et al.*, 1998). ABCG2/BCRP was first isolated from the MCF-7 breast cancer cell line as late as 1998 (Doyle *et al.*, 1998), therefore gaining the name BCRP. ABCG2/BCRP is a half transporter of approximately 75 kDa, forming most likely a homodimer or oligomer (homotetramer) (Xu *et al.*, 2004). Placenta is among the tissues with the highest ABCG2/BCRP expression (Maliepaard *et al.*, 2001), where it localizes predominantly in the apical surface of syncytiotrophoblast (Ceckova *et al.*, 2006).

ABCG2/BCRP was originally identified to confer drug resistance against anthracycline antitumour drugs and mitoxantrone (Doyle *et al.*, 1998). Currently, it is known that ABCG2/BCRP is able to transport a broad spectrum of substrates, ranging from chemotherapeutic agents to organic anions, with various other functions, like differentiation, as shown in BeWo cells (Evseenko *et al.*, 2007b) and protection of stem cells against hypoxia (see Sarkadi *et al.*, 2004). ABCG2/BCRP is possibly important in the protection of placental cells (Shiverick *et al.*, 2007) and it protects trophoblasts from tumour necrosis factor (TNF)-induced apoptosis, and its expression is down-regulated by TNF-α (Evseenko *et al.*, 2007a). Many drugs that may be used during pregnancy such as nitrofurantoin (Merino *et al.*, 2005), cimetidine (Pavek *et al.*, 2005) and glyburide (Gedeon *et al.*, 2008; Pollex *et al.*, 2008) are ABCG2/BCRP substrates (see also Mao, 2008). Interestingly, ABCG2 substrate specificity seems to partially overlap with the substrate specificities of other ABC transporters, P-gps and ABCC2/MRP2 (Table 1). ABCG2/BCRP limits the transfer of drugs from maternal to fetal circulation in the same way as the ABCB1/P-gp. In mouse placenta, Bcrp1 has been shown to limit the transfer of nitrofurantoin (Zhang *et al.*, 2007), topotecan (Jonker *et al.*, 2000), genistein (Enokizono *et al.*, 2007) and glyburide (Zhou *et al.*, 2008a). The functional role of ABCG2/BCRP has also been studied in models utilizing human placental tissue. Gedeon *et al.* (2008) recently showed that inhibition of ABCG2/BCRP by novobiocin increases the intra-vesicular accumulation of [(3)H]-glyburide into the inside-out membrane vesicles isolated from human term placenta. This was confirmed by a human

placental perfusion study showing that nicardipine, also an inhibitor of ABCG2/BCRP, increased the fetal to maternal concentration ratio of glypuride (Pollex *et al.*, 2008). In our recent study, we showed that ABCG2/BCRP decreases the fetal : maternal concentration ratio of a food carcinogen, PhIP, in perfused human placenta (Myllynen *et al.*, 2008).

As with P-gp expression, ABCG2/BCRP expression may also change as gestation advances. In the study by Meyer zu Schwabedissen *et al.* (2006), both mRNA and protein levels of ABCG2/BCRP in human placenta at preterm were approximately twofold higher than at term. However, two other studies report contradictory results. In a study by Yeboah *et al.* (2006), the mRNA levels of ABCG2/BCRP in the placenta did not change significantly as gestation progressed, while the protein levels increased towards the end of gestation. On the other hand, another human study with a limited number of tissue samples (Mathias *et al.*, 2005) suggested that ABCG2/BCRP protein and mRNA expression do not significantly change in placenta during gestation.

Pregnancy-related steroid hormones, growth factors and cytokines have been suggested as regulators of ABCG2/BCRP expression in human placenta (Mao, 2008). BCRP can also be induced by some environmental chemicals. The promoter region of the ABCG2/BCRP gene contains a functional oestrogen response element (Ee *et al.*, 2004; Staud and Pavek, 2005) and progesterone response element (Wang *et al.*, 2008a). In a recent study by Wang *et al.* (2008a), progesterone induced ABCG2/BCRP expression in BeWo cells via progesterone receptor isoform B, but not via the A isoform. In mouse placenta, the regulation of Bcrp1 seems to differ from that in human placenta, because in the study by Kalabis *et al.* (2007), exogenous progesterone did not induce Bcrp1, the murine homologue of ABCG2/BCRP. It has been reported recently that oestriol, human placental lactogen and human prolactin can induce ABCG2/BCRP expression in BeWo cells at physiological concentrations (Wang *et al.*, 2008b). Earlier reports were contradictory about the effects of 17-β-oestradiol in BeWo cells: Yasuda *et al.* (2006) found ABCG2/BCRP mRNA and protein levels to be increased, while in the hands of Wang and coworkers (2006), 17-β-oestradiol down-regulated ABCG2/BCRP expression. Up-regulation of ABCG2/BCRP is seen in primary trophoblasts (Evseenko *et al.*, 2007a). Also, other factors such as cytokines and growth factors may affect ABCG2/BCRP expression levels in the placenta (see Mao, 2008). ABCG2/BCRP in other tissues is regulated also by the Hif-1 pathway, peroxisome proliferator-activated receptor-γ and retinoid X receptor, all of which are also active in human trophoblasts and may thus have a regulative role in placenta (Vore and Leggas, 2008 and references therein). Another level of regulation is probably the coordinated expression of different transporters. Grube and coworkers (2007) have recently described a correlation between the mRNA expression of ABCG2/BCRP and SLCO2B1/OATP2B1 in human placenta. They also gained support for functional interaction between these proteins in the transport of steroid sulphates in a transfected kidney cell model where OATP2B1 was expressed in the basolateral membrane and ABCG2/BCRP in the apical surface, as they are localized in syncytiotrophoblast.

ABCG2/BCRP is highly polymorphic and the frequency of variants is different in different ethnic groups (Kobayashi *et al.*, 2005). At least one non-synonymous polymorphism (C421A; Gln141Lys) seems to lead to a lower expression of ABCG2/BCRP protein both *in vitro* (Imai *et al.*, 2002) and in human placenta (Kobayashi *et al.*, 2005). A C376T (Gln126 to STOP codon) polymorphism may have significance also in the placenta, because the 376T allele does not produce a protein (Imai *et al.*, 2002).

ABCC family (MRPs) in placenta

Placenta expresses several ABCC family transporters in apical and basal membranes of syncytiotrophoblast as well as in fetal capillary endothelium. However, compared with ABCB1/P-gp or ABCG2/BCRP, even less is known about their functional significance in the placenta. ABCC2/MRP2 is enriched in the apical membrane of syncytiotrophoblast facing maternal blood (St-Pierre *et al.*, 2000; Meyer zu Schwabedissen *et al.*, 2005b) similarly to ABCB1 and ABCG2, while ABCC1/MRP1 has been found in the basal membrane of syncytiotrophoblast (Figure 1B; Atkinson *et al.*, 2003; Nagashige *et al.*, 2003). ABCC3/MRP3 has been suggested to localize to capillary endothelium as well as in the apical membrane of syncytiotrophoblast (St-Pierre *et al.*, 2000). ABCC5/MRP5, a transport protein for cyclic nucleotides and putatively antiviral nucleoside drugs, has been found especially in the basal membrane of syncytiotrophoblast and in fetal vessels near them (Meyer zu Schwabedissen *et al.*, 2005a). Also, other ABCC transporters have been detected at mRNA level in human placenta (Nishimura and Naito, 2005) but their expression at protein level and localization is still unknown.

The expression of ABCC/MRP proteins varies with the stage of gestation. The mRNA and protein expression of ABCC5/MRP5 is highest in early preterm placentas and decreases towards term (Meyer zu Schwabedissen *et al.*, 2005a). Also, there is larger interindividual variation in preterm placentas. In isolated trophoblasts, ABCC5/MRP5 expression is associated with human chorionic gonadotrophin (hCG) production of the cells. The expression of ABCC2/MRP2 mRNA and protein is a total mirror image of ABCC5/MRP5 with an increase by gestation (Meyer zu Schwabedissen *et al.*, 2005b).

Substrate specificity of ABCC1/MRP1 is extremely broad and includes many drugs, including antineoplastics, antivirals and antibiotics, and toxic compounds like aflatoxin B1 and methoxychlor (Deeley and Cole, 2006). The major difference in substrate specificity between ABCCs and P-gp is the known ability of MRPs to actively transport conjugates of steroid hormones and some foreign compounds in addition to the parent compound. It has been suggested that the physiological function of ABCC/MRP proteins in the placenta could be removal of metabolic end products from the fetus (Leslie *et al.*, 2005). While both ABCC1/MRP1 and ABCC3/MRP3 transport glucuronide conjugates, only ABCC1/MRP1 transports glutathione (GSH) and GSH conjugates, and the major contributor to this activity has been localized to Tyr440, which is substituted by Phe in ABCC3/MRP3 (Grant *et al.*, 2008). Saquinavir induces both P-gp and ABCC1/MRP1 in trophoblastic cells at mRNA and protein level, and its transport towards basolateral direction is increased by

cyclosporin, an inhibitor of both P-gp and ABCC1/MRP1 (Parry and Zhang, 2007). However, more information about the transporter specificity of saquinavir is necessary to show that saquinavir is a substrate of ABCC1/MRP1 in human placenta.

ABCC2/MRP2 has been shown to have functional significance in human placenta. Recently, May and coworkers (2008) demonstrated, in human placental perfusion, that materno-fetal transfer of talinolol is inhibited by functional inhibition of ABCC2. Because the expression of ABCC2 increases towards late pregnancy, they discuss the possibility that placental ABCC2 may be more important than ABCB1 for drug transport in late pregnancy. Genetic polymorphisms of ABCC2/MRP2 have been found and at least one of them (G1249A) results in lower ABCC2/MRP2 expression.

OATs (SLC10, SLC21A3-A15, SLC22A6-A12)

OATs are anion exchangers, which take up one organic anion to the cell while liberating another outside from the cell, and have very broad substrate specificity, including many drugs (Rizwan and Burckhardt, 2007). The typical structure of OATs include 12 transmembrane domains, intracellular N-terminal and C-terminus, and a long extracellular loop between the transmembrane domains 1 and 2 (with glycosylation sites) and a large intracellular loop between the transmembrane domains 6 and 7 (with phosphorylation sites).

The SLC22 family (see Rizwan and Burckhardt, 2007) includes the OAT4 (SLC22A11/OAT4), which is the only transporter specific for humans and expressed mainly in placenta and kidney. There are discrepancies in the published values for the SLC gene number as well as the chromosomal localization of OAT4 [SLC22A11 at 11q13.1 (Rizwan and Burckhardt, 2007), SLC22A9 at 11q12.3 (Wang *et al.* (2007) or SLC22A11 (Ugele *et al.*, 2003)]. Eight non-synonymous changes have been described in the OAT4 gene (see Rizwan and Burckhardt, 2007). OAT4 interacts with apical scaffolding protein (NHERF1) and PDZK1 through the so-called PDZ motif, which anchors OAT4 in the plasma membrane (see Rizwan and Burckhardt, 2007). In human trophoblastic BeWo cells, OAT4 activity is down-regulated by progesterone, because it is translocated into cytoplasm and the activation of protein kinase C, while oestradiol has no effect (Zhou *et al.*, 2006; 2007).

Both OATP2B1 (OATP-B) and OAT4 are localized on the basolateral surface of syncytiotrophoblast and membranes of cytotrophoblasts, and their physiological function is the uptake of fetus-derived steroid sulphates (Ugele *et al.*, 2003; 2008). Different functions of these two transporters in the placenta are suggested by the finding that the affinity of OAT4 towards dehydroepiandrosterone sulphate (DHEAS) is 10 times higher than the affinity of OATP2B1, implying that OAT4 is the major transporter providing C-19 steroids for placental oestrogen synthesis (Ugele *et al.*, 2008). On the other hand, the putatively coordinated function in the transport of steroid sulphates by ABCG2/BCRP and OATP2B1 (Grube *et al.*, 2007) would suggest OATP2B1 as an important player as well.

Many more of the OATs may be involved in placental transport. There is evidence of OATP-E being expressed at protein level in human placenta, predominantly in the apical surface of syncytiotrophoblast (Sato *et al.*, 2003). OATP-E transports thyroid hormones in various tissues and may thus be involved in the transport of thyroid hormones also in the placenta. OATP-8 mRNA is expressed in human placenta and trophoblast cells, and mRNA from these sources can confer the ability to take up OATP-8 substrates, such as unconjugated bilirubin and 17-β-D-glucuronyl oestradiol, on *Xenopus laevis* oocytes (Briz *et al.*, 2003). Sodium-dependent OAT (SLC10A6) is highly expressed in testis and relatively highly expressed in placenta and pancreas (Geyer *et al.*, 2007). In addition to sulphates of some hormones, for example, oestrone-3-sulphate, sulphates of methylpyrenes found in cigarette smoke are proven substrates. Additionally, expression of other OATPs like OATP-8 and OATP-D, which may carry frequently prescribed drugs as digoxin or fexofenadine, was described in the human placenta (Kullak-Ublick *et al.*, 2001; Briz *et al.*, 2003; Ugele *et al.*, 2003; Shimizu *et al.*, 2005). Because many drugs are substrates of OATPs, the role of OATPs in human placental transport should be characterized further.

Organic cation transporters (OCT3 or extraneuronal monoamine transporter (EMT), OCTN1, OCTN2)

Sixteen members of the polyspecific organic cation transporters have been identified in humans (see Koepsell *et al.*, 2007). Typical structures of organic cation transporters from the SLC22 family, like many OATs, include 12 transmembrane domains, intracellular N-terminus and C-terminus, a long extracellular loop between the transmembrane domains 1 and 2 (with glycosylation sites) and a large intracellular loop between the transmembrane domains 6 and 7 (with phosphorylation sites) (Koepsell *et al.*, 2007). Three organic cation transporters are found in human placenta: OCT3 (SLC22A3), OCTN1 (SLC22A4) and OCTN2 (SLC22A5) (Table 1). Two of these, OCT3 and OCTN2, are very strongly expressed in the placenta, and there is evidence that from OCT1-3, only OCT3 is expressed in the placenta (Sata *et al.*, 2005). Polymorphisms of the human OCT3 gene are known (Lazar *et al.*, 2003; 2008; Ayoama *et al.*, 2006), but their significance for placental expression and function are unknown. Genetic variation in OCTN2 leading to carnitine deficiency is well known (see Koepsell *et al.*, 2007). Polymorphisms with more subtle effects on OCTN2 function have also been described. However, there are no published data on the effects of these polymorphisms on the transplacental transport.

SLC22A5/OCTN2, a 63 kDa high affinity carnitine uptake protein, is most probably localized in human placenta in the apical surface of syncytiotrophoblast (Grube *et al.*, 2005). This carnitine transporter has been shown to transport L-carnitine also in BeWo cells (Rytting and Audus, 2005; 2008). Fluoroquinolones (Hirano *et al.*, 2008), as well as levamisole and progesterone (Rytting and Audus, 2008), inhibit L-carnitine transport in human trophoblastic BeWo cells. Drugs which are known substrates (amiloride, cimetidine) or inhibitors (quinidine) of OCT1-3 were shown to inhibit acetylcholine release in pieces of human placental tissue by Wessler and

coworkers (2001), indicating that OCTs are active in human placenta and sensitive to drugs.

5-HT and noradrenaline transporters (monoamine transporters)

5-HT and noradrenaline transporters (SERT and NET, respectively) belong to a large superfamily of Na/Cl-dependent transporters, which consist of about 600 amino acids with 12 transmembrane domains (see Gill *et al.*, 2008). Both are expressed in human placenta: Bottalico and coworkers (2004) found high expression of NET mRNA in anchoring villi and lower levels in chorionic villi, while SERT mRNA was localized mainly in chorionic villi. Human placental SERT and NET transporters have been characterized in a series of papers (Ramamoorthy *et al.*, 1993a,b,c,d; 1995; Jayanthi *et al.*, 1994). They showed that brush border membrane vesicles accumulate noradrenaline Na/Cl dependently and demonstrated two NET mRNA of different sizes in the vesicles (Ramamoorthy *et al.*, 1993d). In the same study, human placental choriocarcinoma cells (JAR cells) did not accumulate noradrenaline, nor did they contain the relevant mRNA. Although only one SERT gene exists in human genome in chromosome 17, three mRNAs hybridize to the probe in human placenta (Ramamoorthy *et al.*, 1993a). SERT expression in human choriocarcinoma cells can be induced by compounds, increasing intracellular cyclic adenosine monophosphate (cAMP) levels (Ramamoorthy *et al.*, 1993b). The expression of NET mRNA was highest in human placenta among 23 tissues assayed, including spinal cord, but no other neuronal tissues (Table 2; Nishimura and Naito, 2005). Except for testis and adrenal gland, other tissues contained only minute amounts of the transcript.

The placental 5-HT transporter (SERT), originally cloned by Lee and coworkers in 1986 (see Ramamoorthy *et al.*, 1993b), is identical to that in neurons and platelets (Ramamoorthy *et al.*, 1993a; Jayanthi *et al.*, 1994). Human SERT is a glycoprotein with 630 amino acids that localizes either on cell membrane or intracellularly, depending on the extracellular concentration of its substrates (Ahmed *et al.*, 2008 and references therein). The function of SERT in the placenta has been proposed to be the control of the concentration of vasoconstrictive 5-HT in the intervillous space to ensure sufficient blood flow to the placenta (see Kekuda *et al.*, 2000). Because 5-HT regulates the development of the nervous system during early embryogenesis, the SERT system in placenta may be also an important provider of this neurotransmitter to the embryo (see Unal *et al.*, 2007).

The regulation of SERT is probably both transcriptional (Ramamoorthy *et al.*, 1993b) and post-translational (Jayanthi *et al.*, 1994) in human placenta. Placental SERT is predicted to have 12 membrane-spanning domains, with both ends of the protein in the cytoplasmic side of the membrane. The C-terminus of the protein is important for the localization of the transporter in the cell membrane (Larsen *et al.*, 2006) and is the site where a small T and regulating protein (Rab-4), in its active guanosine triphosphate (GTP)-bound form, binds to SERT to retain it in cytoplasm (Ahmed *et al.*, 2008). Many other proteins, including neuronal nitric oxide synthase (nNOS), also bind the C-terminus of neuronal SERT to regulate its localization and function (Chanrion *et al.*, 2007), but these have not been studied in the placenta. In JAR choriocarcinoma cells constitutively expressing SERT, interleukin (IL)-1 activates transcription of SERT through IL-1 receptors, which occur in normal human placenta more abundantly than in JAR cells (Kekuda *et al.*, 2000). It was also shown quite recently that diabetic concentrations of glucose down-regulate SERT in JAR cells (Unal *et al.*, 2007).

Although the function of placental SERT is not known, the similarity of SERT in different tissues (see Kekuda *et al.*, 2000) suggests similar effects of drugs on placental SERT. On the other hand, studies on intestinal SERT suggest species differences in the expression and localization (Gill *et al.*, 2008 and references therein). The primary function of SERT is to transport 5-HT back to neurons from the synaptic cleft, which would imply that tricyclic antidepressants and serotonin specific reuptake inhibitors (SSRI) drugs may inhibit SERT function also in the placenta.

Some drugs of abuse have been shown to affect monoamine transporters. Ramamoorthy and coworkers (1995) showed that both placental SERT and NET are inhibited by cocaine and amphetamines, so that SERT is inhibited more by cocaine and NET is inhibited more by amphetamines. Whether medicinal drugs, illegal drugs or other chemicals, the inhibition of cellular uptake of 5-HT and/or noradrenaline putatively leads to their increased concentration in the intervillous spaces, decreasing blood flow from uterus and affecting, thus, placental function (Ramamoorthy *et al.*, 1995; Kekuda *et al.*, 2000). It is not inconsistent with this theory that mRNAs of NET and one of the organic cation transporters, EMT, are decreased in pre-eclamptic placentas compared with controls (Bottalico *et al.*, 2004).

Conclusions

A significant number of known functional drug transporters, and even more at mRNA level, have been found in human placenta. However, not very much is known about the regulation of their expression in human placenta, or about the significance of drug transport to the fetus. Animal studies strongly suggest the importance of placental drug transporters in placental function and fetal development and safety. However, the structure of human placenta is complex and there are significant differences in placental structure and function between different species, creating difficulties in the extrapolation of animal results to humans. Results from various *in vitro* models are partly discrepant, which raises the question of how to best study transport to the fetus. Experimental models utilizing human placental tissue, especially human placental perfusion, offer valuable possibilities, which have been inadequately assessed so far. Clinical use of transporters and their inhibitors in drug treatment requires more detailed studies. As important are the mechanistic studies, hopefully creating ideas and possibilities to utilize the knowledge in drug development and fetal protection.

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Conflict of interest

Neither of the authors has any conflicts of interest.

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